workbench Software

bms bio molecular systems

Made for extraordinary life

Workbench User Manual

Version	Revision	Language	Release Date
2.0.0	**A**	**English (EN)**	**July 2025**

Contents

Legal Notice and Disclaimer

Intended Use

Workbench is integrated software designed for use with Bio Molecular Systems' Myra, Myra9, Myra+, and Mic qPCR instruments. It is intended to assist in the setup, execution, and analysis of molecular biology and biochemical workflows.

The Myra, Myra9, and Myra+ instruments are intended for automated liquid handling in molecular biology applications, including those in medical, agricultural, forensic, pharmaceutical, and life science research.

The Mic and the on-deck Mic component of Myra+ are intended for performing real-time PCR and melt curve analysis for similar research applications.

All instruments and software are intended for use by trained laboratory personnel—including research staff, technicians, and scientists experienced in molecular biology or biochemical engineering—and are designated for Research Use Only.

Licensing and Patents

The purchase of the Mic qPCR Cycler with HRM capabilities includes a limited, non-transferable license for research use (excluding human and veterinary in vitro diagnostics) under specific claims of the following U.S. patents:

6,174,670; 6,569,627; 7,387,887; 7,582,429; 7,670,832; 7,803,551; 8,068,992; 9,093,002; 8,296,074; and 9,273,346.

These patents are owned by the University of Utah Research Foundation and licensed to BioFire Defense, LLC, and Roche Diagnostics GmbH.

Certain polymerase chain reaction (PCR) methods may involve technologies protected by additional third-party patents. It is the responsibility of the user to ensure all necessary rights and licenses are obtained for their intended applications.

Safety Information

Before using any BMS instrument, it is important to read this user manual to familiarise yourself with its operations. Follow all instructions to ensure proper operation. Do not use any accessories or external equipment other than those specified. Safety warnings must be always adhered to avoid risk in personal injury and/or damage to the instrument. If the equipment is used in a manner not specified by BMS, the protection provided by the equipment may be impaired.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements established in the user's country.

Warning Symbols

The following safety warnings appear throughout this manual.

WARNING 	Electrical hazard.
WARNING 	Follow instructions to avoid risk in personal injury.
CAUTION 	Follow instructions to avoid damage to the instrument.
PINCH POINT 	Keeps fingers and hands clear of sliding parts to avoid personal injury.
HOT SURFACE 	The temperature of the rotor may be above 40°C (104°F). To avoid personal injury, do not touch the rotor during a run or for 5 minutes following an aborted run.
BIOLOGICAL HAZARD 	There is potential for exposure to infectious agents when working with equipment used in molecular biology. To avoid exposure to such hazards, ensure proper personal protective equipment is worn, and laboratory best practise is adhered to.
ATTENTION 	Follow instructions to ensure optimal instrument performance.

Proper Use Warnings

WARNING 	**Malfunctioning Lid Open Sensor:** Do not use the Myra instrument if the lid sensor is not functioning. There is a high risk of personal injury from parts that are moving rapidly.
WARNING 	**Lethal Voltages Inside Instrument:** When the instrument is connected to line power, terminals may be live. Opening covers or removing parts is likely to expose live parts.
WARNING 	**Power Supply Grounding:** Power supply must be connected to an outlet with appropriate grounding means.
WARNING 	**Main Supply Cord:** Do not replace detachable main supply cord with an inadequately rated cord.
UV HAZARD 	**UV Radiation Hazard:** Use only with Myra lid down. Protect eyes and skin from exposure to UV light. Do not use if lid windows are damaged or lid safety switch is malfunctioning.
CAUTION 	**Positioning the Instrument:** Do not position the instrument in a manner making it difficult to operate and access the device.
CAUTION 	**Do Not Obstruct Myra Back Vents:** Keep the back vents free from obstruction to prevent interference with the HEPA filter.
CAUTION 	**Do Not Move Instrument During Operation:** Movement may impair the proper function of the instrument resulting in poor performance.
CAUTION 	**Remove Travel Locks Before Powering Instrument:** Remove the travel locks before powering the instrument on. Failure to remove the travel locks may result in damage to the unit if the axis drivers try to move.
CAUTION 	**Authorised Service Only:** There are no user serviceable parts inside the instrument. Service should only be performed by an authorised party.
CAUTION 	**Transport Instrument Using Travel Locks and Approved Packaging:** Always transport the Myra with travel locks applied and in the original shipping container. Failure to apply travel locks and using incorrect shipping container when moving the Myra will void your warranty.
CAUTION 	**Power Disconnection:** The power cord set is to be used as a means of power disconnect. Pull the equipment power cord only in case of emergency.

WARNING 	**Do Not Clean the Chamber with Flammable Liquids:** The Mic chamber can reach temperatures above 100°C (212°F). Any flammable liquids in the chamber could be a fire risk.
WARNING 	**Damaged Lid:** Do not use the Mic if the lid is broken or if the lid lock is damaged. There is a high risk of personal injury to the user through parts that are moving, electrically live, or are hot.
HOT SURFACE 	**Hot Surface:** In the event of a user aborted run do not open the lid until the instrument has cooled. The rotor within the chamber could be above 40°C (104°F). To avoid personal injury, do not touch the rotor for at least 5 minutes.
CAUTION 	**Magnetic Tube Clamp:** Ensure the magnetic tube clamp is in place before starting a run to prevent caps and tubes from coming out of wells during a run. The magnetic tube clamp must be compatible with the tubes being used, e.g., a *V-tube* compatible clamp should be utilised for *V-Cap tubes*.
CAUTION 	**Avoid Spilling Liquid into the Chamber:** Any solution that spills onto electronic boards could cause a short circuit, damaging the instrument.
CAUTION 	**Do Not Obstruct Cooling Vents:** Keep the cooling vents free from obstruction to prevent interference with the cooling of the Mic instrument.
CAUTION 	**Power Connection:** Avoid removing the power connector from the instrument before the power indicator light is off on the power adaptor. Failure to do so may result in electrical arcing.

Biological Safety

Handle biological material with care and in accordance with required safety regulations. Always wear proper personal protection equipment (PPE). The user must take necessary precautions to ensure the surrounding workplace is safe and instrument operators are suitably trained and not exposed to hazardous levels of infections agents (HHS, 2020).

BIOLOGICAL HAZARD 	There is potential for exposure to infectious agents when working with equipment used in molecular biology. To avoid exposure to such hazards, ensure proper personal protective equipment is worn, and laboratory best practise is adhered to.

Decontamination of Instrument

Surfaces of the instruments, including the Myra deck, Mic chamber and tube clamp, can be decontaminated using a solution of sodium hypochlorite (NaOCl). A solution containing 1 gL^{-1} available chlorine will be suitable for sanitation in a general lab environment; stronger solutions (5 gL^{-1}) are recommended when dealing with high-risk situations (WHO, 2020).

Disposal of Waste

Disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

Type Plate Symbols

	**Regulatory Compliance Mark:** This device is compliant with applicable ACMA technical standards for EMC.
	**FCC Declaration of Conformity:** This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.
	**UL Listing:** UL has tested representative samples of the product and determined that it meets UL's requirements for Laboratory Equipment.
	**CE Marking:** The device is in compliance with the essential requirements and other relevant previsions of Low Voltage Directive 2014/95/EU
	**WEEE:** Waste Electrical and Electronic Equipment Directive 2012/19/EU. Do not dispose of the instrument with general waste.

Introduction

Workbench is the unified control software for Bio Molecular Systems' portfolio of instruments: the Mic qPCR Cycler, the Myra and Myra9 liquid handlers, and the fully integrated Myra+ system. From assay design through data analysis, Workbench lets you configure, run, and interpret your experiments—all from one intuitive application.

Summary of Changes in Workbench v2.0 from Workbench v1.0

Instrumentation

- Introduction of the Myra9
- New 20 µL pipette head for low volume work
- New multi-channel head to reduce pipetting times.
- Multi-dispense to further speed up loading times

MyraIntelligence

- New AI applications
- MyraSim: new simulator to allow you to test your protocols before a run
- MyraVision: Plate scanning option introduced
- MyraSense: using the pressure sensor to determine liquid classes

User Interface

- Updated to a modern Windows 11–style theme
- Run Types renamed for clarity
- New Start Screen layout introduced

qPCR Workflow Updates

- "Multi-Tube Assay" renamed to Panels
- "Prep and Setup" renamed to qPCR Setup, now the recommended primary setup workflow
- Added support for reaction grouping (by Sample, Assay, or Panel)
- Redesigned Standard Curve configuration for improved usability
- New Run Layout options: Default, One Reaction Group Per Run, Avoid Splitting Groups
- Sample Preparation Enhancements
- Added Independent Prep step for pre-processing (e.g. magnetic bead workflows)
- Support for foil piercing
- Advanced options under Sample Prep and Reaction Setup
- New feature to allow outer wells of a plate to be avoided
- Can position controls at the end of a reaction list for automated qPCR setup reactions

Deck Layout Improvements

- Unified deck interface across all run types
- Enhanced Plates, Liquids, and Operations tabs:
- Quick-Add Plates, Adapters, and Delete tools
- Liquid Sets (e.g. Dropper/Fill Bucket) with configuration warnings
- Improved Operations grouping, playback (MyraSim), timing arrows, and volume control
- Enhanced Well Properties and Actions

Script-Driven Workflows

- MyraScript API updated:
- More flexible parameter handling and UI integration
- Supports multi-channel pipette heads
- Now based on Python 3
- Parameter pages can be designed to eliminate need for user script editing
- Existing scripts may require minor updates (support available from BMS)

qPCR Run & Project

- Minimal changes; retains full compatibility with Mic runs and analysis tools

Other Functional Enhancements

- Improved normalization, pooling, and transfer workflows
- Expanded transfer settings between source and destination wells

Support and Assistance

At Bio Molecular Systems, we are committed to providing responsive and effective support to help you get the most from your instrument and software. Whether you are setting up, troubleshooting, or optimizing your workflow, our expert team is here to assist.

Contact Details

Email: support@biomolecularsystems.com

Address: 5 – 7 Tonka Street, Yatala QLD 4207

Support Portal: www.biomolecularsystems.com/support

On our website, you will find:

- Software updates
- MyraScripts for various applications
- FAQs, application notes, and troubleshooting articles

Please register online as a customer to receive notification of new software updates and product developments.

https://biomolecularsystems.com/login/

Technical Support

Support Packages

For software or hardware-related issues, a Support Package is typically required to help us diagnose the problem efficiently. To generate a Support Package:

1. Open the Workbench software.
2. Click the Help icon in the toolbar.
3. Follow the prompts to generate and send the package to our support team.

Submitting a Support Package ensures accurate and timely assistance. If the size of the file exceeds your email limits let our support team know and they will find an alternative method.

Assay Compatibility Issues

If you experience issues with assay compatibility, please provide:

- A clear description of the issue
- Any relevant Information for Use (IFU) documents associated with the assay or reagents

This allows our team to review assay conditions and provide targeted recommendations.

Warranty Claims and Repairs

If your instrument requires replacement under warranty or repair:

1. Contact BMS Support to discuss the issue and obtain a Return Merchandise Authorization (RMA) form.
2. Complete and return the RMA form, confirming that the instrument is free from contamination by hazardous or biohazardous materials.
3. Ship the instrument to the designated service location as instructed.

Please note that instruments must be returned clean and decontaminated. BMS reserves the right to refuse service on any equipment deemed unsafe due to contamination risk.

Getting Started with Workbench Software

Workbench is the unified control software for Bio Molecular Systems' portfolio of instruments: the Mic qPCR Cycler, the Myra and Myra9 liquid handlers, and the fully integrated Myra+ system. From assay design through data analysis, Workbench lets you configure, run, and interpret your experiments—all from one intuitive application.

Software Installation

Install the Workbench software, located on the provided USB Flash drive, onto a PC.

Ensure the PC meets the following minimum requirements:

- Windows® 10, 64-bit (English version) Operating System
- Intel® Core™ i5, Core™ 3, Core™ Ultra 3, or equivalent AMD processor (ARM CPUs like the Snapdragon family are not supported)
- 8 GB RAM (16 GB recommended)
- 3 GB free hard drive capacity
- 1080p screen resolution
- One USB port
- Adobe® Reader® must be installed to be able to view reports in PDF format.

Double-click Workbench.msi. Follow the instructions that appear in the Setup Wizard.

If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your system administrator.

When the software has been successfully installed, the Workbench software icon will appear on the PC desktop.

The Workbench software is currently not compatible with Macintosh systems. For more information on how to run it with the Macintosh system, refer to Appendix A – Running BMS Workbench on MacIntosh Operating Systems.

User Permissions

The software offers three levels of **User Permissions** to allow different users varied levels of access when it comes to the control of the qPCR instrument and software features. This is a particularly useful feature in diagnostic laboratories, where changes to templates may have implications on regulatory related affairs, such as accreditation.

For information on how to setup user permissions, refer to Appendix B – User Permissions.

Software and Firmware Updates

Software and firmware updates are available for download at https://biomolecularsystems.com/media-downloads/.
You will need to login using your username and password, which is provided when registering your instrument(s) online.

Please check the website periodically to see if new software and firmware updates are available. Registered users will be notified via email upon release of a new software version.

Click on the instrument type that you own and wish to update the software to.
If you own multiple BMS instruments, it is not necessary to download software updates separately.

Download the Workbench software update setup file.
Release notes are also provided with each new build.

To initiate the installation, double-click on the setup file and follow the prompts.
Some new releases of software will require a firmware update. The software will notify the user of the requirement to upgrade firmware upon selection of an instrument following the software update.

Select the Upgrade Firmware option in the drop-down list after selecting the Instrument icon.
The instrument will begin to flash the front LED indicator red to notify the user the firmware is being updated. The LED will turn to blue once the update has completed.

Mic qPCR Cycler Hardware Overview

The Mic is the world's first magnetic induction real-time PCR instrument, engineered in Australia for ultimate speed, accuracy, and portability. Mic's advanced software meets MIQE guidelines and offers built-in analysis modes (Relative Quantification, HRM, Absolute Quantitation, Genotyping, Allelic Discrimination, Identifier), plus the ability to combine up to 10 instruments or runs into a single project for high-throughput consistency.

Mic General Features

1	Rotor	Aluminium rotor with 48 sample positions and a tube location label to ensure correct loading of tubes.
2	Tube clamp	Safeguards against the tubes and caps from coming out of the rotor during a run.
3	Lid Lock	Mechanism that locks the lid shut during a run, to protect the user from moving parts.
4	Lid	Provides access to the rotor.
5	LED indicator	When illuminated blue, indicates that the instrument is powered **On**. When green, indicates the instrument is **Running**. When flashing red, indicates a run has been **Aborted**.
6	Extraction fan	Ensures cooling of electronics.
7	Power switch	Powers the instrument on/off.
8	USB cable inlet	Provides USB connection to a PC.
9	Bluetooth® antenna	Provides wireless connection to a PC.
10	Power inlet	Connects to the power adaptor.

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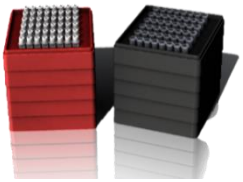
Mic Specifications

Physical	Dimensions	W: 150 mm, L: 150 mm, H: 130 mm (265 mm lid open)		
	Weight	2.1 kg		
Electrical	AC Input	100-240 VAC, 50/60 Hz 4.0 A		
Thermal Performance	Temperature Accuracy	± 0.25°C		
	Temperature Uniformity	± 0.1°C (MIC-2 and MIC-4 models) ± 0.05°C (MIC-4+HRM model only)		
	Ramp Rates	Heating: 4°C/s (fast mode)		
		Cooling: 3°C/s (fast mode)		
	Temperature Input Range	35 – 99°C (min 40°C when cycling)		
Optical	Detectors	High sensitivity photodiode per channel		
	Excitation Sources	High energy light emitting diode per channel		
	Channels	Green	Ex. 465 nm	Em. 510 nm filters
		Yellow	Ex. 540 nm	Em. 570 nm filters
		Orange	Ex. 585 nm	Em. 618 nm filters
		Red	Ex. 635 nm	Em. 675 nm filters
	Acquisition time	1 second		
Reaction Vessels	Samples per Instrument	48		
	Reaction Volume Range	5 – 30 µL (Standard Caps) 5 – 25 µL (V-Caps[1])		
Operating Environment	Temperature	18 – 30°C		
	Relative Humidity	20 – 80%		

[1] V-caps are only used with Myra+.

Mic Spare Parts, Consumables and Accessories

Please contact your local BMS Representative for more information on pricing and ordering.

Consumables		
	Mic Tubes and Caps **(MIC-TUBES)**	Strip of four reaction vessels with volume range of 5 – 30 µL. Preloaded with silicone oil. Pre-packaged into a rack of 48 tubes, stacked together in a row of 5, and boxed as 4 x 5 stacks. Mic caps supplied loosely in a bag.
	Mic Tubes and Racked Caps **(MIC-TUBES +RACKEDCAPS)**	Same as Mic Tubes and Caps, but the strip of four Mic caps are pre-packaged into a rack of 48 caps, stacked together in a row of 5, and boxed as 4x 5 stacks. Can be used with MIC-Rapid Cap Tool.

Spare Parts		
	Mic Capping Tool **(MIC-CT)**	Allows for easy capping of caps to Mic tubes.
	Mic Power Adaptor **(MIC-PA)**	External power supply for the instrument.
	Mic Tube Clamp **(MIC-TC)**	Magnetic tube clamp to ensure all Mic tubes are level and secure.
	Mic Bluetooth® Antenna **(MIC-BA)**	Enables Bluetooth® communication with PC

Accessories[2]		
	Mic Rapid Cap Tool **(MIC-RAPIDCAP)**	Allows for simultaneous capping of 48 Mic caps onto a rack of 48 Mic tubes.
	Mic SBS Robotic Loading Block **(MYRA-LBMIC)**	SBS dimension loading block to fit most liquid handling systems. Loads 2 x 48 well Mic racks. Talk to your liquid handling instrument manufacturer about incorporating the block into their software.

[2] List is not exhaustive and new products will be added continually. For more details on a more comprehensive list please contact your distributor.

Mic Installation

What's In the Mic Box

The following items are packaged within the Mic shipping container:

- Mic instrument (with magnetic tube clamp inside)
- Power adaptor
- Power cable
- 2 m USB cable
- Bluetooth® antenna
- Capping tool
- Mic tubes and caps (960 reactions)
- USB flash drive containing copy of Workbench software and manual
- Mic Quick Start Guide

Mic Hardware Installation

Place the Mic instrument on a level surface.

Screw in the Bluetooth® antenna at the back of the instrument; or connect the instrument to a PC using the provided 2 m USB cable.

CAUTION	
	Instrument is not to be used with a USB cable greater than 2 m.

Plug the power cord into the adaptor and insert the adaptor into the back of the instrument.

CAUTION	
	To avoid electrical arcing, ensure that the power adapter is not plugged into a wall socket before plugging into the instrument.

Plug the power cord into a wall socket and switch the power on at the socket.

Power the instrument 'On' using the power switch at the back of the instrument.

An illuminated blue light at the front of the instrument will show the instrument is powered on. For information on different illuminations refer to Mic LED Indicator Colours.

Upgrading to HRM

An HRM activation key, linked to an instrument serial number, will be provided upon purchase of the HRM upgrade module.

Start the Workbench software and ensure the instrument is in communication with the PC.

Select the instrument requiring HRM activation then select Properties.

Enter the activation key in the provided field and select OK.

The instrument will now have HRM enabled. Only data collected on this instrument can be analysed using HRM.

Firmware updates

Some new releases of software will require a firmware update. The software will notify the user of the requirement to upgrade firmware upon selection of an instrument following the software update.

To achieve the firmware upgrade, the instrument must be connected to the PC via USB cable. Firmware upgrades cannot be achieved through Bluetooth®.

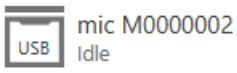
Select the Upgrade Firmware option in the drop-down list after selecting the Instrument icon.

The instrument will begin to flash the front LED indicator red to notify the user the firmware is being updated.

Getting Started with Mic

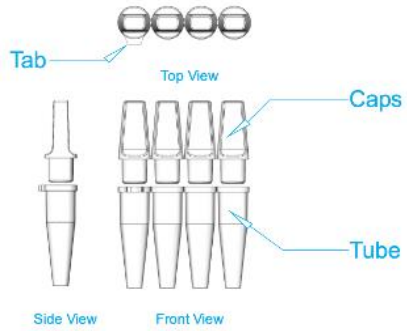
Open the Workbench software from the desk top icon.
The software will recognise the instrument via Bluetooth® or USB by displaying the Instrument icon in the tool bar (top right).

Multiple instruments can be recognised by the software and will be displayed.

	Instruments connected to a PC via USB (left) and Bluetooth® (right).

Loading Mic Tubes into the Rotor

Each **Mic tube** is part of a strip of four, with the first tube having a small tab to ensure the strip is loaded into the instrument correctly. Each tube is preloaded with silicone oil, which acts as a barrier to prevent evaporation and condensation, thereby improving reaction performance and removing the need for a heated lid. The allowable range of total reaction volume is 5 - 30 µL.

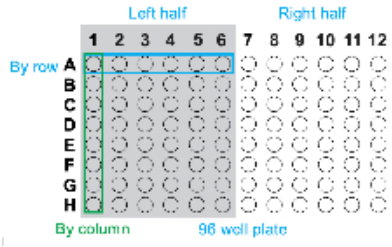
	The caps are designed to fit tightly into the tubes. A tab on tube one is used as a marker to ensure the tubes are correctly oriented into the loading block and instrument.

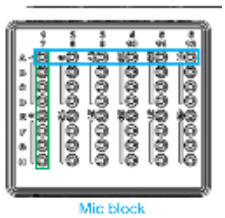
Use the provided loading rack to pipette reagents and samples into each tube.

The tubes are pre-packed into a loading rack, with the tube tab matching the tab on the loading rack.

The loading rack is also compatible with a multichannel pipette (8-channel). For this strategy, the loading orientation switches to across the top of the loading block (A1, A2, A3…) instead of the standard down orientation (A1, B1, C1…). The software has provision to change the display of samples based on the type of layout selected (see Well Layout).

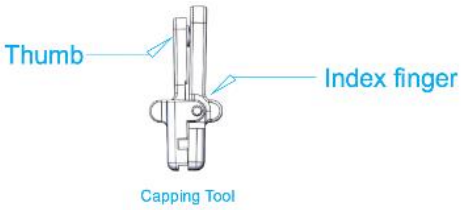
Note: Ensure that only the first six tip positions are utilised and not the full eight. For more information on using the multichannel pipetting strategy.





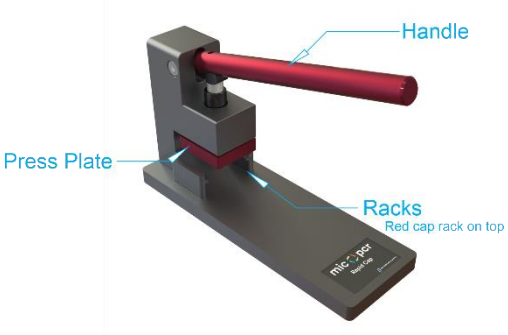
Wells highlighted in green follow the standard loading orientation (A1, B1, C1…) and wells highlighted in blue follow the multi-channel pipette loading orientation (A1, A2, A3…). The loading strategy will allow you to load half a 96- well plate per loading block.

Once tubes are loaded, properly fit caps to ensure tubes are sealed.
Use the provided **Capping Tool** to help with fitting caps properly and avoid cross contamination.



The tool is designed to clamp down on a strip of four caps. With your thumb and index finger on either side of the tool, press the caps down into the strip of four tubes until firmly in place. Remove the tool by unclamping the caps.

Alternatively, if you are completing a full Mic qPCR run you can use the Mic **Rapid Cap tool** to quickly cap a full rack of Mic tubes.



This tool is designed to clamp down a rack of 48 caps onto a rack of 48 Mic tubes. Flip over the cap rack and align the caps with the Mic tubes. Apply some pressure to loosely attach the caps to the tubes. Slide the racks into the Mic Rapid Cap tool, with the red cap rack on top, and align with the red press plate. Using the handle, press down the caps firmly into the tubes. Release the racks by raising the handle.

Caps can be removed later to access the post PCR reaction for downstream applications such as gel electrophoresis or DNA sequencing. Refer to Appendix F – Post-PCR Sample Recovery from Mic Tubes.

ATTENTION

Ensure post PCR amplicons are handled away from a pre-PCR environment to avoid contamination issues.

Place the reaction tubes into the rotor keeping the tube tab in line with the marker located on the rotor label.

Load Water Tubes in unused wells.

No tubes, empty tubes, and tubes with different volumes of liquid all have different thermal loads on the metal rotor. Variations in thermal load around the rotor can cause significant thermal gradients both at static temperatures and during ramping, resulting in increased variability in results.

As the oil overlay prevents evaporation, these Water Tubes can be stored and reused for over a week.

ATTENTION	
	To achieve optimum temperature uniformity, it is important to load tubes pre-filled with water into unused wells of the rotor using the same volume as reaction tubes.

After loading all the tubes, place the Tube Clamp at the top of the rotor.
The tube clamp will safeguard against any of the tubes or caps coming out of the rotor during a run.

Once the lid is closed the instrument is ready to be run.
Failure to close the lid will prevent the run from starting. This is to prevent injury to the user and/or damage to the instrument.

When the run begins, the lid will be locked into place to prevent it being opened.

Removing Tubes

Once the run has completed and the instrument has cooled down, the lid lock will disengage allowing you to open the lid.
The LED at the front of the instrument will flash green.

WARNING	
	If the lid lock has disengaged due to a power failure or fault prior to the run stopping, please do not open the lid for at least 5 minutes until the chamber has cooled to avoid personal injury. The rotor within the chamber could be above 40°C (104°F).

Remove the tube clamp.

Pull the tubes out of the wells.

Ensure you put the tube clamp back into the chamber before closing the lid to prevent loss or damage.

Myra System Hardware Overview

The **Myra** delivers class-leading pipetting precision (≤ 1% CV at 5 µL) through closed-loop axis control and integrated high-precision camera–guided calibration. Its lightweight, < 10 kg footprint and internal HEPA + UV decontamination options make it ideal for busy labs.

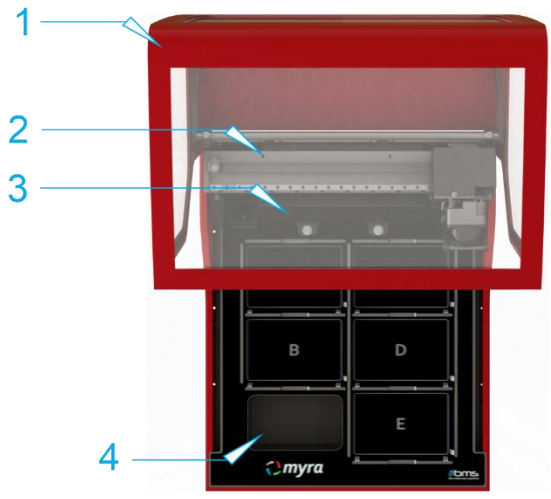
With **Myra9**, you gain three extra deck positions (nine total), enabling 384-well plate workflows and expanded throughput without sacrificing Myra's signature compact design.

Myra+ merges Myra's automated sample prep with the Mic cycler in one seamless platform. Using specialized V-Cap tubes and a precision loading portal, it automates tube transfer, capping and thermal cycling—streamlining small-to-medium throughput qPCR workflows into a single, cost-effective device.

Myra and Myra9 General Features

1	Lid	Provides access to the deck and keeps contaminants out during a run.
2	Travel locks	Used to hold the axis drivers in place during transport to prevent damage. Locks must be removed prior to turning on the system.
3	HEPA filter	Minimises contamination from the external environment. Filter can easily be replaced by the user when required.
4	Waste tub	Captures used pipette tips that are discarded by the pipette head. Smart-Tip-Capture™ ensures the tips are evenly distributed across the tub. A lid with small aperture holes is designed to reduce the potential for contamination after the tips are discarded.
5	Pipette head	Stainless steel interchangeable pipetting head with on-board control unit. The head contains all the calibration information making it easy to swap out. The standard head is compatible with our 50 µL robot tips in a 384 well format and can be used for volumes as low as 1 µL.
6	Axis drivers	Control the movement of the pipetting head in x, y, and z direction.
7	UV light	LED light at 280 nm wavelength used to decontaminate the internal deck. The UV light is mounted onto the axis drivers so that it can move around the whole deck area to maximise exposure to the UV.
8	Camera	Used by the robot to visualise the deck to simplify calibration and ensure proper deck layout.
9	Deck	Six SBS positions including waste tub. Contains Easy-Fit-SBS™ positioning clips that ensure optimum and sturdy positioning with minimal effort.
10	LED indicator	When illuminated blue, indicates that the instrument is powered 'On.' When green, indicates the instrument is 'Running.' When red, indicates there is an issue. When amber, attention is required.

11	USB cable inlet	USB connection to a PC.
12	Power switch	Powers the instrument on/off.
13	Power inlet	Connects to the power adaptor.
14	Air vents	Airflow for HEPA filter.



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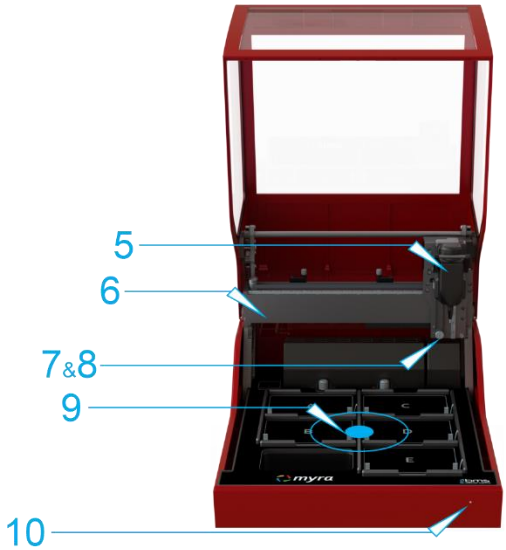

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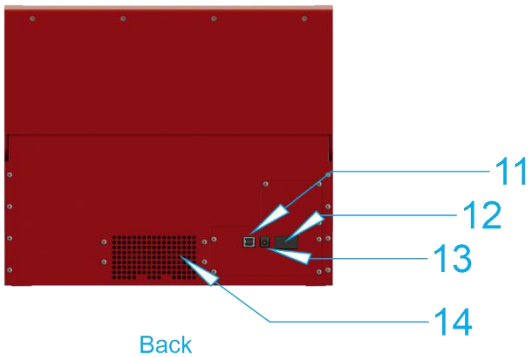

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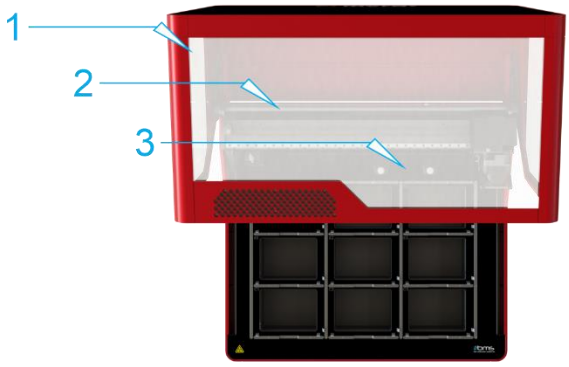

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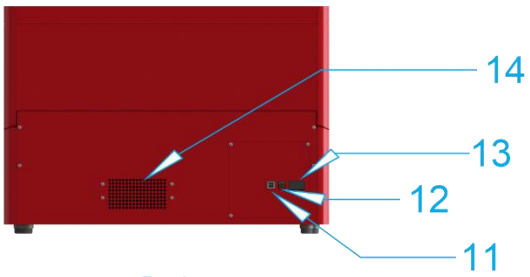

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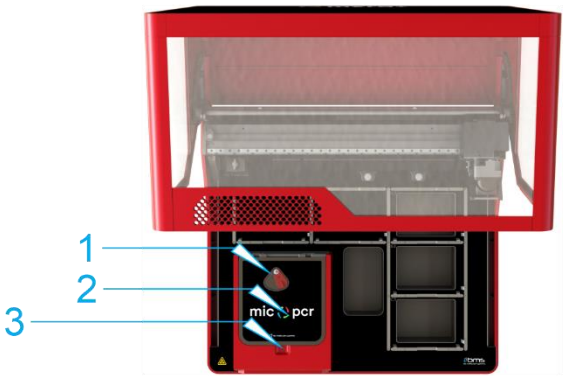
Myra+ General Features

Most of the features found on the Myra and Myra9 can be found on the Myra+. The following table lists additional features to the Myra+ over those found in the Myra and Myra9.

1	On-deck Mic Lid Portal	Used by the Myra+ instrument during **qPCR Setup** to load the on-deck Mic.
2	On-deck Mic Lid	Provides access to the rotor.
3	On-deck Mic Transport Lock	Mechanism that locks the lid shut during travel, to prevent lid from opening during transport.
4	On-deck Mic qPCR Cycler	On-deck Mic qPCR cycler that can be used as a standalone instrument or in conjunction with automatic liquid handling functions in **qPCR Setup**.
5	Cycler Shroud	Ensures cooling of electronics.
6	Air Vents	Airflow for HEPA filter and on-deck Mic qPCR cycler.
7	Mic Cable Inlet	USB connection to a PC for the on-deck Mic instrument.
8	Myra+ Cable Inlet	USB connection to a PC for the Myra+ instrument.
9	Myra+ Power Inlet	Connects the liquid handling instrument to the power adaptor.
10	On-deck Mic Power Inlet	Connects the on-deck Mic qPCR cycler to the power adaptor.



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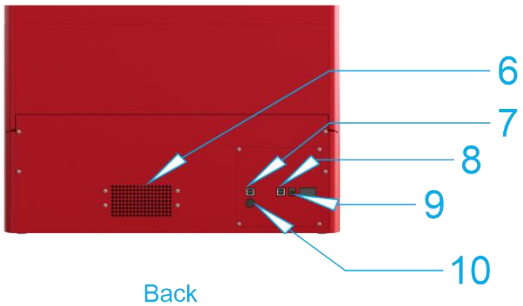

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Myra System Specifications

Myra System		Myra	Myra9	Myra+
Physical	Dimensions	W: 360 mm, L: 460 mm, H: 310 mm (610 mm lid open)	W: 520 mm, L: 468 mm, H: 356 mm (635 mm lid open)	
	Weight	10 kg	17 kg	19 kg
Electrical	AC Input	100-240 VAC, 50/60 Hz 1.4 A		Myra: 100-240 VAC, 50/60 Hz 1.4 A Myra+ Cyler: 100-240 VAC, 50/60 Hz 4.0 A
Technical	Position control	Closed loop, 100 µm resolution		
	Level detection type	Pressure sensing		
	Calibration	High precision camera		
	Pipetting Strategy	Single or multi-dispense		
Contamination Control	Tip disposal	Internal waste tub with capacity for up to 1000 tips		
	UV light	High intensity 70 mW, 280 nm UV LED		
	HEPA	99.98% at 0.3 µm		
Operating Environment	Temperature	18 – 30°C		
	Relative Humidity	20 – 80%		
Additional Instruments	On Deck Cycler	None	None	Mic-4 Channel qPCR cycler (see Mic Specifications)

Pipette Head Specifications

Pipette Head Type		20 µL single Ch.		50 µL single Ch.		50 µL multi-channel	
							
Technical	Volume Range	TBD – 20 µL		1 – 50 µL		1 – 50 µL	
	Tips per rack	384					
	Precision[3]	TBD	TBD	1 µL	5% CV	1 µL	TBD
		1 µL	TBD	2 µL	2.5% CV	2 µL	TBD
		2 – 20 µL	TBD	5 – 50 µL	1% CV	5 – 50 µL	TBD
	Accuracy[4]	0.2 µL	TBD	1 µL	10%	1 µL	TBD
		1 µL	TBD	2 µL	5%	2 µL	TBD
		2 – 20 µL	TBD	5 – 50 µL	1%	5 – 50 µL	TBD

The 20 µL single channel and 50 µL multi-channel heads have not yet been released.

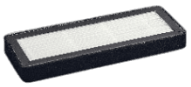
[3] Measured as percentage coefficient of variation ($\frac{\sigma_{n-1}}{\bar{x}}$) of ten consecutive dispensations of water.

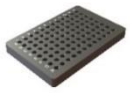
[4] Measured as percentage variation of mean volume from expected volume of ten consecutive dispensations of water.

Myra System Consumables, Spare Parts, and Accessories

Please contact your local BMS Representative for more information on pricing and ordering.

Myra System Consumables		
	Myra 384 Well Tips **(MYRA-TIPS384-50)**	Sterile filtered tips are provided in 384 well format, pre-racked. For volumes between 1 and 50 µL.
	V-Cap Tubes (Compatible with Myra+ only) **(MIC-TUBE+V-CAPS)**	Strip of four reaction vessels containing a V-Cap tube. Volume range of 5 – 25 µL. Preloaded with silicone oil. Pre-packaged into a rack of 48 tubes, with caps, stacked together in a row of 5, and boxed as 4 x 5 stacks. Each stack comes with a Myra+ Tube Detection Dye, which is a pre-filled self-standing 2mL amber screw cap tube with a white lid.

Myra System Spare Parts		
	Myra Power Adaptor **(MYRA-PA)**	External power supply for the instrument.
n/a	Myra USB Cable **(MYRA-USBCABLE)**	2.0m USB cable allows for connection to PC.
	Myra HEPA Filter **(MYRA-HEPAFILTER)**	Minimises contamination from the external environment. Filter can easily be replaced by the user when required.
	Myra+ V-Cap compatible Magnetic Tube Clamp **(MIC-TC-VCAP)**	Magnetic tube clamp to ensure all V-Cap tubes are level and secure.

Myra System Accessories[5]		
	Myra 2x Mic Racks Adapter	SBS dimension loading block for Mic tubes. Loads 2 x 48 well Mic tube racks.
	Myra Multipurpose Loading Block	Designed to hold various generic tube formats sufficient for most qPCR applications. Includes up to 4 x 10 mL bottles, 4 x 5 mL tubes, 24 x 1.5/2.0 mL tubes (tapered or flat bottom), 24 x 0.5/0.6 mL tubes, and/or 12 x 0.2 mL tubes. Comes supplied with 4 x Blue 5 mL Tube Adaptors and 18 x Purple 0.5 mL Tube Adaptors.
	Aluminium Blocks	Precision machined out of high-quality aluminium and anodised in a mat black finish. These blocks are designed to hold various tube and plate formats. Being machined out of aluminium ensures a higher degree of precision, while the blocks can also be cooled.
	Plastic Blocks	3D printed from PLA+ polymer; the blocks are designed for various tube formats. We also offer 3D design and printing services for your own specific tube types.
	Tube Cap Holders	Designed to hold the caps of tubes that are loaded into the various block types. 3D printed from PLA+ polymer, these cap holders ensure you can correctly match the cap to the tube used on the block.
	Tube adaptors	Allows the use of various tube sizes in the aluminium or plastic blocks.
	Specialist Blocks	Examples include the Magnetic Station used for protocols such as bead clean up.
	Cooling Blocks	Designed to provide passive cooling for up to 2 hours for various plate formats including 384 well.

[5] This list is not exhaustive, and additional products are continually being added. For a more comprehensive and up-to-date catalogue, please contact your distributor or visit our website at www.biomolecularsystems.com.

Myra System Installation

What's in the Myra System Box?

The following items are packed within the shipping container of each type of Myra System. A separate accessories box will be shipped with the Myra9 and Myra+.

Myra	Myra9	Myra+
Myra instrument	Myra9 instrument	Myra+ instrument
Power adaptor		Myra+ power adaptor and On-deck Mic power adaptor
Power cable		2 x power cables
2 m USB cable		2 x 2 m USB cables
		1 rack of V-Caps and tubes, including Myra+ Tube Detection Dye in 2 mL Screw Cap Tube
Waste tub with lid		
1 rack of 384 tips		
USB flash dive containing copy of Workbench software and manual		
Myra Type Quick Start Guide		

Myra System Hardware Installation

Carefully follow the instructions on the shipping container to remove the instruments out of the box.

Place the instrument on a level surface.

Connect the instrument to a PC using the provided 2 m USB cable (Myra+ requires 2 USB cables).

CAUTION

Instrument is not to be used with a USB cable greater than 3 m.

Plug the power cord into the adaptor and insert the adaptor into the back of the instrument.

For the Myra+, plug the other power cord into the Mic power adaptor and insert the 4-pin adaptor into the back of the Myra+ instrument.

Plug the power cord/s into a wall socket and switch the power on at the socket.

Power the instrument 'On' using the power switch at the back of the instrument.

An illuminated blue light at the front of the instrument will show the instrument is powered on.

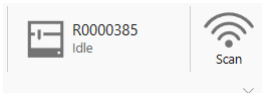
Getting Started with a Myra System

Connecting to a Myra Instrument for the First Time

Open the Workbench software from the desktop icon.

The software will recognise the instrument via USB by displaying the Instrument icon in the tool bar (top right).

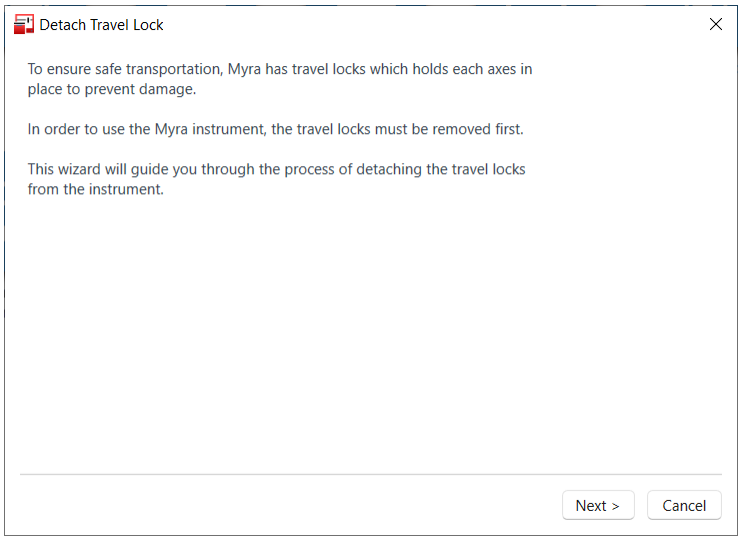
Multiple instruments can be recognised by the software and will be displayed.



Myra connected to a PC via USB.

To get started select the Properties option from the Instrument icon drop down list.

On first start up the instrument will begin a **Myra Installation Wizard** that will walk you through how to unlock the instrument and have the pipette head connected.



Travel Locks

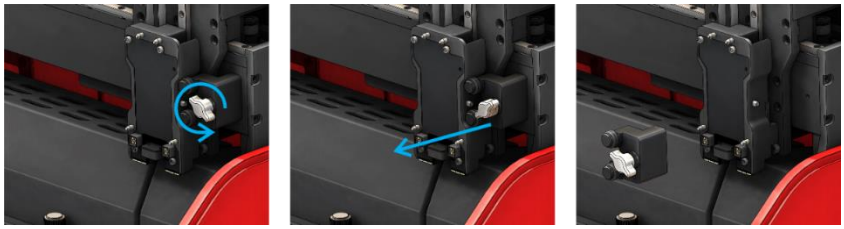
The travel locks prevent the axis drivers from moving during shipping. It is important to remove the locks before proceeding further. The software will prompt you to follow the process of removing the travel locks.

Remove the main driver arm travel locks.

Unscrew the two back locks holding the main driver arm. Screw them into the provided threads at the back of the instrument.

Carefully unscrew the z-axis travel lock.

Store the z-axis travel lock in the provided area labelled on the deck to avoid losing it.



For Myra+, unscrew the on-deck Mic travel lock.

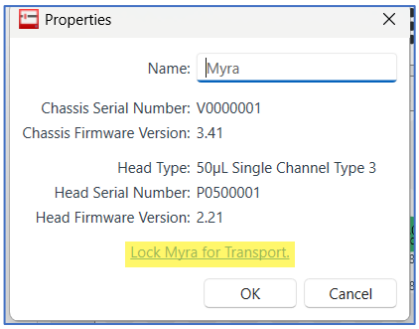
There is also an additional space beside the HEPA filter to store the on-deck Mic travel lock.

CAUTION	
⚠	Remove the travel locks before running the instrument. Failure to remove the travel locks may result in damage to the unit if the axis drivers try to move.

Re-locking

Use the travel locks whenever moving the instrument over a distance greater than 5 meters to avoid damaging the axis drivers.

To place the axis drivers into place to allow the travel screws to be applied; select Properties from the options in the Instrument icon then select Lock Myra for Transport. Follow the on-screen instructions to lock the arms into place.

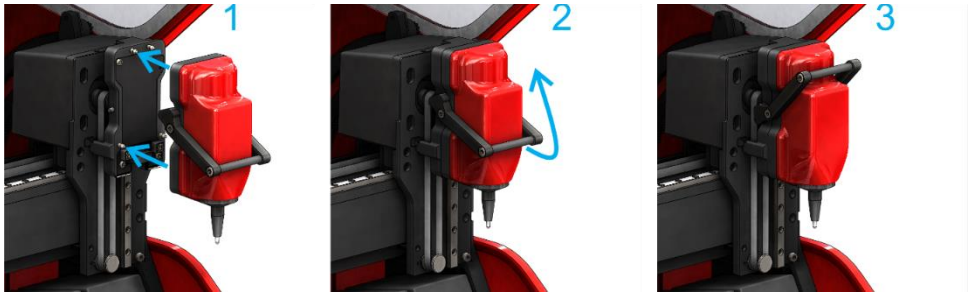


CAUTION	
⚠	Always transport the instrument with the travel locks applied and in the original shipping container. Failure to apply travel locks and use the correct shipping container when moving the instrument will void your warranty.

Connecting the Head

Connect the pipette head to the z-axis driver.

1. Align the pipette head with the z-axis connector platform pins.
2. Once flush, pull the handle up to lock the head into place.
3. Head is now inserted and ready for use.



All connected instruments are now ready to be used.

On the first use you will need to calibrate consumables and blocks that you will use.

Loading the Deck

Each Myra system deck has a different orientation and number of SBS positions.

Instrument	Myra	Myra9	Myra+
			
Number of Positions	6	9	6 + Cycler
SBS Position ID	A, B, C, D, E	A1 – A3, B1 – B3, C1 – C3	A1 – A3, B3 and C3

Use the Easy-Fit-SBS loading clips to orient and insert tip racks and loading blocks or plates.

Place the tip rack or SBS loading block/plate up against the back strip of the SBS position. Then simply slide it down into the slot. The Easy-Fit-SBS clips will help you orient the plate/block into position while ensuring the plate/block is held firmly in place to improve positional accuracy for the pipette head. To remove the plate/block, grab the front of the plate/block and pull it up and out of the deck position.

Some positions of the Myra9 and Myra+ have deeper well sockets. BMS manufactures loading blocks compatible with these positions, to allow for longer tube types, particularly with tubes containing VTM. However, tubes with lower volumes may still be out of reach for the pipette head.

Insert the Waste Tub into the desired socket position and place the lid on top.

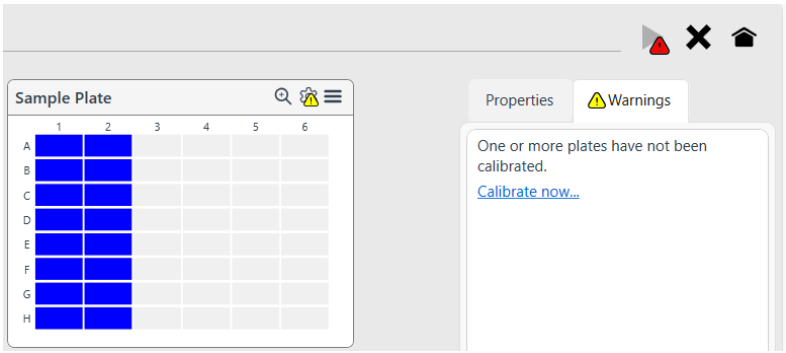
Tips will be discarded evenly across the holes using our Smart-Capture-Tip program. The lid is designed to reduce potential for contamination after tips are discarded. Always empty the waste tub at the end of a run.

CAUTION	
	Do not allow the waste tub to fill up to more than 75% of the tub volume. Over filling the waste tub will prevent the tips from being discarded and can cause damage to the head.

BIOLOGICAL HAZARD	
	Handle biological material with care and in accordance with required safety regulations. The waste tub can be decontaminated with a solution containing 1 gL^{-1} available chlorine.

Calibrating Blocks, Plates and Adaptors

When using a block, plate, or adaptor for the first time you will need to calibrate the position and/or height. This is achieved prior to the start of a run. A notification is displayed on the run summary banner on the play button. The run cannot begin until calibration is completed.



Click on Calibrate now... to begin the calibration.

A warning indicating that the lid safety interlock is disabled will appear. Although the speed of the axis drivers is reduced significantly for safety, you must exercise caution while calibrating with the lid open.

The instrument will initiate calibration by preparing the pipetting head, turning on a white light and moving to the first position (default will be the tip rack in position A).

You can enter calibration any time using the gear icon on top of each plate in the deck layout.

Position Calibration

The software will display an image of the block or plate that requires calibration. Typically, this will be the first well of the tip, block, or plate type being used. It will also highlight the well position required in the deck layout display.

Position the centre of the white cross hairs to the centre of the well that is being calibrated.

You have the option to zoom in on the position to help orient the cross hairs to the centre. Once you click on the targeted position, the red cross hairs will move to the set position, indicating the new calibration point.

Click the green tick to accept the new position.

You can select the red cross × to revert to the previous coordinates.

ATTENTION	
⚠	When calibrating Myra 384 Well Tips, it is important to remove the first tip in the rack to find the centre of the empty hole. This will ensure uniformity across all tip racks and avoid the need to calibrate each new rack.

Height Calibration

You will need to perform height calibration for all the different plate types used in the run. The software will determine which wells need calibrating and will display their positions on the deck layout. You should use empty wells for this process.

CAUTION	
⚠	Avoid wells with liquid in them to prevent contamination of the pipette head, as the process requires the pipette head to reach the bottom of the well.

Click on Calibrate Selected to begin height calibration of all the required wells of a plate or Mic tubes.

The head will pick up a tip and probe the depth of the selected well. A new tip will be used for each required well.

For Mic tubes, the pipette head will use level detection to locate the oil level rather than the base of the tube. This is to ensure the pipette does not enter the oil during pipetting.

At completion, the instrument will display the height calibrated values and will prompt the user to confirm.

Tips and individual tubes do not require height calibration. The system will detect the heights of individual tubes during the run.

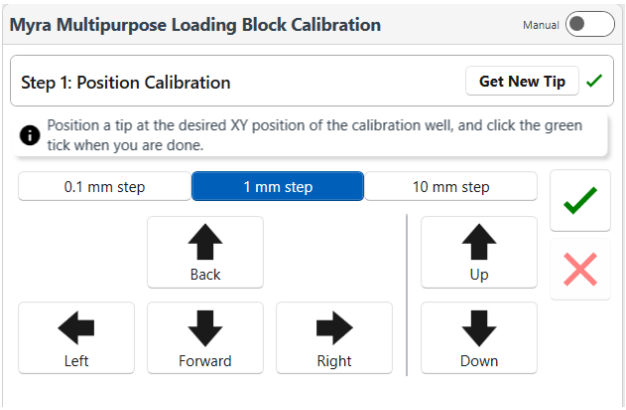
Select Next Plate to move through the calibration process.

Alternatively, select a plate in the deck layout.

Select the Finish button when done or to abort calibration.

You will not be able to start a run until calibration is completed successfully.

You can also use Manual calibration by toggling the switch on the top right. This is not recommended; using the Auto calibration option ensures the pipette head does not hit the bottom of the deck repeatedly.



ATTENTION

For Myra+, the position in the on-deck Mic cannot be calibrated and can only performed at the BMS factory.

HEPA Filter

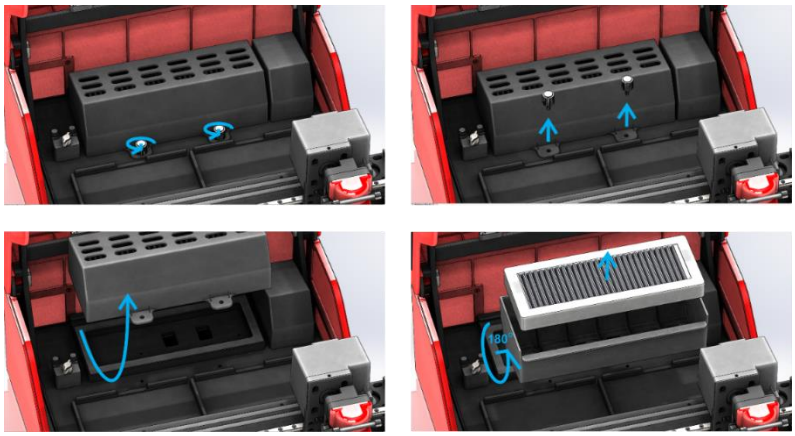
The HEPA filter is used to ensure the air inside the robot is free from most contaminants that could affect a reaction. It is rated to keep out 99.98% of all particulates greater than 0.3 µm in size. The HEPA filter will turn on automatically at the start of each run.

CAUTION

Avoid blocking the air vents to the HEPA filter. Do not obstruct the air vents at the back of the instrument. This will prevent airflow required for the HEPA filter resulting in poor contaminant control.

Replace the HEPA filter when required.

Simply unscrew and place aside the panel screws, pull the panel out and up, turn over and remove the old filter and replace it with a new one. Then reconnect the panel and screw back in place.



UV Light

The UV light is a high-powered LED (70 mW) with a peak wavelength of 280 nm. It is used to decontaminate the internal surfaces of the deck by inducing covalent linkages between the carbon double bonds of adjacent thymidylate residues, producing thymine dimers that can kill or disable microorganisms and render DNA un-amplifiable by inhibiting the polymerase during extension. During UV treatment, the main axis arm moves around the deck to ensure all surfaces within the deck are exposed.

For your safety, the instrument has been built with a clear polycarbonate lid to prevent UV exposure. Ensure the lid is closed prior to starting UV decontamination and do not open it during the UV procedure.

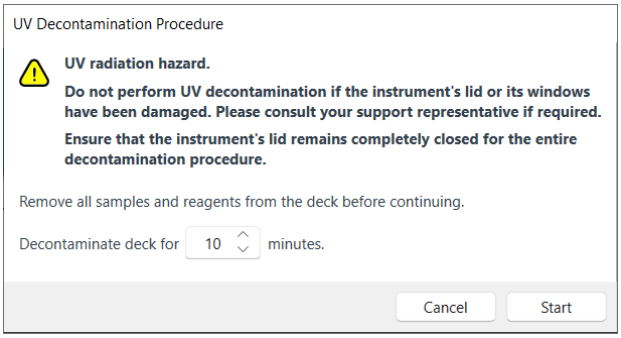
UV HAZARD	
	**UV Radiation Hazard.** Use only with lid down. Protect eyes and skin from exposure to UV light. Do not use if lid windows are damaged or lid safety switch is malfunctioning.

To apply UV light decontamination, select the instrument icon and then UV Decontamination.

Enter the desired time to apply the UV light; the default is 10 minutes.

The software will ask you to open the lid to test the lid sensor is working to ensure safety during use.

After closing the lid, you will be able to start the UV decontamination procedure. The head will move across the deck in segments for the nominated time. The UV light will turn off at any point the lid is opened during this period.



Myra System LED Indicator Colours

The LED indicator at the front of the Myra instrument will change colour and flash during various instrument operations.

Blue, constant: The instrument is switched on and *Idle*.

Blue, flashing: The instrument has been selected to *Start* a run. This instrument can no longer be selected by another user until the designated run has completed.

Green, constant: The instrument is *Running*.

Green, flashing: The instrument has completed the run successfully.

Red, flashing: The run has been *Aborted* or the instrument has had an issue during the run.

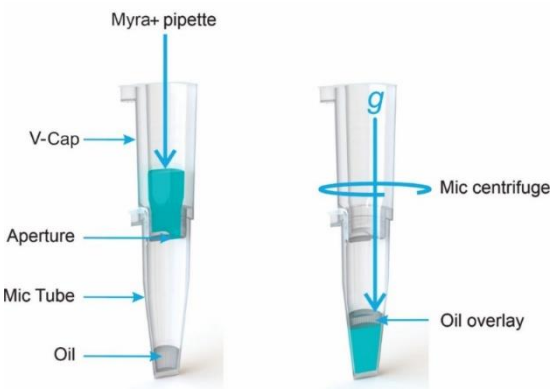
Yellow, flashing: Attention required (e.g., ran out of tips or liquid during the run).

ATTENTION	
	Myra+ LED indicator colours are used to indicate liquid handling and transfer functions only. When the on-deck Mic is in use as a standalone device, the Myra+ LED indicator will remain blue and will not change colours.

V-Cap Tubes for Myra+

V-Caps are a specialised consumable for the Myra+ allowing direct loading of tubes already inside the on-deck Mic rotor. The V-Cap provides a reservoir for loading liquid from the top, which remains in the cap prior to running the on-deck Mic instrument. The loaded liquid inside the V-Cap will flow through a small hole at the bottom and enter the tube during the centrifugation step when the run is initiated.

Each tube is part of a strip of four, with the first tube having a small tab to ensure the strip is loaded into the instrument correctly. As with the standard Mic tubes, each V-Cap tube is preloaded with silicone oil at the bottom of the tube, which acts as a barrier to prevent evaporation and condensation. The allowable range of total reaction volume is 5 - 25 µL. The V-Cap tubes are pre-packed into a loading rack, with the tube tab matching the tab on the loading rack.



Place the reaction tubes into the rotor keeping the tube tab in line with the marker located on the rotor label.

ATTENTION	
⚠	Always remove all tubes from the Myra+ Cycler and ensure the entire rotor is filled with new, empty V-Cap tubes prior to commencing a run. Failure to follow these instructions could lead to invalid results, instrument damage and contamination.

After loading all the tubes, place the special V-Cap tube magnetic clamp at the top of the rotor.

The tube clamp will safeguard against any of the tubes or caps coming out of the rotor during a run. It is slightly smaller than a standard Mic magnetic clamp; to allow the pipette access to the V-Cap tubes so they can be loaded by the Myra+.

ATTENTION	
⚠	Do not use a standard Mic tube magnetic clamp. The larger diameter size of the clamp will prevent the Myra+ from pipetting into the V-Caps tubes. This can result in the liquid spilling throughout the rotor.

Once the lid is closed the instrument is ready to be run.

When the run begins, the lid will be locked into place to prevent it being opened. As the Myra+ loads the V-Cap tubes, a small access port will open at the top of the on-deck Mic lid to provide access to the pipette. The V-Cap tubes will be moved into position through indexing of the rotor motor.

Water Load Tubes and Myra+ Tube Detection Dye

As with all Mic instruments, no tubes, empty tubes, and tubes with different volumes of liquid all have different thermal loads on the metal rotor. Variations in thermal load around the rotor can cause significant thermal gradients both at static temperatures and during ramping, resulting in increased variability in results. The Myra+ will automatically load water into unused tubes around the rotor to control thermal load.

To avoid potentially over loading into previous reaction tubes – especially water load tubes – the Myra+ is designed to detect the difference between empty and full tubes. To assist the Myra+ in the detection of water load tubes used in a previous run, a special dye called the **Myra+ Tube Detection Dye** is loaded into these tubes. The dye must be present on deck prior to starting a run on the Myra+ using the on-deck Mic instrument. Each stack of V-Cap tubes comes with a vial of dye.

Removing V-Cap Tubes in Myra+

Once the run has completed and the on-deck Mic has cooled down, the lid lock will disengage allowing you to open the Mic lid.

Importantly, it is still possible to open the lid of the Myra+ instrument while the Myra+ Cycler is running.

HOT SURFACE 	**Hot Surface.** If the lid lock has disengaged due to a power failure or fault prior to the run stopping, please do not open the lid for at least 5 minutes until the chamber has cooled. The rotor within the chamber could be above 40°C (104°F). To avoid personal injury, do not touch the rotor for at least 5 minutes.

Remove the tube clamp and place it to the side.

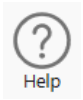
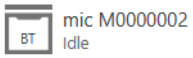
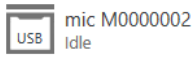
Pull the tubes out of the wells. Ensure that you put the tube clamp back into the chamber before closing the lid to ensure it is not lost or damaged.

General Features of Workbench Software

Workbench is a single user-friendly interface and is intended for use with all BMS instruments, including Myra, Myra9, Myra+ and Mic. This section is a high-level overview of these features in the Workbench software, with definitions pertaining to respective instruments.

Tool Bar

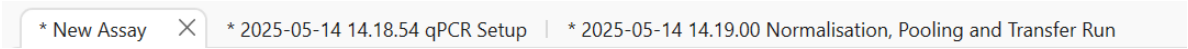
The top section of the user interface is referred to as the Tool bar and consists of the following:

	New	Begin a new document and bring up the Start Page.
	Open	Open a saved Assay, Run, or Template from a file directory.
	Save	Save an open Assay or Run.
	Save As	Save an open Assay or Run under another file name or as a Template.
	Help	Access to Workbench Manual, MyraScript Manual, Settings, Create Support Package and About Workbench. **Settings** contains the option to disable graphics acceleration off (Appendix E – Disabling Hardware Acceleration Graphics for the Mic for details). It also contains the option to enable user permissions, which is explained further in Appendix B – User Permissions. About Workbench displays information about which version is installed on the PC.
	Instrument	BMS instruments connected to the software are displayed in the tool bar. Multiple BMS instruments can be connected simultaneously on a PC for integrated laboratory workflows.
		**Myra and Myra9**
		**Mic (connected via Bluetooth®)**
		**Mic (connected via USB cable)**
		**Myra+**

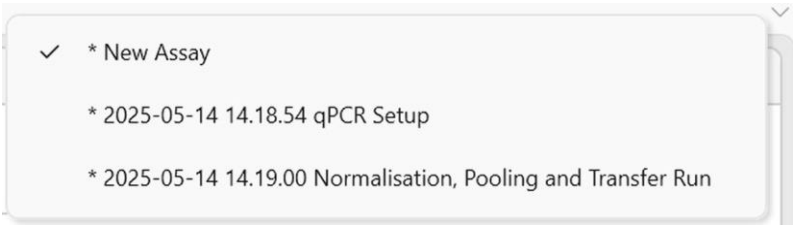
		Status of the instruments is also displayed beneath the name of the instrument, including: **Idle**: Instrument is ready to start a run. **Setup**: A run is being configured, but has not yet started. **Running**: Instrument is running and cannot be used until run is completed. **Lid Open**: Myra, Myra9 and Myra+ status of lid. **Reconnecting**: The instrument has lost connection with the PC and the software is attempt to reconnect to it. **Offline**: The instrument has lost communication with the PC and the software was unable to reconnect to it. Click the **Scan for Instruments** button to attempt to see if the instrument has been reconnected to the PC to use it again. Clicking on instrument icons displays options to **Connect** (for Myra) or **Start Run** (for Mic), **Hide Instrument** and **Properties**. On the liquid handling systems, there is an additional setting to commence **UV Decontamination**. Refer to Using the UV Light for more details.
	Scan	Detects available instruments. Instruments that respond to the software will be displayed next to the communication symbol, using the icons above.

File Tabs

Every open file will be displayed with its name on a tab. Multiple files can be open at the one time. The file being displayed in the main window will be highlighted white. Files that need to be saved will have an asterisk just before the file name. If a file is linked to a run in-progress, selecting the instrument running it will open the associated tab.



Use the down arrow to view files that might be out of view if multiple tabs are open at the one time. You can also use the left and right arrows to scroll across to view open files.



Common Functions in Workbench Software

The following functions are commonly found throughout the Workbench software.

	Copy to clipboard	Copy data from a table to clipboard so that you can paste it into another file format.
	Tool tips	Hover over the tool tip to show details regarding a particular field.
	Caution symbol	Indicates an issue with the run setup or post run that requires your attention. Hover over the caution symbol to see more details regarding the issue.
	Add button	Select this to add another analysis, function, or variable.
	Remove button	Removes a selected function or variable.
	Delete button	Deletes a selected function or variable.
	Magnify	Magnifies the view of a particular graph or SBS position.
	Submenu	Contains a selection of menu items that can be seen once the submenu icon is selected.

Start Page

Application Icons

Application icons are presented on the **Start Page** when opening Workbench or opening a **New Document**. Clicking on each icon opens a new document specific to that application. The yellow-shaded applications below are specific to the Mic qPCR cycler.

qPCR		**qPCR Assay** defines targets, reaction components and cycling profiles for a qPCR assay. See more on qPCR Assay.
		**qPCR Setup** prepares qPCR reactions either from pre-prepared samples or by performing upstream preparation of raw samples, with the reaction output plate auto-populated by Workbench. See more on qPCR Setup.
		**Reaction Driven qPCR Setup** prepares qPCR reactions from pre-processed samples, allowing for customisation of the reaction output plate. See more on Reaction Driven qPCR Setup.
		**qPCR Run** sets up runs and associated analyses on the Mic qPCR cycler. See more on qPCR Run.
		**qPCR Project** combines and analyses multiple qPCR Runs using the Mic qPCR Cycler in the one file. See more on qPCR Project.
General		**Normalization, Pooling, Transfer** transfers volumes from a source to a destination, including sample normalisation and pooling. See more on Normalisation, Pooling, Transfer.
		**MyraScript Run** allows for Python-based creation of customized workflows for enhanced flexibility. See more on MyraScript Run.

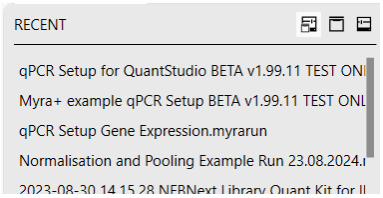
Run Templates from Start Page

A useful location to find all existing templates can be found to the bottom left of the Start Page.

The type of template files can be easily toggled using the Myra and Mic icons next to the heading. You can view all templates, Mic qPCR Cycler templates only, or Liquid Handling Systems templates only. See more on Templates.

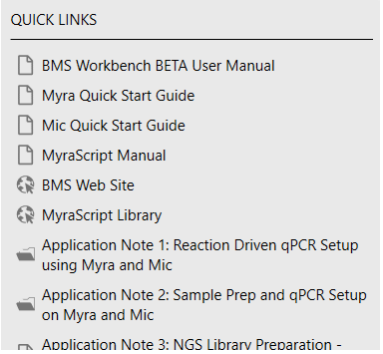
Recently Opened Files

Additional useful shortcuts on the left-hand side of Start Page includes **Recent** files that have been opened. The same toggle function for file type can also be used to find Mic or Myra specific files.



Quick Links

On the right-hand side of the Start Page are the **Quick Links**, which include Quick Start Guides, User Manuals, links to the BMS website and MyraScript Library, as well as additional information on common applications used in Workbench. These are a great resource to understand the software better.



Navigator Bar

To the left-hand side of the main user interface is the Navigator Bar. The **Navigator Bar** allows you to view the different sections for an Assay or Run Setup.

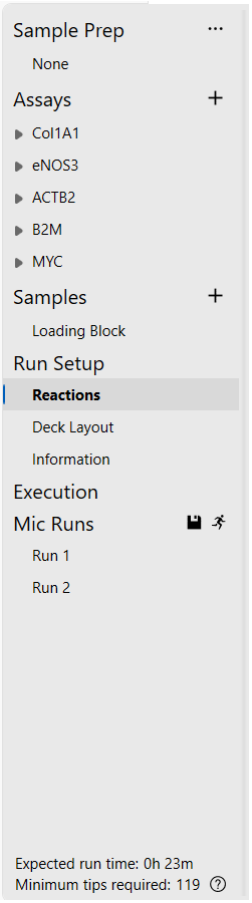
Some sections contain subsections that can be viewed by expanding the navigator tree.

Sections open in the main window will be highlighted in dark grey and a blue strip.

Add New items by using the plus (+) button.

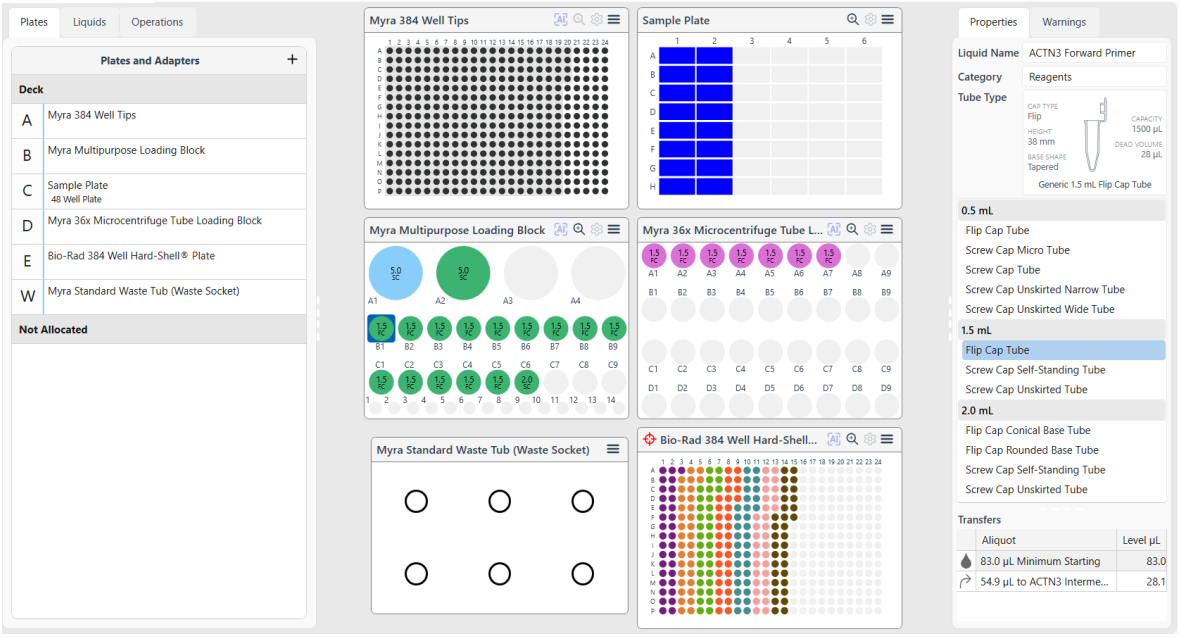
Remove user-created sections by using the delete (x) icon.

Expected run time and the **Minimum tips required** for the run are reported at the bottom of the Navigator bar. These will update when any of the variables for the run are changed that impact either time or tips required.



Active Windows

In the central area of the user interface are segmented windows that are active for a specific section of the Navigator Bar. Toggle between available tabs to open different elements.



Templates

Templates allow the user to set-up runs that will be used frequently. For example, you may want to:

- Set pre-defined assays for each run;
- Set pre-defined controls or standards in each run.
- Set pre-defined plates and tubes.
- Use of created MyraScripts

There is one default Template provided with the software for use with the BMS Myra Demo Kit.

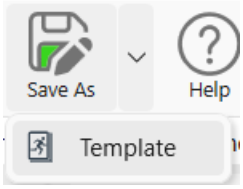
To create a new template, first click on New Document.

Create your run or MyraScript as per your desired parameters.

At least one sample needs to be present in the run for the software to allocate components for reactions on the deck layout; BMS recommends the first sample be named ‘Click Here to Start.’

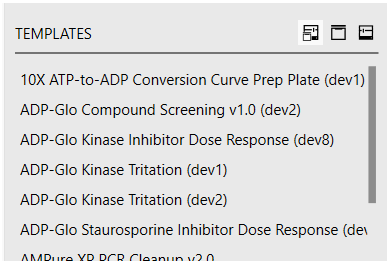
Select the down arrow next to the Save As button, then select Template.

Save the template into the Template library located in Documents/Bio Molecular Systems/Workbench/Templates. Templates that are downloaded and opened will automatically be added to the Templates library.



To use a template, select the desired Template on the Start Page.

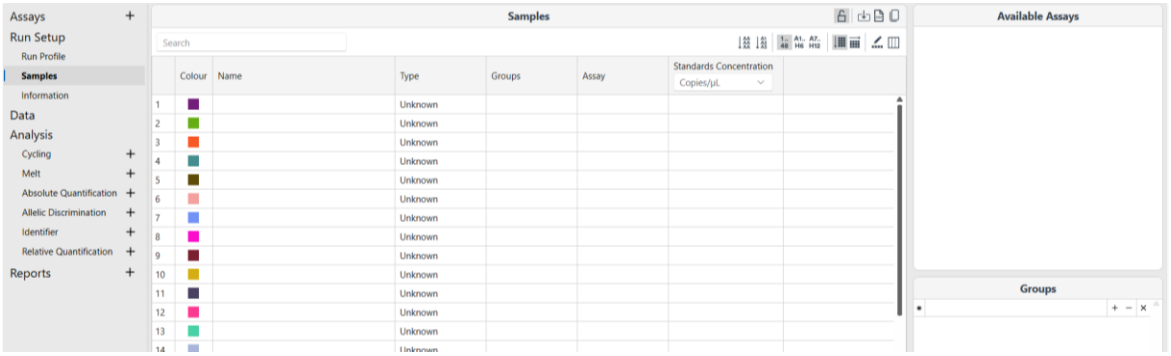
The run will open with all the saved parameters, including sample annotation.



Mic Basic Functions

Samples Editor

The Samples editor is displayed in a table format and allows you to annotate your samples. For Mic specific runs the editor is different to that for Myra and/or Mic setup runs. More details about this editor can be found in qPCR Run.



Importing Sample Data for Mic

You can import sample information from several file types for your Mic runs.

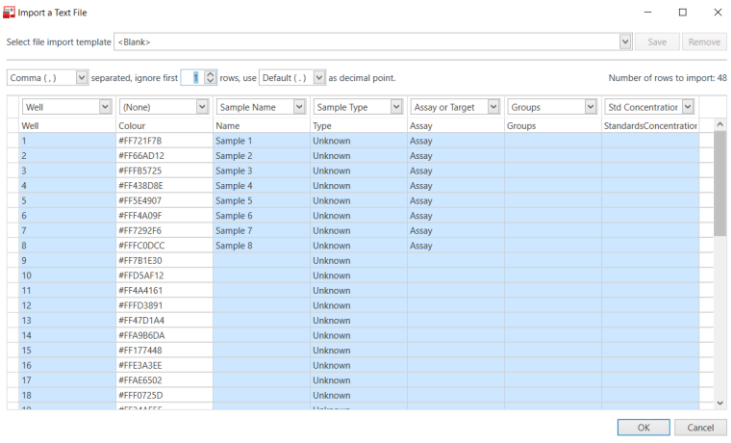
	A	B	C	D
1	Well	Sample ID	Concentration	
2	1	Sample 1	82	
3	2	Sample 2	158	
4	3	Sample 3	569	
5	4	Sample 4	924	
6	5	Sample 5	717	
7	6	Sample 6	731	
8	7	Sample 7	199	
9	8	Sample 8	110	
10	9	Sample 9	184	
11	10	Sample 10	812	
12	11	Sample 11	113	
13	12	Sample 12	246	
14	13	Sample 13	711	

To do this, select the Import Samples icon to import sample information from another source.

You can import from any comma delimitated, tab delimited or space delimited files.

Browse and select the file to import.

Select both the fields to import and which column to important into the Sample Editor.



Once the run file is selected, a table will display all the fields in the file. You have the option to select the type of delimitation (e.g., comma) and number of rows to be ignored before capturing the data.

Next, choose the fields to import by linking the column from the files to one of the columns in the sample's editor using the drop-down menu.

You have the option to save the import style as a template. This will allow you to complete the import faster without having to re-do the matching when conducting repetitive runs.

Run Profile

When thermal cycling conditions are programmed into the Mic, it is referred to as creating the **Run Profile**. This can be modified independently of assays.

Reasons for changing the assay profile include the testing of a new polymerase with a different activation time, or adding an extra cycling step to improve template yield. Modifying the Run Profile will save time, compared with changing individual assay profiles when using multiple assays in the run. A warning will pop up if selected assays are not compatible with the assay profile, which can be safely ignored.

NOTE	Test any modified profiles using inexpensive reagents or water before, and on a small subset of samples to confirm these changes are valid, before running experiments with many samples to avoid major loss of valuable samples and reagents. The consequences might be suboptimal performance of the qPCR leading to poor results.

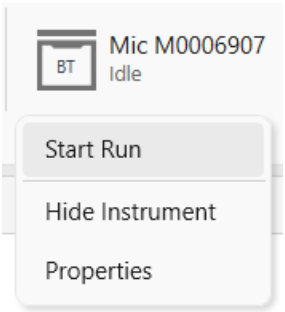
Starting a Mic Run

Select the Instrument you wish to use for the run in the tool bar.

Only Idle instruments can be selected to start a run.

A drop-down box will appear with options to Start Run, Hide Instrument and view Properties.

Select Start Run.



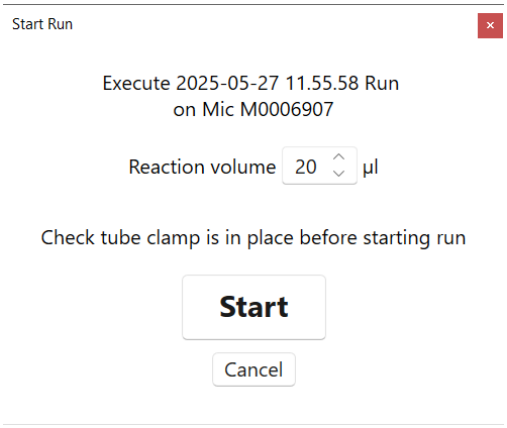
A confirmation dialogue box will appear.

Ensure the tube clamp is in place to prevent the tubes and caps from coming off during a run.

Ensure the lid is closed prior to starting the run.

A lid sensor will detect if the lid is open and will prevent the instrument from starting, while a warning will notify you of the fact.

Double check that the volume displayed is correct; if not, select the appropriate volume.



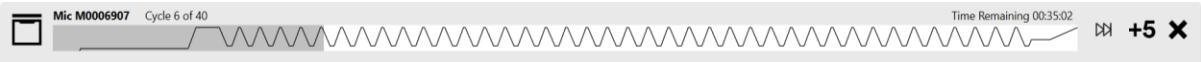
At this point the instrument LED indicator will be flashing blue indicating to other users the instrument has been ‘booked’ to start a run. No other user can start a run on the instrument until it has completed this run. On another PC, the instrument will be displayed as Busy.

To execute the run, click the Start button in the Start Run dialogue box.

The instrument will automatically lock the lid, centrifuge the samples down, and then the run profile will begin. The LED indicator will turn green to notify a user the instrument is running.

During a Mic Run

A Run Summary banner will appear as soon as the run commences. The raw data is displayed for the channels selected under the Data section. Once the run has completed the raw data will always be available to view at any time.



In the **Run Summary** banner, the hold temperature or cycle number is displayed to the left side of the banner next to the name of the instrument performing the run.

A graphic of the **Profile Summary** is also displayed. The section at which the run has progressed through is highlighted in grey.

The **Time Remaining** to completion of the run is displayed to the right side of the Run Summary banner.

The Run Summary banner also has three functions to control the run:

⏭	**Skip:** Use the skip function if you wish to move to the next section of the profile. You may choose to skip the last number of cycles of a run profile if you believe there has been enough cycles to generate amplicon and would like to commence the melt.
+5	**Add 5 Cycles:** Use this function if you believe you require more cycles to thoroughly complete amplification. You can only add another five cycles while the cycling is still running, up to a maximum of 60 cycles total.
✖	**Abort:** You can stop the run at any point by selecting the Abort function. The instrument will cool to a safe temperature before disengaging the lid lock.

HOT SURFACE 	In the event of a user aborted run do not open the lid until the instrument has cooled. The rotor within the chamber could be above 40°C (104°F). To avoid personal injury, do not touch the rotor for at least 5 minutes.

Any warnings about the run will be displayed under **Messages** along with the time it occurred.

Common messages include the run start time, instrument name and firmware version. Some messages may be warnings such as assay incompatibility with run profile or communication loss with instrument and time at which communication was restored. All messages will appear in any report generated.

Autogain values, which are used to determine the fluorescent baseline, will also be reported.

During analysis, all parameter changes will be logged once the run file is saved. This procedure allows Workbench to conform to the 21CFR11 code, by creating audit trails. For more information, see Appendix K – 21CFR11.

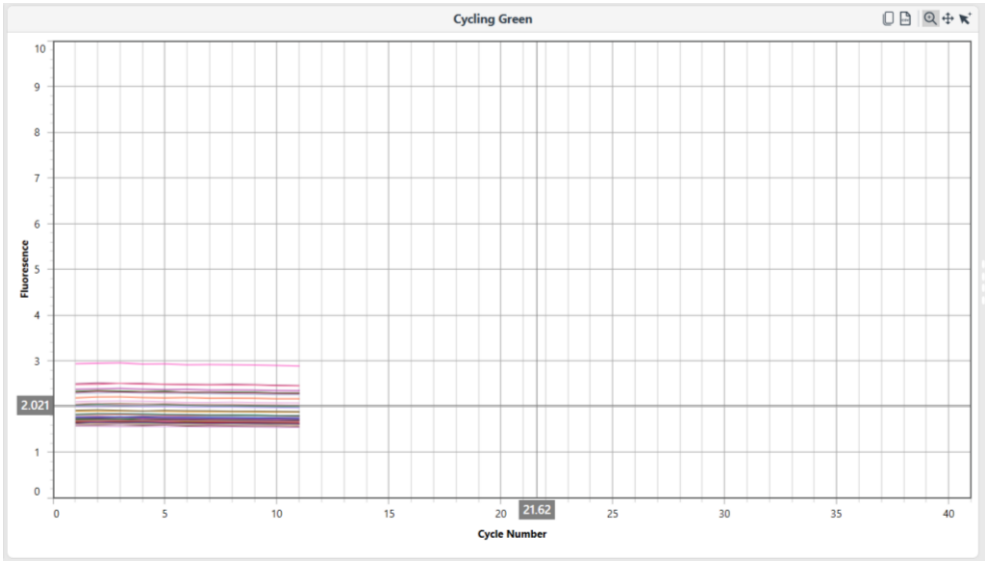
Messages

Time	Priority	Category	User	Message
19/04/2023 11:43:55 AM	Information	Run	Operator 1	Run started via Bluetooth on "mic M0000002" S/N M0000002 F/W v2.28 S/W v2.12.6
19/04/2023 11:46:11 AM	Information	Run	Operator 1	Reconnecting instrument to run
19/04/2023 11:57:27 AM	Information	Run	Operator 1	Autogain completed for Green using Sample: 15, Detector gain: 2x, LED power: 559, Scale: 1.05
19/04/2023 11:57:30 AM	Information	Run	Operator 1	Autogain completed for Yellow using Sample: 15, Detector gain: 2x, LED power: 534, Scale: 1.01
19/04/2023 11:57:31 AM	Information	Run	Operator 1	Autogain completed for Orange using Sample: 15, Detector gain: 1x, LED power: 300, Scale: 1.11
19/04/2023 11:57:34 AM	Information	Run	Operator 1	Autogain completed for Red using Sample: 37, Detector gain: 2x, LED power: 207, Scale: 0.99
19/04/2023 12:27:30 PM	Information	Run	Operator 1	Run complete

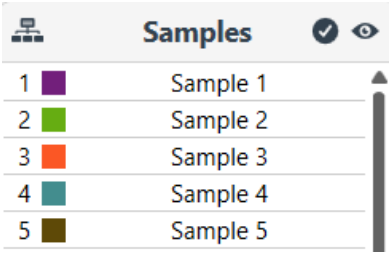
Raw Data

Raw data is displayed for each acquired channel and is listed in the Navigator Bar. Each channel is named "Cycling", followed by one of four colours: Green, Yellow, Orange or Red.

Cycle data is plotted as fluorescence value (y-axis) against cycle number (x-axis) with a maximum fluorescence value of 100 units. At the start of the run the fluorescence will be scaled from 0 – 10 units or 70 – 90 units depending on the Adjust Gain Settings chosen in the Run Profile. As the real-time curve grows beyond 10 units or drops below 70 units, the graph is auto-scaled to ensure the maximum curve takes up 90% of the visualised graph.



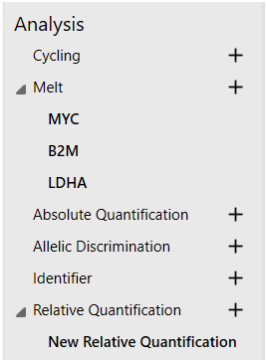
Samples can be highlighted, or selected or deselected during a run using the **Samples list** found to the left of the raw data graph.



Melt data is plotted as fluorescence value (y-axis) against temperature (x-axis) with a maximum fluorescence value of 100 units. Again, the graphs are auto-scaled depending on the Adjust Gain Settings option chosen.

Analysis

Workbench offers commonly used qPCR analyses for application to raw data generated by the Mic. These can be accessed under the **Analysis** heading in the Navigator Bar.



a. **Cycling analysis:** Determines quantification cycle (Cq) and reaction efficiency based on desired parameters.

b. **Melt analysis:** Studies the melting behaviour of double-stranded DNA molecules, most used in conjunction with PCR reagents such as intercalating dyes.

c. **Absolute Quantification analysis:** Determines the exact number or concentration of a target molecule, most often performed using a series of standard dilutions.

d. **Allelic Discrimination analysis:** Genotypes samples, using real-time kinetic data from two or more channels.

e. **Identifier analysis:** Uses a set of rules to auto-call presence or absence of a target(s) in a sample.

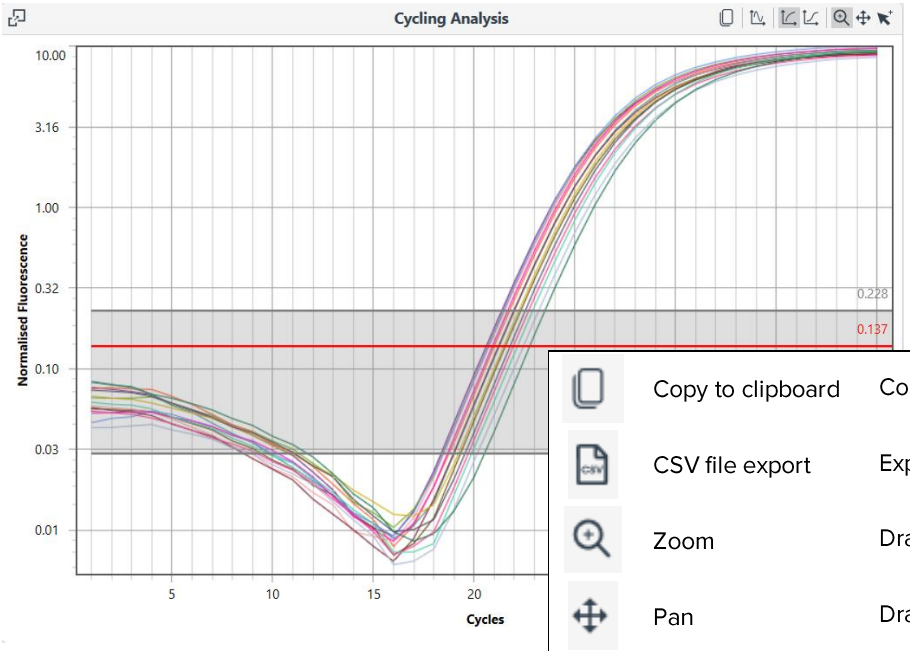
f. **Relative Quantification analysis:** Analyses differences in gene expression for a given group of samples relative to another control group; for example, measuring gene expression in response to a drug.

g. **High Resolution Melting (HRM) analysis:** (optional, requires a purchased license) Determines differences in sample melting characteristics by using normalised melt curve data.

See qPCR Run to learn more.

Graph Toolbar

All raw cycling data and analysis type will generate a graph toolbar for ease-of-access. Although there is a graph toolbar generated for each unique analysis type, all share the same common features, located on the top right:



	Copy to clipboard	Copy chart to the clipboard
	CSV file export	Export as a CSV file.
	Zoom	Drag to zoom in on one area
	Pan	Drag to pan chart
	Select	Drag to select samples

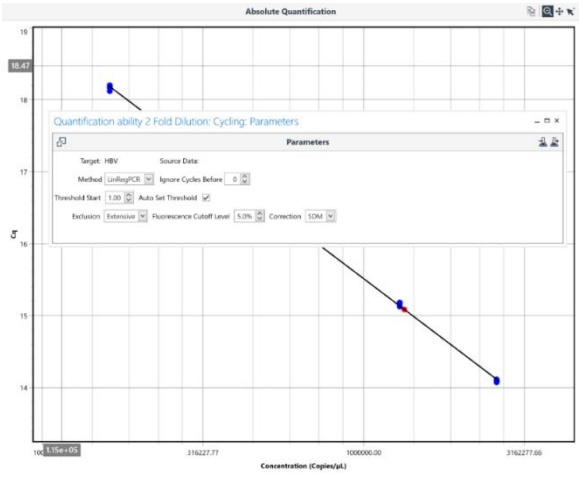
Floating Windows

Under some circumstances, it may be necessary to view either the cycling analysis graph or cycling analysis parameters in parallel with another analysis type. For example, you may want to observe what a change in cycle threshold has on reaction efficiency using a standard curve. The floating window option allows you to achieve this multiple analysis window view. To use this option, select the Pop out copy of panel into floating window icon, located in the top right-hand corner of either the cycling analysis graph or parameters windows.

You can move the floating window into any position of the screen.

Once the window is floating you can select the parent analysis (e.g., Absolute Quantification).

Any change made to the floating window will be represented in the associated parent analysis.



Use the close icon × to close the floating window.

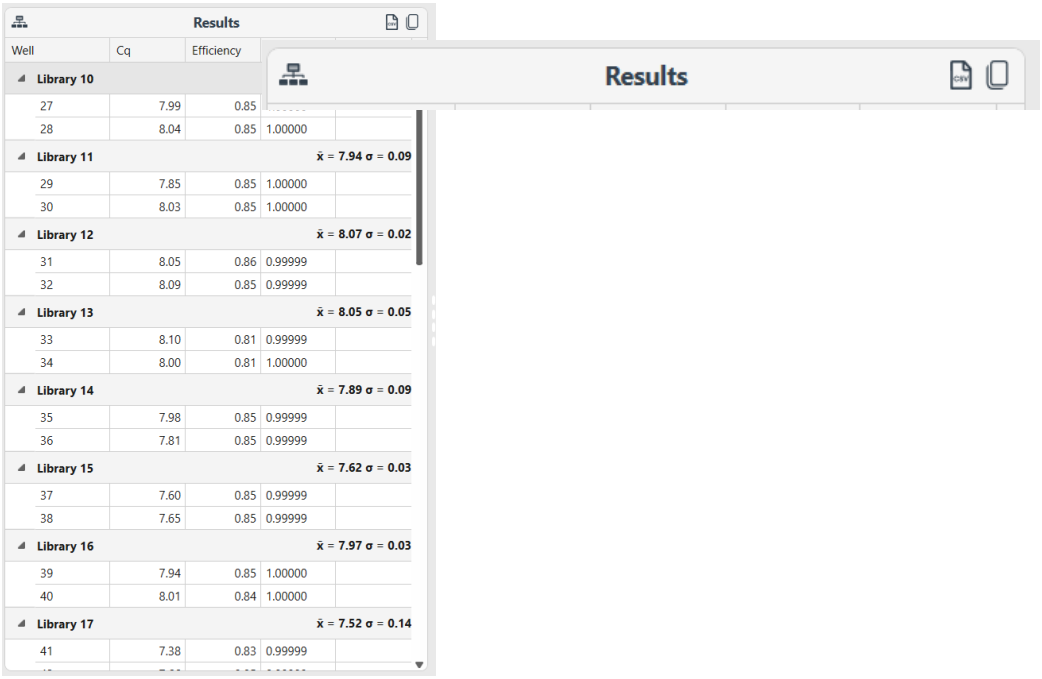
Parameters Toolbar

Each analysis type also generates a Parameters toolbar; as with the Graph toolbar, each analysis type has a parameters toolbar with unique features, sharing only 2 commonalities found on the top right allowing for analysis settings to be saved to an assay target and loading analysis settings from a target.

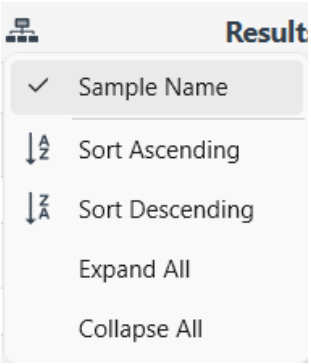
For a more comprehensive explanation of each Parameters toolbar, refer to Analysis under qPCR Run.

Results Toolbar

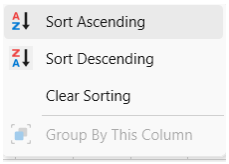
Each analysis type will generate a results toolbar for ease-of-access. All Results toolbars have the same options to use the Sample selector, save the table as a CSV file or copy the table to the clipboard.



The results table can be compressed by hiding the individual sample results using the triangle to the right of each sample name or selecting the **Collapse All** option under the Sample selector. Click the Samples table selector to view the different table sorting options.



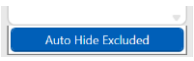
Right-clicking on a column heading displays options to sort samples. To sort the samples in replicates using an alphanumeric order use the Group by this column option.



The individual samples can be sorted in Ascending or Descending order for any of the columns for both grouped and ungrouped tables. Use this option to determine the range of Cq or efficiency values by displaying the highest to the lowest values.

Clicking on a column heading will also display each column in ascending or descending order. To display the samples in well number order, click on the Well column heading. If a melt has been performed, click on the Tm column heading to display samples in melt temperature order.

For Cycling Analysis, there is also an option to **Auto Hide Excluded** found at the bottom of the results table. Deselecting this will display any samples excluded from analysis in the graph.



Reports for Mic

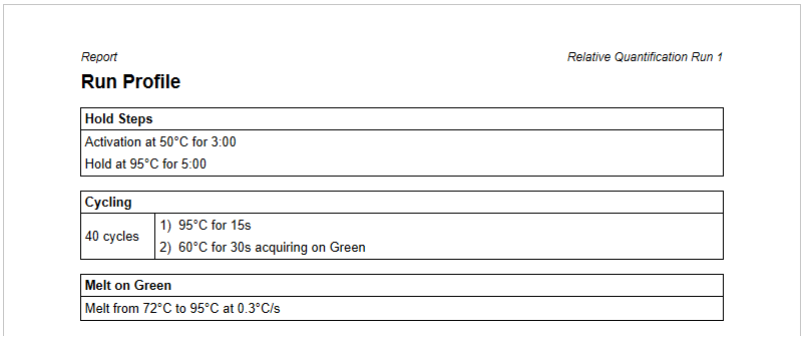
Each selected section will be displayed in the report Preview. A new page will begin following each section. Each page will have a number in page footer along with the version of software used. The run name will be displayed in the page header.

The Run Properties section displays the following:

- **Name**: The name of the run.
- **File**: The directory location of the run file.
- **Status**: Displays the status of the instrument at the end of the run.
- **Operator**: Name of the individual that completion the run.
- **Notes**: Any notes regarding the run completed by the Operator.
- **Started**: Date and time the run began.
- **Completed**: Date and time the run finished.
- **Event Log**: A report of important messages generated during the run, including any issues such as loss of communication.

The Samples editor is replicated in the report preview including Sample Name, Type, Standard Concentration and Assay. All 48 samples will be displayed.

All the segments of the Run Profile are reported for any Holds, Cycling and Melts. The channels used are reported along with the step at which fluorescence was acquired.



Report *Relative Quantification Run 1*

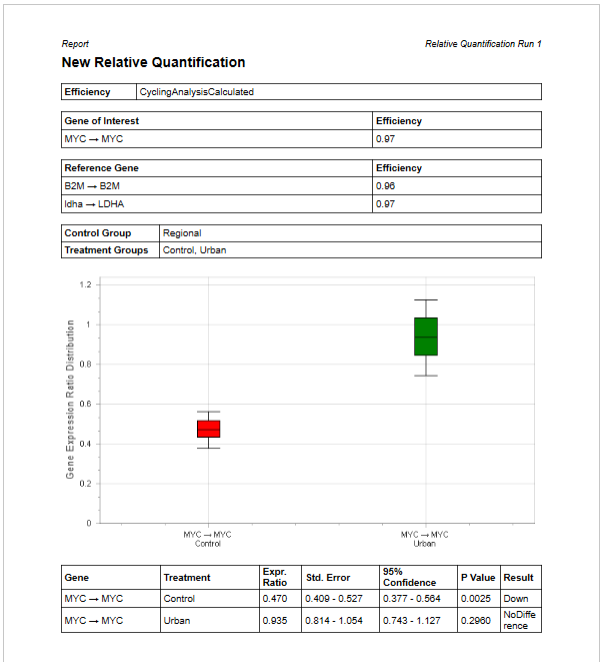
Run Profile

Hold Steps
Activation at 50°C for 3:00 Hold at 95°C for 5:00

Cycling	
40 cycles	1) 95°C for 15s 2) 60°C for 30s acquiring on Green

Melt on Green
Melt from 72°C to 95°C at 0.3°C/s

Each analysis will be displayed depending on the information provided for the analysis. Only the targets selected will be displayed. To display multiple targets, open a new analysis for the next targets required. The analysis parameters are displayed at the top of the page followed by a graph of the results. The results table is reported below the analysis graph.



Report *Relative Quantification Run 1*

New Relative Quantification

Efficiency	CyclingAnalysisCalculated

Gene of Interest	Efficiency
MYC → MYC	0.97

Reference Gene	Efficiency
B2M → B2M	0.96
Idha → LDHA	0.97

Control Group	Regional
Treatment Groups	Control, Urban

Gene	Treatment	Expr. Ratio	Std. Error	95% Confidence	P Value	Result
MYC → MYC	Control	0.470	0.409 - 0.527	0.377 - 0.564	0.0025	Down
MYC → MYC	Urban	0.935	0.814 - 1.054	0.743 - 1.127	0.2960	NoDifference

Other report options include:

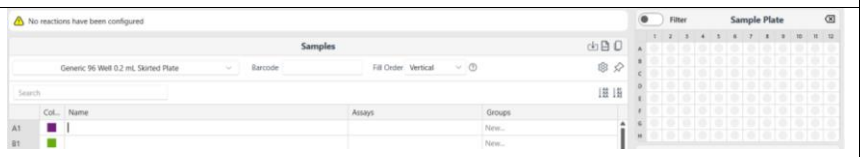
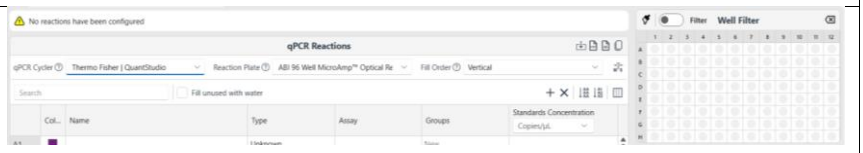
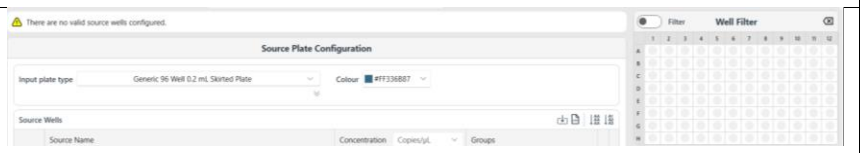
(from left to right)

- **Search**: find a word or string of characters in the report preview. Enter the search word(s) to find them in the report. The located words will be highlighted in the preview.
- **Print**: print the report using user defined settings.
- **Quick Print**: print the report using default print settings.
- **Page Setup**: select the paper type and orientation and adjust page margins.
- **Zoom**: Use the zoom in or zoom out to best view the report preview.
- **Page selection**: navigate through the pages using the page selection buttons; First page, Previous page, Next page, and Last page.
- **Export**: export the report using one of the available file formats including PDF, XLS and Text file. Each export will have a set of options to choose from.
- **Send**: email a report using one of the available file formats. A report generated in the selected file format will be attached to an email using your default email client, which will open automatically (if available).
- **Watermark**: add a watermark to your report. The water mark can be either a text or image. This option can be used to embed text such as Confidential to the report. A list of default text is provided or you can enter your own. Alternatively, add an image to the report such as a company logo. The direction and position of the text or image can be configured as well as which pages to apply the watermark to.

Liquid Handling Basic Functions

Samples, Reactions and Sources Editors

The Editors are displayed in a table format and allow you to annotate your samples, sources, or reactions. The following types are available for Myra associated setup:

Application	Editor Type	
qPCR Setup	Samples	
Reaction Driven Setup	Reactions[6]	
Normalisation, Pooling, Transfer	Sources	

There are a few common functions used for all the editors.

Enter the Name of each sample/source/reaction.

Each Name will be used as a template by the Myra. Samples with the same characters and assay will be treated as replicates and reported with a mean ($\bar{x}$) and standard deviation ($x\sigma_{n-1}$) for most Mic qPCR analyses.

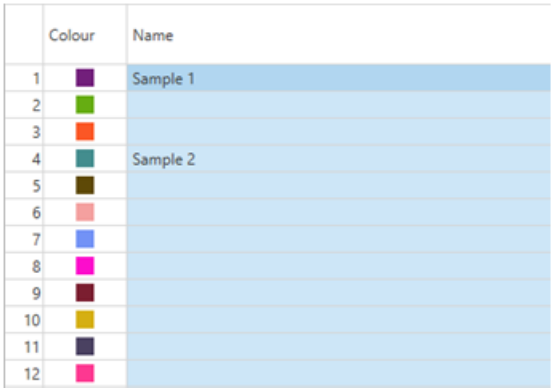
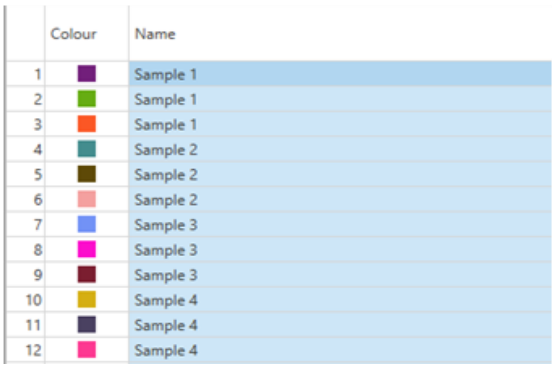
You can highlight multiple cells within a column and enter the same characters to annotate replicates.

Alternatively, enter the name in one cell, highlight that cell and other cells that will be part of the replicates (use Ctrl + Click to highlight non-adjacent cells), and then select the **Fill Down icon** to give all the selected cells the same name.

Use the **Auto Fill icon** to annotate sequential characters (e.g., sample 1, sample 2, sample 3...). To allow for replicates, follow this process:

Enter the first set of characters for the first name (e.g., Sample 1).	

[6] The Reactions editor for **Reaction Driven qPCR Setup** differs from **qPCR Setup** in that the setup is configured for the output reactions, not the sample source. All reactions, including controls have to be manually configured to be included in the setup of output reactions.

Leave the same number of rows blank as the number of replicates required below the first name. Enter the second name of the sequence (e.g., Sample 2).	
Now highlight all the cells required to complete the filling of the names and replicates.	
Click on the Auto fill icon.	
The names will be sequential based on the first two inputs and the replicates for each will be automatically filled in too.	

To delete data from table cells you will need to deactivate the editor by selecting the escape key. Once deactivated, you can use the delete key to clear the contents of the selected cells.

You can use a Barcode reader/scanner to simplify the sample editing process.

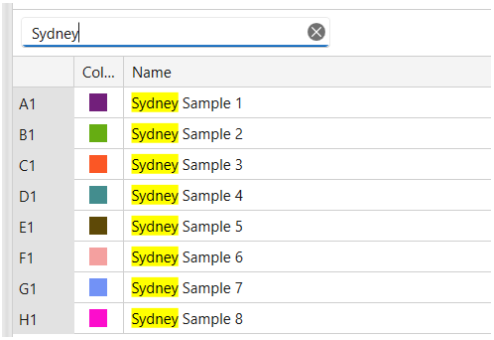
Any reader that can be connected to the computer running the BMS Workbench software can be used for this.

To use the barcode reader, have the cell of the Samples table that you would like to scan the data for active and then start scanning. The active cell will automatically move down the table as you scan.

Some barcode readers may need the reader output re-configured for it to move automatically to the next cell. You may have to scan a barcode in the barcode reader's manual to change the output function, where the option may be called carriage return. Refer to your barcode reader manual for more information.

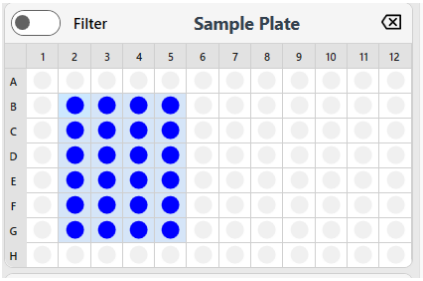
Use the Search function to find specific samples in larger lists.

Simply type the sample name or common characters to locate a specific sample or group of samples with common text. These specific samples will only be displayed in the table and the characters used in the search highlighted.

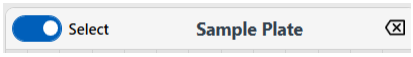


A Well Filter display is provided for plates and loading blocks other than the Mic or Rotor-Gene® Disc.

This feature enhances the editing process by allowing you to select specific parts of the plate or loading block to edit. For example, you could choose to only edit wells within the centre of a 96 well plate to avoid edge effects on your block-based cycler. By highlighting the centre wells in the Well Filter, only these wells will be displayed in the editor table. Once the wells are edited, they will be coloured in the Well Filter.



You can choose individual wells by toggling to use the **Select feature** instead of the Filter, or use the row or column feature to quickly select each row or column. Select the **Clear current** selection button, to revert to displaying all the wells in the sample editor table.



Importing Samples for Myra

Sample names and loading positions can be imported using the **Import sample** data from a file option.

In **qPCR Setup** and **Normalisation, Pooling and Transfer**, information about Components can be imported using the Fill wells according to information imported from a CSV file. In **Reaction Driven qPCR Setup**, Components and Templates can be imported using the same option.

In all the above scenarios, the **Import** icon appears in the toolbar:

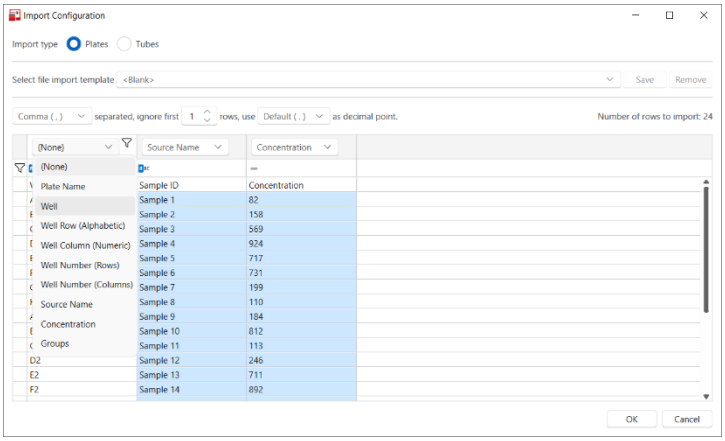
Select the CSV file to import.

Use the Import Configuration to match the CSV file columns to the desired columns.

Pick if the import type is Plates or Tubes.
You have the option to save the options as a template for future imports or select an existing import configuration.

Select the appropriate data separator, and if any rows need be ignored.
The following column options are available:

- **Plate Name**: defines the whole plate ID, this will provide the name for the plate source.
- **Well**: defines the well ID, this will number the individual source wells based on the import ID (e.g., A1).
- **Source Name**: the name of the sample.
- **Concentration**: the concentration of the sample, which can be used for normalisation.
- **Groups**: an ID that represents a group of samples, which can be used for pooling



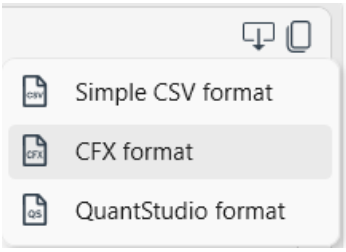
For additional information on using this feature to direct the Myra to randomly select wells using a CSV file (refer to Workbench Application Note 5 – Cherry Picking), found under Quick Links on the Start Page.

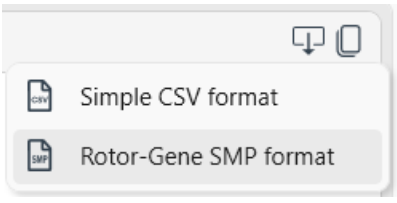
Export Samples for Myra

If a Mic is not being used, the Reaction list can be exported to a file format that can be imported into common cycler software/s. This can be accessed through the Export icon at the end of the reaction's editor bar.

Supported formats include:

- **Simple CSV format**: generates a CSV file that contains data visible in the reaction table.
- **CFX format** generates either a file that is compatible with the spreadsheet import function of the CFX Maestro plate editor or a CFX LIMS import file that will contain additional information such as sample type, target information and standard concentrations. This option is only available if the selected Reaction Plate is from the 96 Well Plates or 384 Well Plates categories.
- **QuantStudio format**: generates a plate import file that is compatible with QuantStudio software v1.5 or v2.6. This includes sample type, target information and standard concentrations. This option is only available if the selected Reaction Plate is from the 96 Well Plates or 384 Well Plates categories.
- **Rotor-Gene SMP format** generates a file that can be imported into the Rotor-Gene software. This includes some options for arranging samples into pages. This option is only available when the selected Reaction Plate is a Rotor-Gene Rotor-Disc or tube strips.





Where there are multiple reaction plates generated by the run settings, the export process will prompt you to select the Reaction Plate for export.

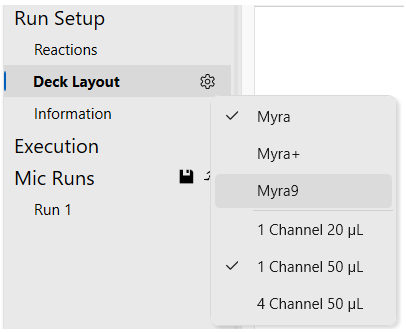
Deck Layout

An important stage of a Myra setup requires you to layout the deck with the various components required for the run, such as any plates, consumables, liquids, and mix tubes. The Deck Layout contains three sections:

- **Inventory List** (to the left)
- **Deck Configuration** (middle)
- **Information** (to the right)

Configure the Robot

Select the type of Myra system and pipette head to be used for the run by clicking on the gear icon.
Expected run time and the minimum tips required can change when another pipette head type is selected.

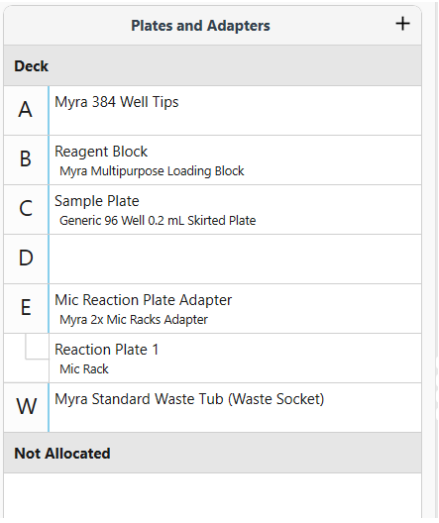


Inventory Lists

In the Deck Layout you will need to configure the deck using inventory list for Plates with required plates/blocks/adapters, allocate Liquids (Mixes, Reagents, Reactions, and Samples), and view the Operations list of the run. Each of these sections are found at the top of the page, in a separate tab for each.

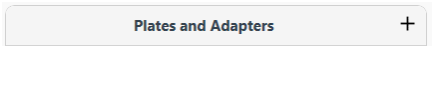
Plates Inventory

The plate list will display all the plates required for the run in the deck position they are currently in. Plates that sit on an adapter are displayed as a sub folder in the list (see Using Adaptors to learn more). By default, the deck contains a Myra 384 Well Tips rack, Myra Standard Waste Tub (Waste Socket), Myra Multipurpose Loading Block, sample loading plates or blocks as defined in Samples and reaction plates defined in Reactions.



ATTENTION	Select plates and tube types that match the exact description of consumables you are using; failure to select the appropriate consumable will result in errors and inaccurate pipetting by the Myra.

To add a new plate, use the + button at the top of the plate list or **Click to add plate** in the specific deck position. A **Plate Library** will appear allowing you to select the appropriate type.



Different methods for adding a new plate or adaptor to the deck layout.

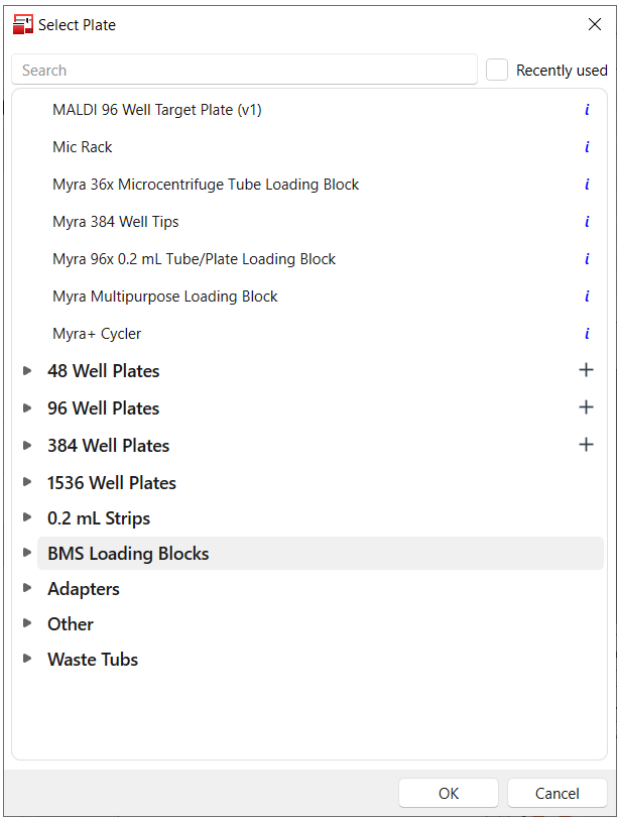
The **Plate Library** consists of several different plate and block type groups. Open the group to show the various types available.

Blocks and tips that are compatible with the Myra will be listed at the top of the Plate Library.

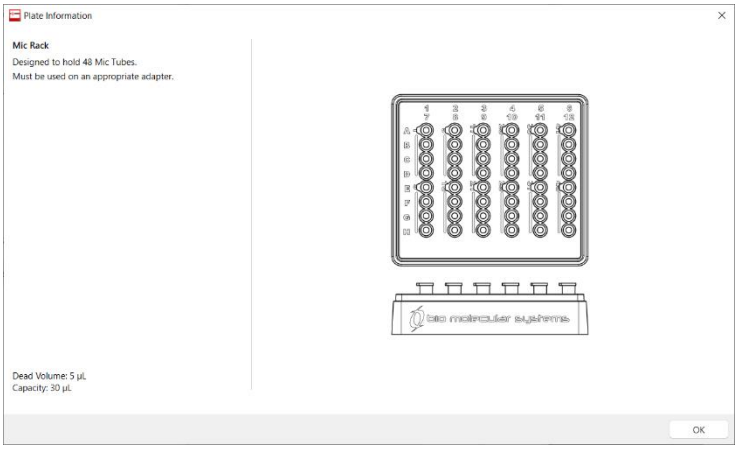
There are a set of generic plate types for each group.

Use the **Search** feature to find plates quickly.

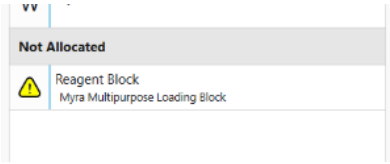
Tick the **Recently used** box to only show you most used plates.



Use the **Information** icon to view details about each plate.



Plates can be Deallocated. These plates are reported in the Plate inventory list under Not Allocated. To deallocate a plate, drag and drop it into the inventory list or use the submenu icon.



Reaction plates cannot be deleted in this page; please refer to the Reactions section for editing. All other plates or blocks can be deleted on this page, but a run cannot be commenced without required plates or blocks being assigned to the deck.

You can rename a plate by double clicking on the plate in the plate inventory list.

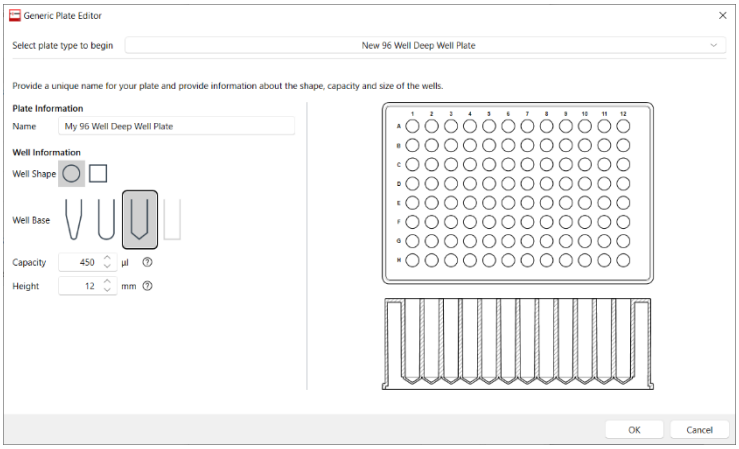
Change the text in the name field and click OK. The name new name will be displayed in both the list and in the deck layout.

Plate Editor

Plate types not already listed in the Plate Library can be manually added.

Create a new plate by using the + icon next to the plate group.

Select from the various plate subgroups (e.g., 96-well deep well plate).



You can use the existing values and simply provide a new name for your plate; or you can define a new profile by entering the values in the fields provided.

Options are based on the well opening shape (round or square), well base (e.g., conical or diamond), and well capacity and height.

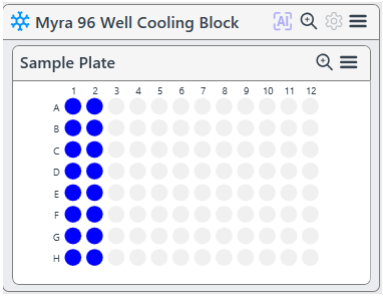
Any new profile will need to be calibrated prior to use (see Calibrating Blocks, Plates and Tubes). The new plate will be stored in the Plate Library. Modify or delete plates in the library through the edit icon.

BMS Support is available for profiling any plate and tube types not currently available in the Myra Plate Library. For details on how to contact BMS Support, please refer to Technical Support.

Using Adaptors

To use Myra Plate Adapters, select the applicable adapter from the Plate Library then drag the adapter from the Not Allocated list on top of the plate on the Deck Layout.

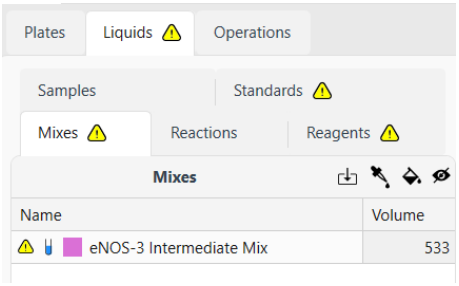
For example, if using a 96-well plate, you can drag the Myra 96 Well Cooling Block on top of the plate. This will change the toolbar to a snowflake, and will require calibration if not used previously. The adapter can be removed at any time using the submenu icon.



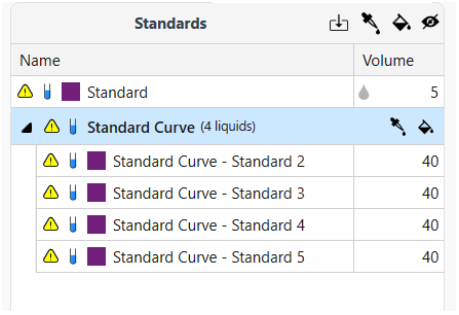
For most MyraScripts the adapter blocks, such as the Myra 96 Well Magnetic Station, will automatically be allocated to the deck.

Liquids Inventory

Liquid classes are divided in to four or five groups; Reagents, Reactions, Mixes, Samples, and Standards if used. Each group list will display all the liquids required for the run, including the volume required. Unallocated liquids will be displayed with a caution symbol next to them. Liquids with no tube type allocated will also be displayed with a blue tube next to the liquid. If using multiple assays, the software will recognise the same reagent name and only provide one instance of it in the list.



Any standard required for the run are displayed along with the dilution series associated with each, along with total volumes. You can expand the details of the standard curves. See Standard Curves to learn more on how to set these up in qPCR Setup or using Reaction Driven setup.



Allocate the Liquids to the Deck Layout.

Drag and drop the liquids from the list into a well located in the Deck Layout. You can use the **Dropper icon** to fill wells with unallocated items one well at a time using the list order; or use the **Bucket Fill icon** to fill consecutive wells with all unallocated items in one click using the highlighted wells as a guide to the orientation.

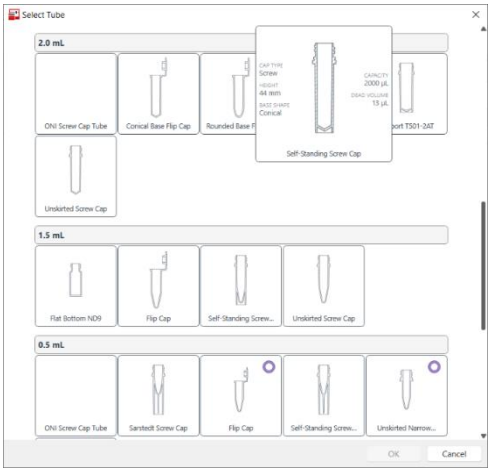
Click the space bar to change from vertical to horizontal orientation. You can also choose to fill wells according to information imported from a CSV icon, as described in Importing Sample Data. There is also an icon to show optional liquids that are not allocated or used in the run but are part of the larger template or assays set.

As individual reagents are selected, positions compatible with the component tube type will be highlighted in light blue.

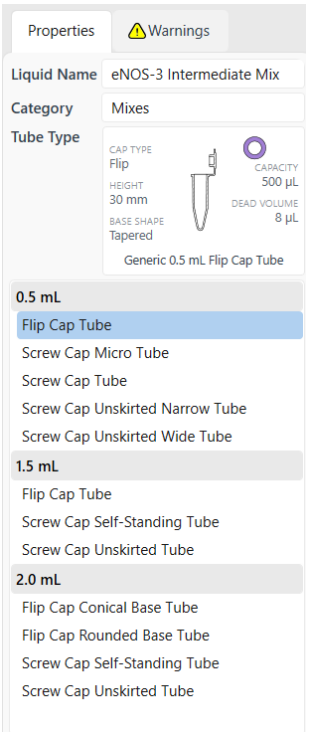
Select the Tube type for each Liquid after allocating it to the deck layout.

The tubes are displayed in groups based on volume (e.g. 2.0 mL). Various tubes types are available from the tube library. Tubes displayed will depend on the block well type (e.g. 5 / 10 mL wells). This makes selection a lot simpler by avoiding an extensive list to choose from.

Hovering over a tube will display information about the tube including; cap type, height, base shape, capacity, and dead volume. For some tube types, like the 0.5 mL tubes, there is the option of an insert for blocks such as the multi-purpose. The circle colours indicate the insert type required.

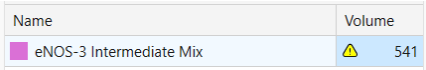


You can right click the well position or use the tube selector in the Properties section of the information section located on the right of the active window.

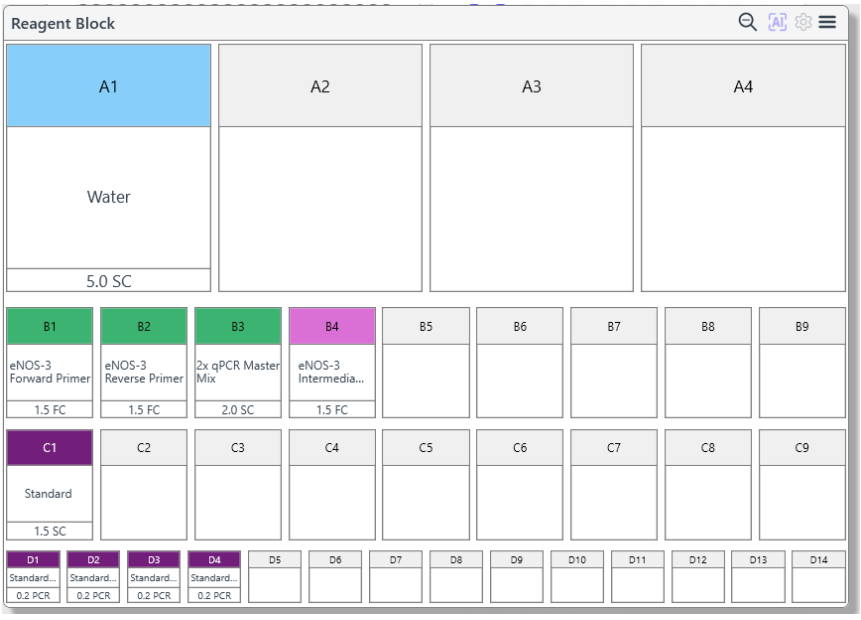


To save time on your runs, select the tube type for each Reagent in the Reaction Setup table under the Assay section.

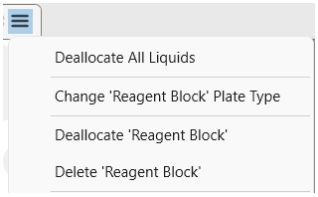
If the capacity of the tube selected is below that of the minimum volume required for the run, a caution will be reported next to the liquid.



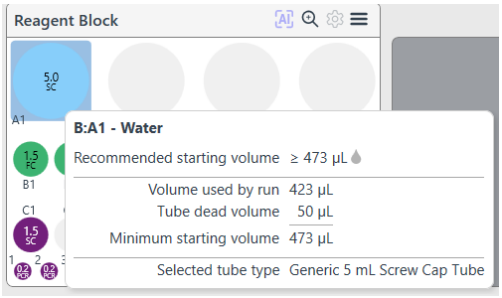
If at any time you would like to double-check your layout, click on the magnifying glass button to zoom in on a specific plate to make sure everything is in the correct position.



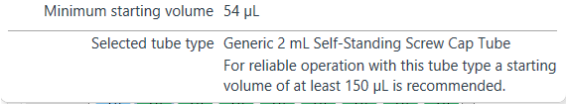
Deallocate a specific liquid from a block using the right-click drop-down menu or Deallocated All Liquids from a block using the submenu icon. There are also options to change the plat type, deallocate a plate or delete it completely from the deck layout.



Hovering over each component well will bring up a tool tip with information on the contents of each well. This includes the **Recommended starting volume** required in the tube to ensure optimal performance. The calculation for the recommended volume is also shown. Tube that are required to be empty at the start of the run are also reported.

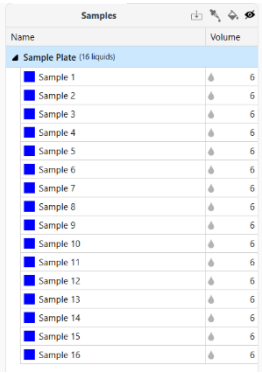


For some tubes, for example 2.0 mL screw cap tubes, a recommended volume is also provided. These volumes are based on the profile of the tube and best practise to ensure optimum performance.



Allocate the Samples onto the Deck Layout, including Assay Controls.

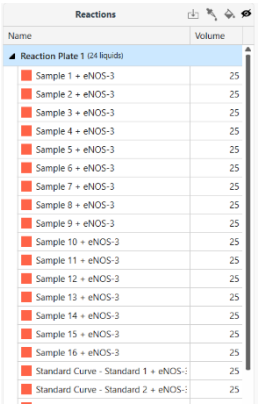
Sample Plates and Loading Blocks are automatically assigned to source plate/block according to the order specified in the Samples editor. To change the order of the source templates, go back to Samples Editor. Any samples defined as Individual tubes, including the source template for standard curves and any Assay Controls must also be allocated to the deck. Samples are listed as groups along with the total number per group. You can expand the list by selecting the triangle expand button.



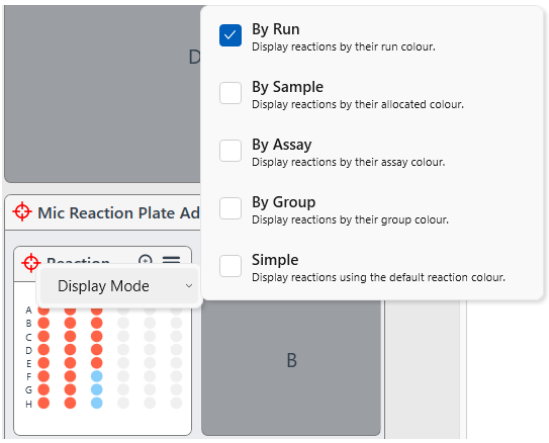
Ensure Reactions are allocated properly.

Reaction Plates will be auto-populated onto the Deck Layout. They can be dragged and dropped to any socket. Any plates that cannot be automatically allocated to a deck position will be marked in the Reaction Liquids group list. All Reaction Plates must be allocated to a deck position to start the run. If there is insufficient space, the number of samples or assays to run must be reduced in the Samples editor.

Reactions are listed as groups along with the total number per group. You can expand the list by selecting the triangle expand button.

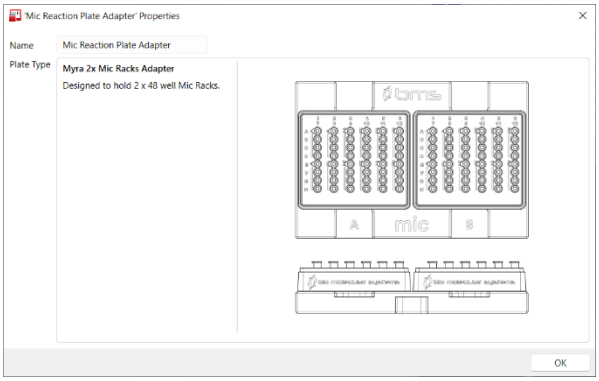


The option to display the reaction output well colours by Run, Sample, Assay, Group or Simple is also available using the red cross hair icon. This is done by selecting the **Display Mode** option and then required type.



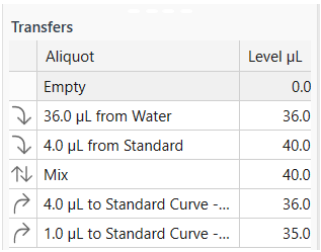
Once components have been allocated, you can drag them across to other wells or plates.

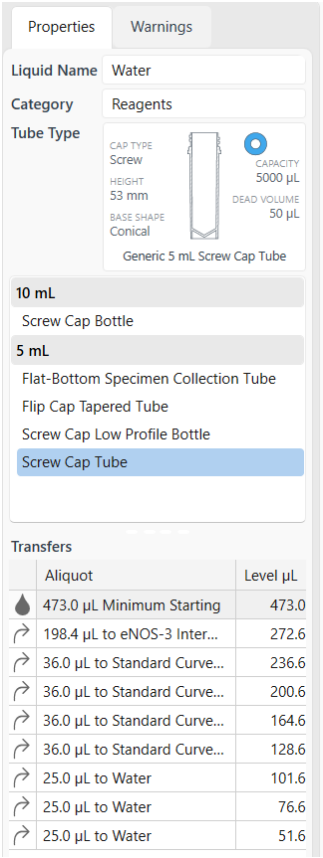
You can use the Properties option in the submenu to view the plate information at each position including adaptor type.



Liquid Transfers List

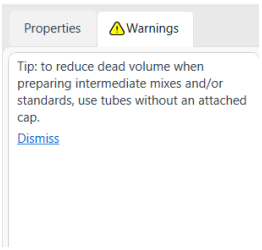
If you click on a particular tube on deck or in a liquid inventory list, it will display the **Transfers** information list in the **Properties** section of the information section to the right of the active window. This will show the order and volume for each liquid transfer for tubes such as master mixes of dilution tubes. This also includes all the transfers of a reagent and the volumes left following each transfer assuming the recommended volumes is loaded at the start.





Warnings

Various warnings will be displayed during the deck layout in the Warnings tab within the information section on the right of the active window. A run cannot begin until all warnings have been handled or dismissed.



Some examples include:

- No reactions have been configured.
- There are no assays included in the run.
- Some samples have no assay configured. No reactions will be generated for these samples.
- Some samples have no assay configured. No reactions will be generated for these samples.
- Assay reaction volumes are not consistent.

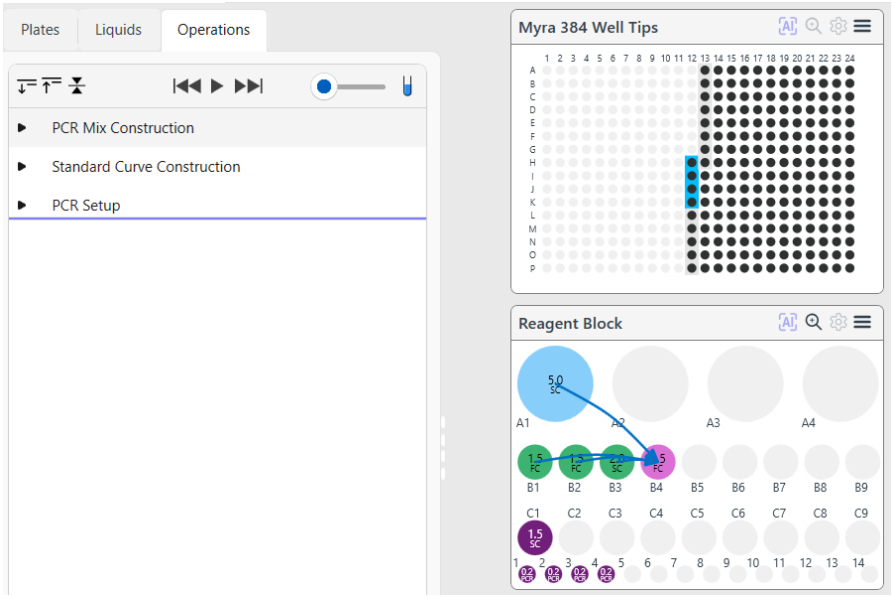
MyraSim

MyraSim makes up the first part of MyraIntelligence and is the expert AI assistant that can help you plan your runs with confidence. The system allows you to:

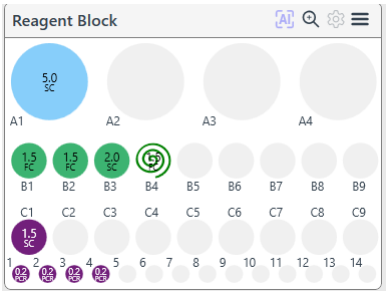
- Dynamically calculate optimal volumes required to complete the run without fail.
- Analyzes every operation in your run to ensure every step will complete as expected - no surprises.
- Provides optimization feedback suggestions for better run performance.

Operations List

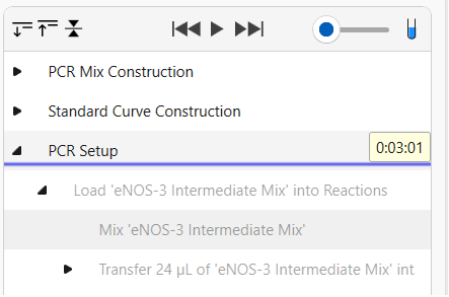
The **Operations list** is in the Deck Layout. Hovering over the list displays the transfer movements across the deck layout using blue arrows. Multiple movements that form a set of transfers are shown all at once. The tips used for each set of transfers is also highlighted in blue in the tip rack. The total number of tips used are highlighted in light grey.



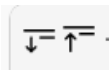
Any mixing operations are shown with a green swirl.



The blue bar shows the time point in the operations list. You can move the bar to see each time point within the list. There is also an icon to allow you to Scroll to rollback bar.

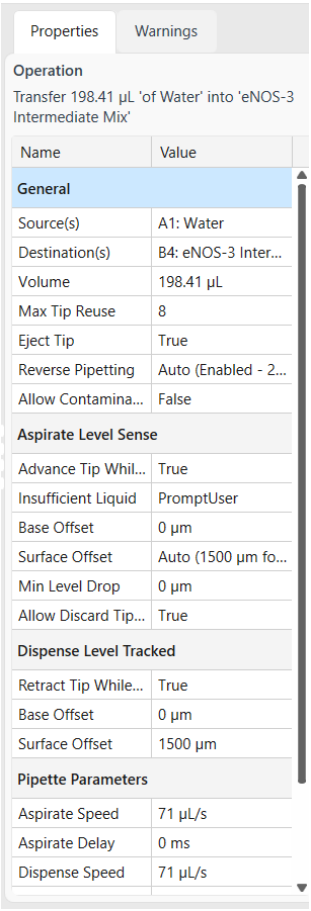


Icons to Expand all operations and to Collapse all operations are available as well. You can expand the operations list to single transfers and steps. Single transfers are shown as single arrow line movements across the deck.



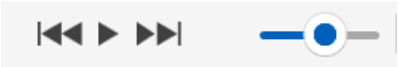
If you click on an individual step the full details are reported in the Properties information tab to the right of the active window. The information provided includes:

- General settings including destination coordinates, volume, tip reuse number, and if you allow for contamination.
- Aspirate level sense settings.
- If dispense level is tracked.
- Pipette parameter settings including speed and air volume.



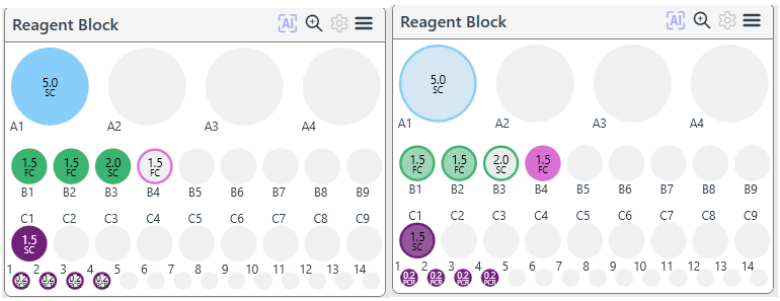
Play the MyraSim

Clicking the Play button allows you to watch the simulation progress through the list.



You have the option to go back to the start or move to the end using the icons on either side of the play button. Adjust the Playback speed using the slider bar.

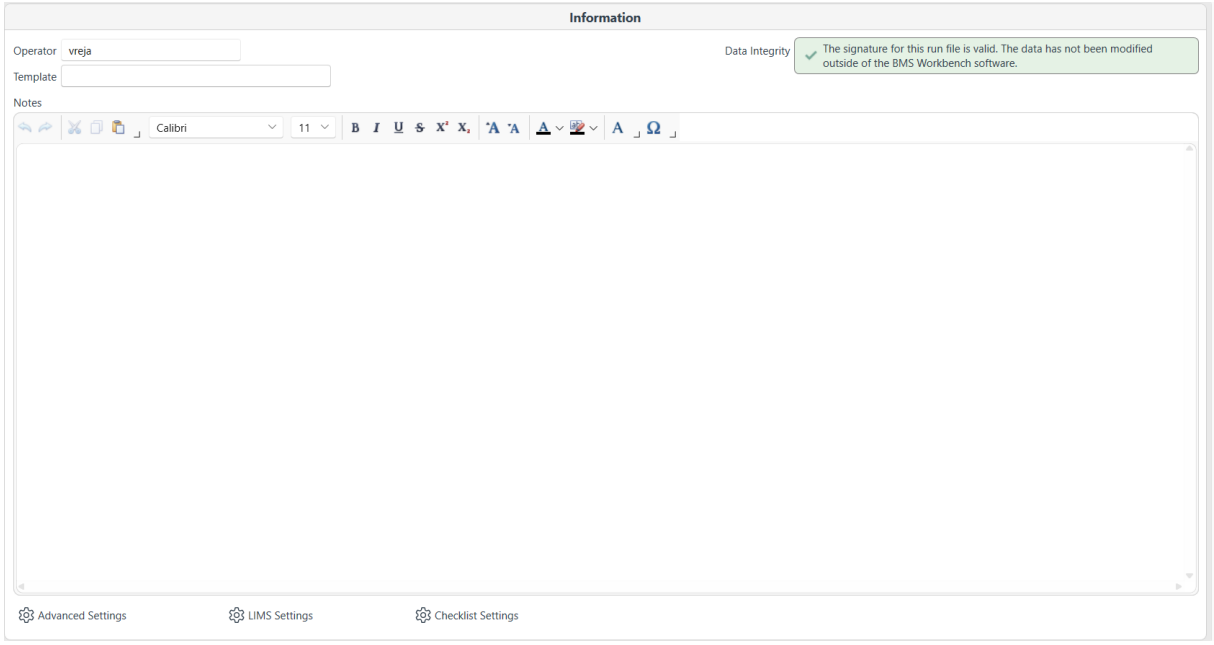
Click the blue tube icon to **Fade the liquid colour** on the deck layout. This indicates the amount of volume relative to the maximum being added or removed from that tube.



As the water in the 5 mL screw cap tube is used the blue colour fades. As the pink master mix and purple stnadard are produced the colour changes to a more darker colour.

Information Page (Additional Settings)

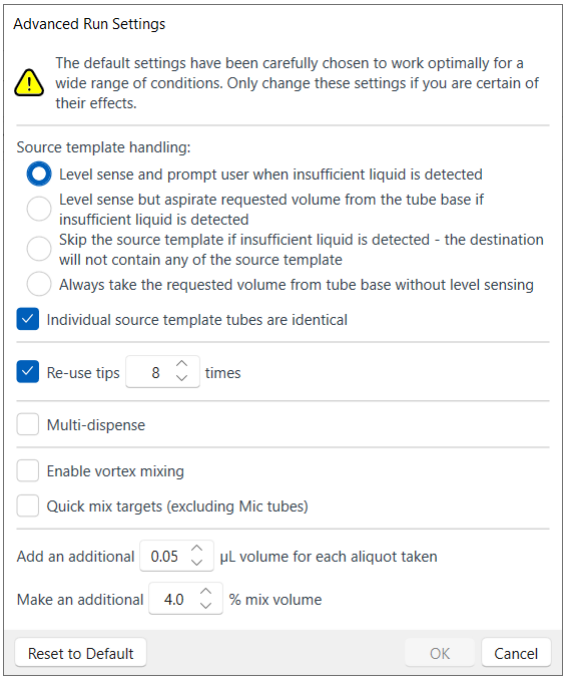
The **Information** heading opens a page where additional notes can be written. There is also an additional field to nominate an **Operator** and a field where a template utilised is auto-populated. A **Data Integrity** message ensures that the file is valid and has not been altered outside of the Workbench software. There are also three additional settings that can be changed in this page, called **Advanced Settings**, **LIMS Settings,** and **Checklist Settings**.



Advanced Run Settings

ATTENTION	
⚠	The default settings have been carefully chosen to work optimally for a wide range of conditions. Only change these settings if you are certain of their effects. You can click on Reset to Default to go back to default settings at any time.

The following four options are available for source template handling:



- **Level sense and prompt user when insufficient liquid is detected**: for accurate level sensing where the run will stop with a warning (and how you want to proceed) if there is insufficient liquid detected.
- **Level sense but aspirate requested volume from the tube base if insufficient liquid is detected**: for automated aspiration from the base of the tube when the system detects insufficient liquid. Use this option if you are certain that the source template wells contain enough liquid to complete the run but often get insufficient liquid warnings due to the shape of the tubes you are using or the composition of the liquid.
- **Skip the source template if insufficient liquid is detected**: if Myra encounters insufficient liquid, it will skip that source template and no template will

be added to the reactions requiring that template. Use this option if you are using source template storage where it is common for some source templates to run out due to differing yield in the extraction process or uneven source template usage. Note that there will be no warning issued if there is insufficient liquid.

- **Always take the requested volume from tube base without level sensing**: for the volume to be taken from the base of the tube without sensing the liquid level. Use this option if your source template tubes contain a shallow pool of liquid that often triggers the insufficient liquid warning or if level sensing sometimes causes bubbles that interfere with the liquid aspiration. Note that there will be no warning issued if there is insufficient liquid.

Individual source template tubes are identical allows the system to assume that all individual source template tubes (tubes in storage blocks or cluster racks are omitted) are of the same brand and shape and therefore have the same tube base position. When this option is enabled, the tubes of the same type will be probed only once for each tube holder. When turned off, Myra will probe each individual tube to ensure maximum accuracy and therefore potentially consume more tips.

The number of times you can Re-use tips can be configured. Untick this condition if you believe you would like a new tip for each new pipetting action. This will result in increased tip usage. The default is to Re-use tips 8 times.

ATTENTION	
	Exercise caution with tip re-use, as this can lead to inaccurate pipetting and drippage onto the deck.

Check the box to use Multi-dispense.

Multi-dispense will reduce run times while still retain high levels of accuracy and precision. The software will apply multi-dispense during reagent or master mix liquid transfers.

The following type of mixing can be enabled:

- **Vortex mixing** is a new function on the Myra, where a vigorous homogenising circular motion is applied by the pipette head, enabling faster mixing.
- **Quick mix targets** allows PCR reactions to be mixed after their construction in PCR plates or tubes.

The following types of additional volumes can be applied:

- Add an **Additional volume** for each aliquot taken will add an additional amount to the required volume for each aliquot taken from the Samples to compensate for liquid retention in the pipette tip. Increase this if you find there is insufficient mix created when running with more samples.
- Make an **Additional percentage of mix volume** if you think you need to compensate more for your master mix creation.

LIMS Settings

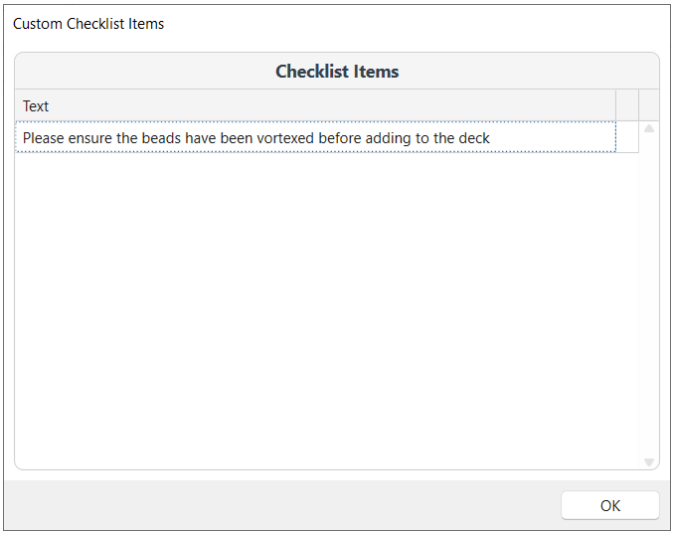
To configure laboratory information management systems (LIMS), refer to Appendix C – Laboratory Information Management System (LIMS) Settings.

Checklist Settings

Workbench generates a checklist that must be completed prior to commencing a run. Additional prompts can be added to this checklist, if needed for your unique laboratory setting.

Add additional settings by typing into the Text box, then hit enter.

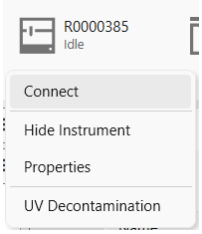
These changes will be reflected in the checklist generated prior to starting a run.



Connecting and Starting a Liquid Handling Instrument

Create a new run and select the Connect option in the drop-down menu of the Instrument icon.

Only instruments that have the Idle status can be used.



Once connected, a run summary status bar will appear.

▶	Start Run	Will start the run.
✖	Abort Run	Abort during a run.
⌂	Home Pipette Head	Will home the pipette head if the run is aborted during a transfer. This ensures the head is parked in a safe place.

CAUTION ⚠	Although rarely needed, if a run is aborted without moving the head to the park position, customers should refrain from physically pushing the pipette head into place to access the deck; using the **Home** icon is sufficient to ensure the pipette head returns to the park position.

Begin the run by selecting the Play icon in the run status summary bar.

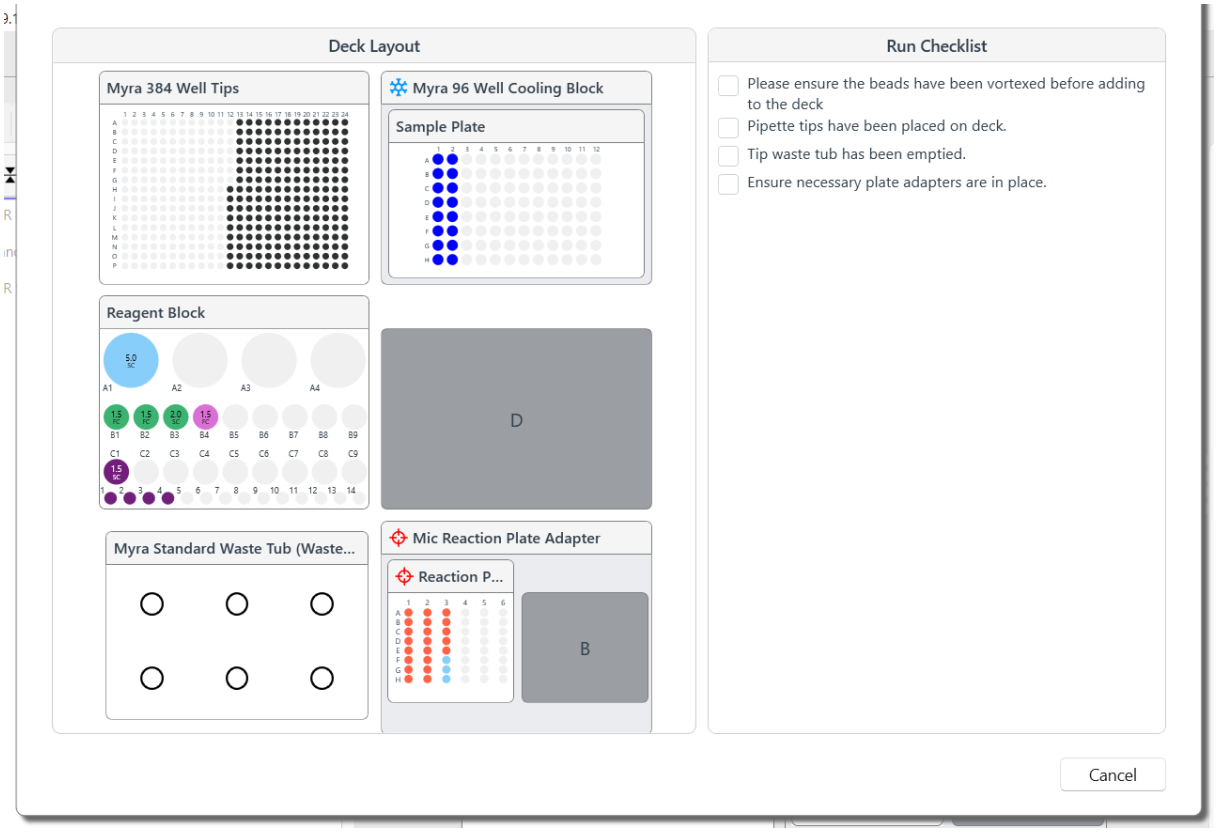
The software will automatically open a Save As dialogue box before proceeding. A default file name is provided; however, you may enter any name you choose.

The instrument LED indicator will flash blue, indicating to other users the instrument has been selected to start a run. No other user can start a run on the instrument until the run is has completed or is cancelled.

After saving the file, a confirmation dialogue box will appear. The box shows the deck layout for the run.

You must tick all items in the Run Check list before you can execute the run.
These can include:

- Pipette tips have been placed on deck.
- Tip waste tub has been emptied.
- Ensure necessary plate adapters are in place.
- Any additional items you have entered in the Checklist Settings of Information page or required for a MyraScript.



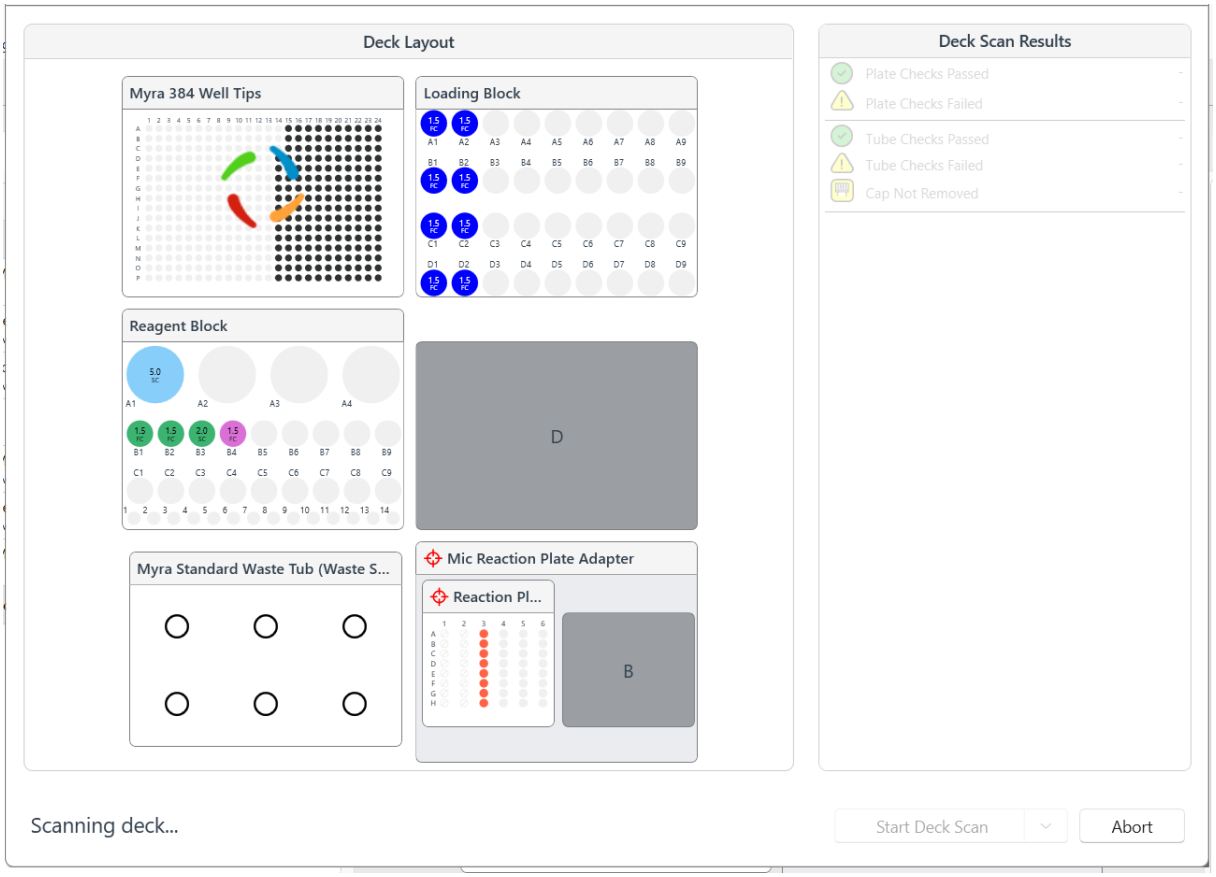
Ensure the lid is closed prior to starting the run. A lid sensor will detect if the lid is open and will prevent the instrument from starting, and a warning will also appear.

MyraVision

MyraVision is the eyes on your setup. Using advanced computer vision and AI learning models, MyraVision understands what is on the deck. It intelligently checks for consumable type, missing plasticware and cap removal, to ensure your run can start with confidence.

To execute the run and start MyraVision, click the Start Deck Scan button in the Start Run dialogue box.

The MyraVision will begin to scan each section of the deck. A colour wheel will appear over the section it is scanning. You will notice a light turn on during the scan and the head move across the section.



Any errors will be reported as either a plate or tube failure:

- **Plate Check** failure is either an incorrect or missing plate.
- **Tube Check** failure is either a missing or incorrect tube, or the tube cap has been left on.

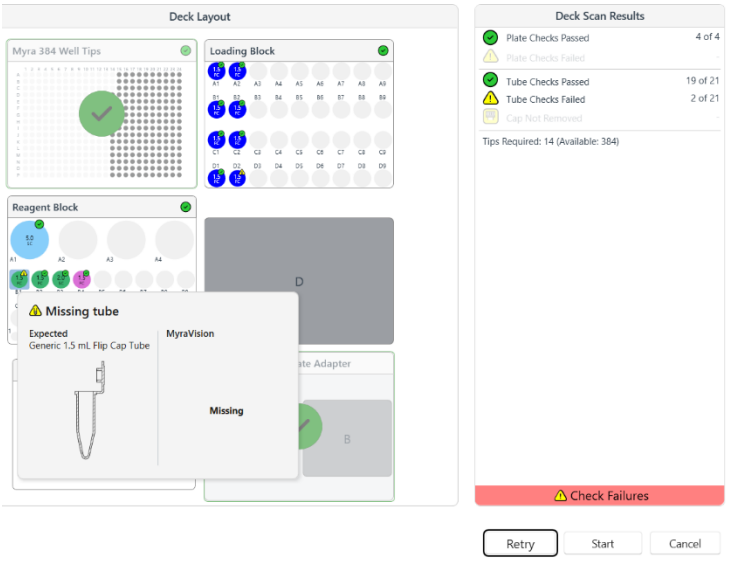
A caution symbol based on the failure type will be reported on the tube or plate in question, on the deck layout image.

Hover over the tube or plate to see a more detailed analysis of the failure.

Images are provided for most expected plate or tube types.

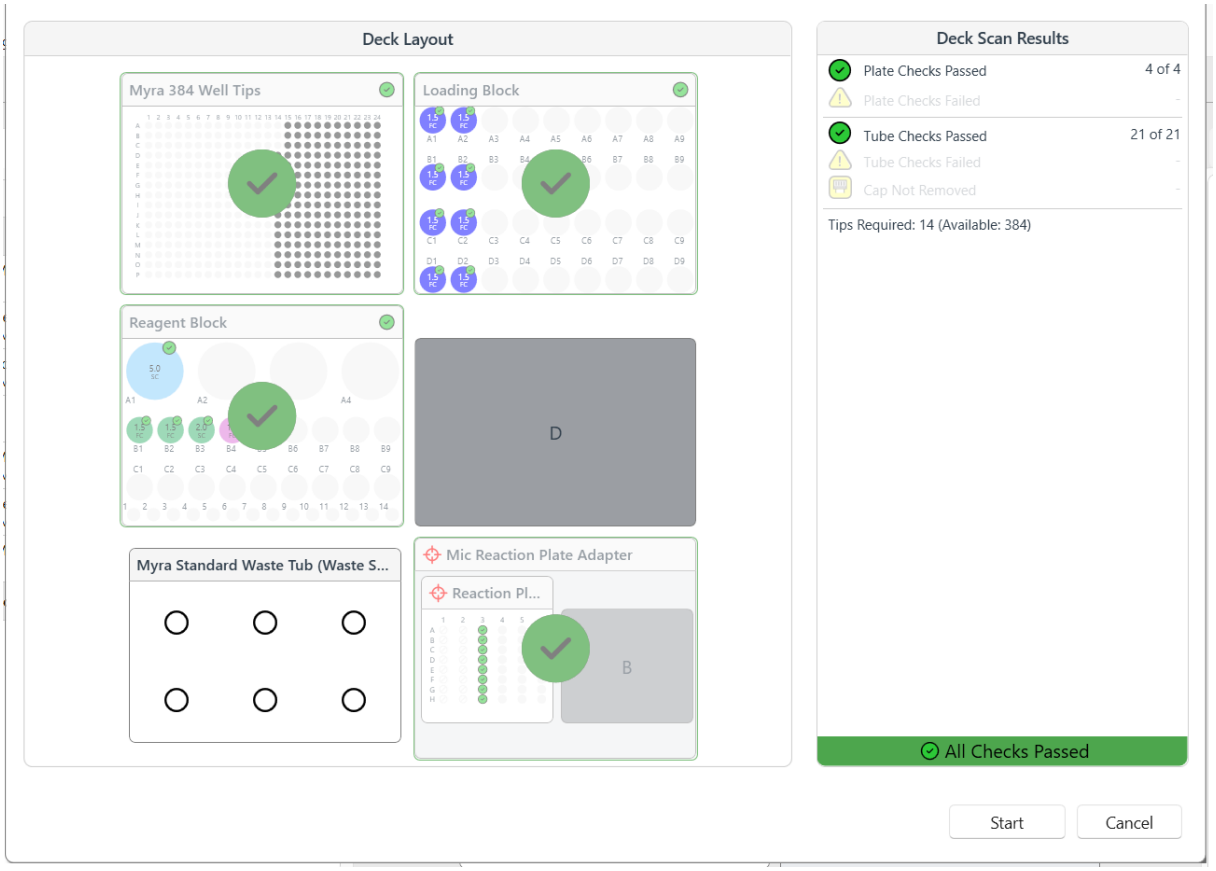
Correct the issue and then Retry the scan.

The scan will be repeated and the result displayed again.



When the MyraVision scan has passed you can commence the run.

Passed scan will show green ticks across all the sections of the deck.

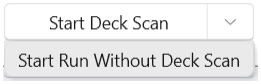


Select the Start Button to begin the Run.

The instrument will automatically activate the lid sensor and then start running the program. The LED indicator will turn green to notify the instrument is running.

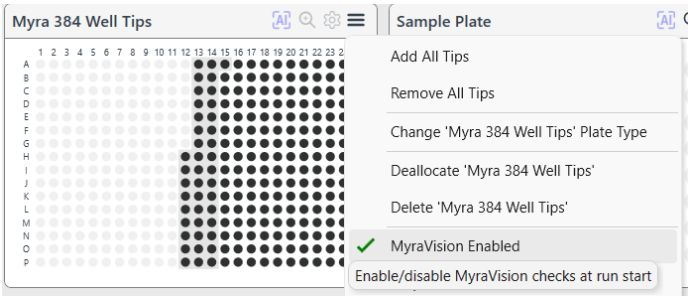
You can also choose to start the run without AI verification using the dropdown menu next to the Start Deck Scan button.

You can choose to do this if MyraVision is having issues correctly imaging one of your consumable types.



ATTENTION	
⚠	If MyraVision is having trouble with your plate or tube type. Please create a Support Package and send it to our Support Team. This will assist us in improving the AI library to ensure we can correctly detect your consumable type.

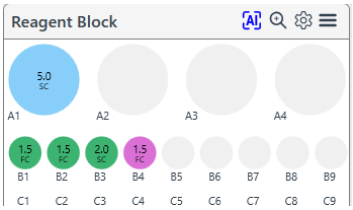
In the submenu there is an option to enable or disable the MyraVision for a particular plate type. This option is typically used if the AI has not yet been properly trained on a new plate type. It will avoid having the MyraVision fail the plate prior to every run. As such you will need to ensure yourself that the plate is correctly in place.



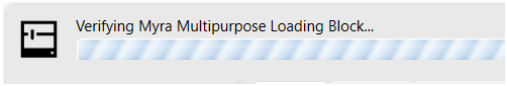
Deck Layout When Connected to Myra Instrument

When connected to a Myra you have the option to calibrate a plate position by selecting the gear icon. See Calibrating Blocks, Plates and Adaptors for more details.

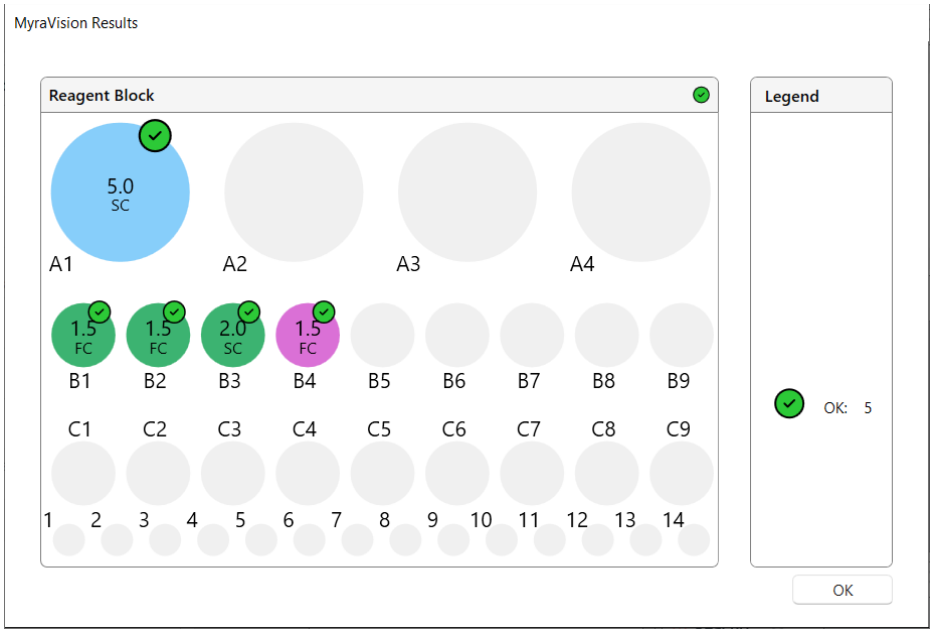
You can also select to. **Perform an AI Verification Scan** of a particular deck position. Click on the AI icon to begin the process. See MyraVision for more details.



The summary banner will show that the AI verification is occuring.



The result of the verification will be reported. Any issues will be highlighted along with the caution symbol associated with the error detected.

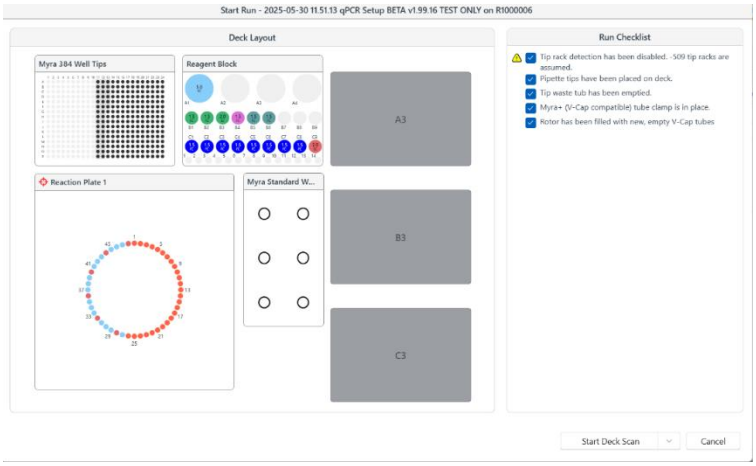


Starting a Myra+ Run

Starting a Myra+ run is slightly different to the Myra or Myra9.

After saving the file, a confirmation dialogue box will appear that will have a specific Checklist for the Myra+, which includes:

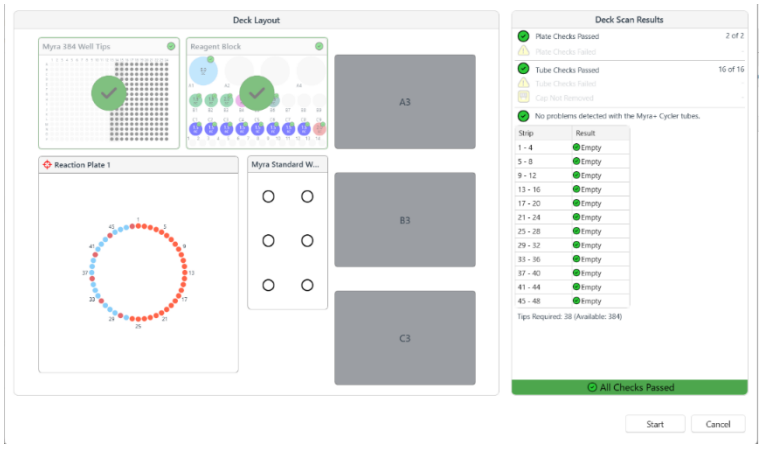
- Myra+ (V-cap compatible) tube clamp is in place.
- Rotor has been filled with V-cap tubes.



ATTENTION

Always remove all tubes from the Myra+ cycler and ensure the entire rotor is filled with new, empty V-Cap tubes prior to commencing a run. Failure to follow these instructions could lead to invalid results, instrument damage and contamination.

For the **qPCR Setup** when the Myra+ loads directly into the Myra+ Cycler, a tube verification is performed during the Deck Scan. The software will initially ensure that the portal to the on-deck Mic chamber is closed. The on-deck Mic will then complete a **V-Cap tube detection test**. During the test, the on-deck Mic will determine if all rotor positions are filled with tubes. The software will then ensure that only empty tubes are detected. Any tubes left over from previous reactions, including water load tubes, will be detected by using the **Myra+ Tube Detection Dye**, as these can result in overfilling tubes. The rest of the scan follows the MyraVision protocol.



The software will issue a warning if it was unable to detect tubes present in all positions or if there are leftover tubes in the rotor. **It is your responsibility to take these warnings seriously and mediate with appropriate actions; failure to ensure that fresh tubes are not loaded into each position in the rotor will affect results and could result in instrument damage.**

Once the run does commence it will initially open the portal to the on-deck Mic chamber. It will then begin the transfer of liquid based on the programmed run. Once all V-Caps are loaded into the on-deck Mic, the access port will close and the Mic instrument will begin its programmed profile.

When using the Myra+ with the integrated **qPCR Setup** option for PCR run using the on-deck Mic, the Run Summary banner that appears automatically combines both the qPCR setup and cycling conditions for total time.

During a Myra Instrument Run

Once the run begins, the instrument will first send the pipette head to the waste tub to ensure there is no tip attached. It will then commence transfer of liquid based on the programmed run.

In the **Myra Run Summary banner**, the current pipetting step is displayed to the left side of the banner next to the instrument icon.

A graphic of the time progressed is displayed in blue.

The time remaining to complete the run is displayed on the right side of the Run Summary banner.

You can pause a run by selecting the **Pause** icon in the run summary banner.

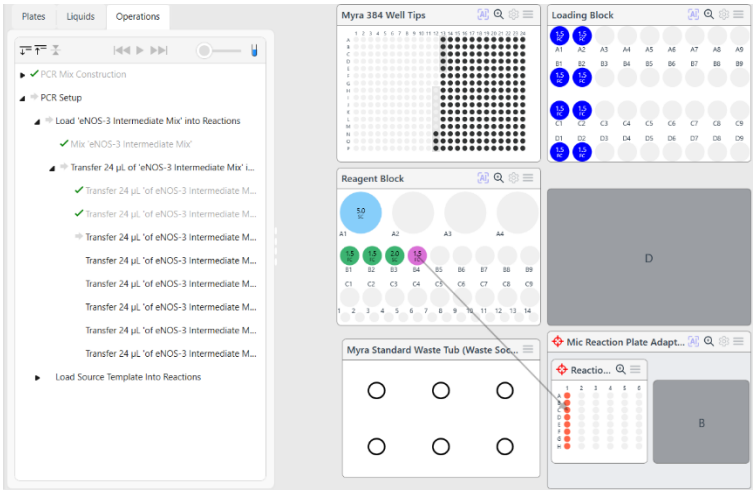
You can stop the run at any point by selecting the **Abort** icon in the run summary banner.

This will prompt the head to move to the park position.

CAUTION	If the run is aborted due to a physical collision of the head with items on the deck, it is generally recommended that the head is not parked. Once the run is aborted and the instrument status has been returned to Idle, power off the instrument and gently manipulate the axis drivers to allow the items on the deck to be removed and any spillage to be cleaned.

The Deck Layout page of the navigator bar contains an Operations display of all volumes and positions liquid is being transferred to during a run. The operation being conducted in real time is highlighted. An arrow on the deck layout displays the trajectory of the transfer. Completed operations in the list receive a green tick.

During the creation of an intermediate mix or loading of reaction tubes, the well will turn from grey to a lighter content colour, defined in the legend. On completion of the mix or reaction, the well will turn into the content colour.

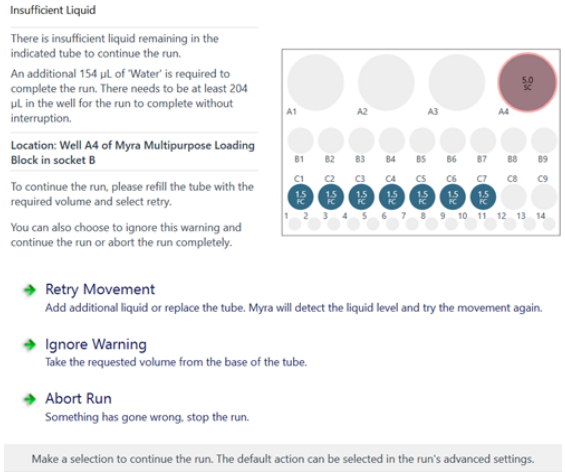


MyraSense

MyraSense is the touch behind every drop. Pressure based liquid level sensing is used for maximum security and precision. Optimized mix type selection, based on liquid levels and tube type, is achieved in real time. Intelligent tube height probing is used to avoid collisions and ensures less time is spent calibrating. Automated run recovery will occur from unexpected collisions. MyraSense will ensure your run completes with confidence

Messages and Error Reporting

Any issues during the run such as running out of liquid will be notified to the user.



All actions achieved by the instrument as well as any warnings about the run will be logged and displayed in **Messages** along with the time it occurred.

Common messages include the start time and instrument name and firmware version.

Some messages may be warnings such as:

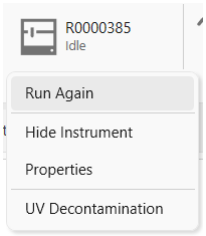
- Running out of tips;
- Not detecting any volume or low volume;
- Lid open events.

Messages

	Time	Priority	Category	User	Message
	30/05/2023 12:27:23 PM	Information	Run	Operator 1	Run started on BMS Workbench v0.9.5 at 30/05/2023 12:27:23 PM (GMT+10:00).
	30/05/2023 12:27:23 PM	Information	Run	Operator 1	Executing on Myra S/N V0000001 F/W v3.41, Head S/N P0500001 F/W v2.21
	30/05/2023 12:27:26 PM	Information	Run	Operator 1	Moving 87.38µL of Probe Mix from B:B7 to B:C9.
	30/05/2023 12:27:31 PM	Information	Run	Operator 1	Moving 87.38µL of Oligo Mix from B:B8 to B:C9.
⚠	30/05/2023 12:27:33 PM	Warning	Run	Operator 1	Run paused.
⚠	30/05/2023 12:29:25 PM	Warning	Run	Operator 1	Run resumed.
	30/05/2023 12:29:26 PM	Information	Run	Operator 1	Moving 48.22µL of Water from B:B9 to B:C2.
	30/05/2023 12:29:27 PM	Information	Run	Operator 1	Moving 44.49µL of Water from B:B9 to B:C3.
	30/05/2023 12:29:29 PM	Information	Run	Operator 1	Moving 37.49µL of Water from B:B9 to B:C4.
	30/05/2023 12:29:30 PM	Information	Run	Operator 1	Moving 24.38µL of Water from B:B9 to B:C5.
	30/05/2023 12:29:32 PM	Information	Run	Operator 1	Moving 48.22µL of Standard from B:C1 to B:C2.
	30/05/2023 12:29:33 PM	Information	Run	Operator 1	Mixing liquid in B:C2.
	30/05/2023 12:30:29 PM	Information	Run	Operator 1	Run finished at 30/05/2023 12:30:29 PM (GMT+10:00).

Run Again Option

Once completed, a **Run Again** option on the Myra icon dropdown menu lets you repeat the selected run without needing to setup the software again. This option is great for repeating experiments.



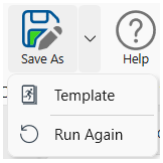
ATTENTION

Discard intermediate mix tubes after completing a run and do not re-use tubes. Failure to do so will result in incorrect pipetting by the Myra.

All aspects of the previous run will be used including reagents, templates, and reaction numbers. Only the tip and Mic tube positions will be updated to reflect the previous run usage.

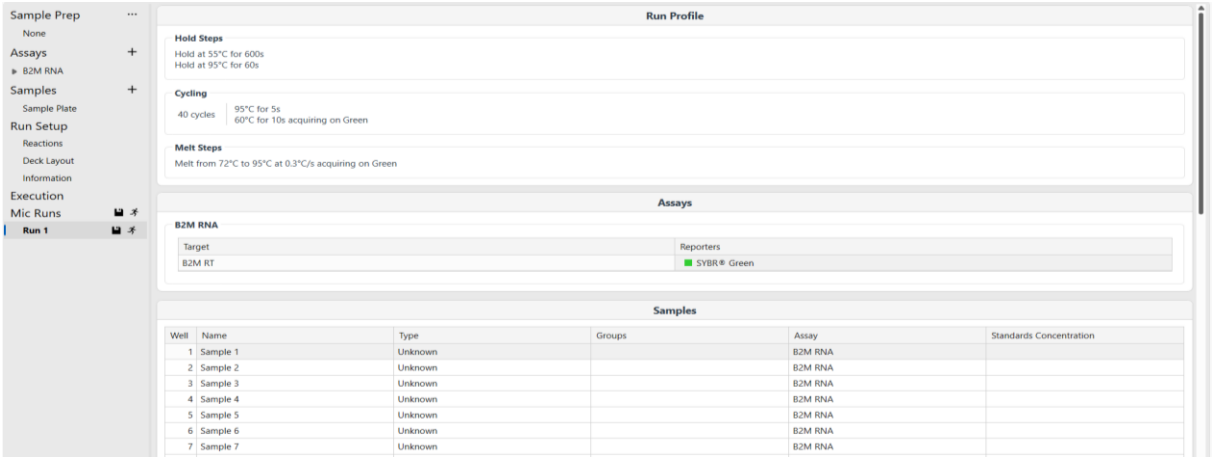
You can modify any of the run parameters prior to starting the run.

If you wish to modify run previously completed and do not have connection to a Myra instrument, you can select Run Again in the **Save As** drop down list.



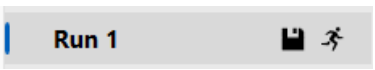
Starting a Mic Run Created by a Myra

You can start a Mic run from either qPCR setup options without needing to export any sample information or start a new Mic run from scratch.



Select the Running man icon next to the Mic Runs in the Navigator Bar.

Workbench will open the Mic run file.



Cap and load the tubes filled by the Myra into the Mic rotor and select the Mic instrument from the tool bar.

Start the run.

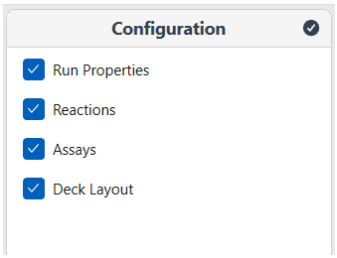
Reports for Myra Instruments

View a Pre-run or Post-run report for each run located in the Run Navigator. A preview of the report will be displayed to the right side of the user interface and can be configured to show only certain parts.

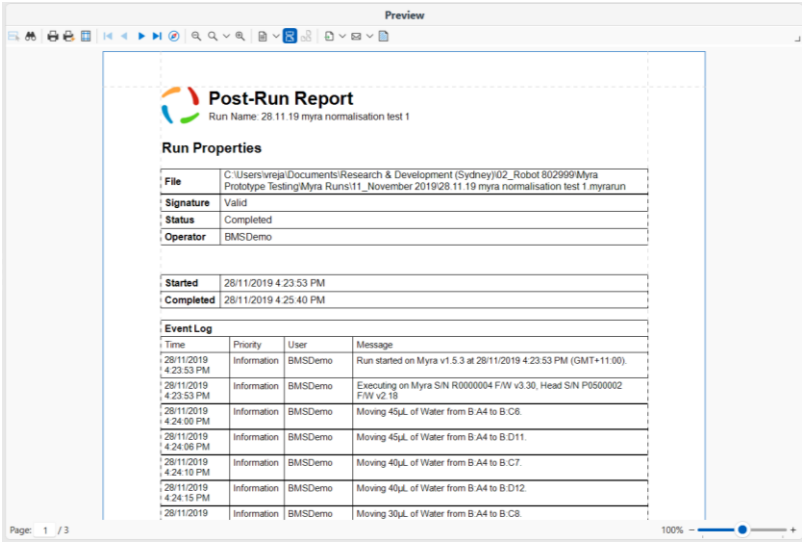
A Pre-run report will display information about Run Properties, Reactions, Assays, and Deck Layout. Pre-Run reports are not available for qPCR Setup.

A Post-run report will display the same information along with the additional logs for the run.

Each report is divided into standard sections: Run Properties, Reactions, Assays and Deck layout. You can choose which sections to display in the report by ticking sections in report Configuration.



Each selected section will be displayed in the report **Preview**. A new page will begin following each section. Each page will have a number in page footer along with the version of software used. The run name will be displayed in the page header.



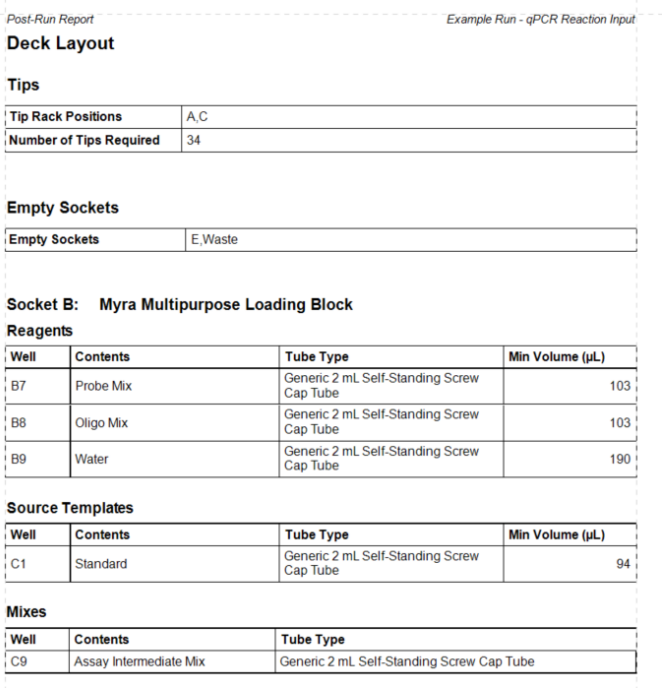
The Run Properties section will display the following:

- The Name of the run.
- The directory location of the run File.
- Status of the run, including whether it was successfully run.
- Operator name.
- Any Notes regarding the run completed, as input by the user.
- The date and time the run Started.
- The date and time the run was Completed.
- An Event Log, which is a report of important messages generated during the run, including any issues such as loss of communication.

The Samples editor is replicated in the report preview including sample Name, Type, Standard Concentrations, and Assay. All samples will be displayed.

The Assays section contains all the information about the targets as well as oligonucleotides and reporter dyes. The reaction components and volumes are also listed. All segments of the run profile are reported, including Holds, Cycling and Melts. Channels used are reported along with the step at which fluorescence was acquired.

A simple representation of the Deck Layout is displayed in the reports. The names and volumes of each reagent, mix or reaction is also shown.



Post-Run Report *Example Run - qPCR Reaction Input*

Deck Layout

Tips

Tip Rack Positions	A,C
Number of Tips Required	34

Empty Sockets

Empty Sockets	E,Waste

Socket B: Myra Multipurpose Loading Block

Reagents

Well	Contents	Tube Type	Min Volume (µL)
B7	Probe Mix	Generic 2 mL Self-Standing Screw Cap Tube	103
B8	Oligo Mix	Generic 2 mL Self-Standing Screw Cap Tube	103
B9	Water	Generic 2 mL Self-Standing Screw Cap Tube	190

Source Templates

Well	Contents	Tube Type	Min Volume (µL)
C1	Standard	Generic 2 mL Self-Standing Screw Cap Tube	94

Mixes

Well	Contents	Tube Type
C9	Assay Intermediate Mix	Generic 2 mL Self-Standing Screw Cap Tube

Other report options include:

- **Search**: find a word or string of characters in the report preview. Enter the search word(s) to find them in the report. The located words will be highlighted in the preview.
- **Print**: print the report using user defined settings.
- **Quick Print**: print the report using default print settings.
- **Page Setup**: select the paper type and orientation and adjust page margins.
- **Zoom**: Use the zoom in or zoom out to best view the report preview.
- **Page selection**: navigate through the pages using the page selection buttons; First page, Previous page, Next page, and Last page.
- **Export**: export the report using one of the available file formats including PDF, XLS and Text file. Each export will have a set of options to choose from.
- **Send**: email a report using one of the available file formats. A report generated in the selected file format will be attached to an email using your default email application, which will open automatically (if available).
- **Watermark**: add a watermark to your report. The watermark can be either a text or image. This option can be used to embed text such as Confidential to the report. A list of default text is provided, or you can enter your own. Alternatively, add an image to the report such as a company logo. The direction and position of the text or image can be configured as well as which pages to apply the watermark to.

qPCR Assay

To set up a qPCR run on the Mic or Myra systems, a qPCR assay must first be defined. This includes details about the target amplicons, as well as the reagent composition and volumes required for the reaction. For the Mic or Myra+ Cycler, additional settings must also be specified, such as the qPCR cycling conditions and selected analysis types (e.g. Relative Quantification) with their associated parameters.

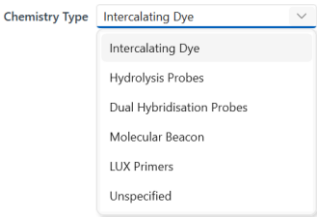
Once an assay is set up, it is saved to the Assay Library, where it can be easily accessed and added to future runs. A new run can be started by selecting the required assay(s), which contain all necessary information to perform the qPCR reaction and, for the Mic or Myra+ Cycler, conduct the corresponding analysis.

Assay Setup

Information

If you are not using a Mic or Myra+ Cycler, we recommend you untick the Show mic compatibility warnings box on the top right to remove any automated error checks required for a Mic or Myra+ Cycler.

Start by selecting the Chemistry Type.
Choices include Intercalating Dye, Hydrolysis Probes, Dual Hybridisation Probes, Molecular Beacon probes, and LUX® Primers.

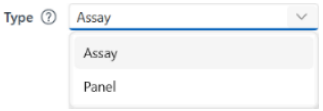


The type of chemistry selected will also set the default Assay Profile (Mic or Myra+ Cycler only).

Specific details for each chemistry type and Mic compatibility are found in the table below:

Chemistry Type	Detail	Notes
Intercalating Dye	Select the reporter dye used	This will set the channel required for acquisition on Mic.
Hydrolysis Probes or Molecular Beacons	Enter the name and sequence information for each probe.	You need to ensure that each probe is labelled with a different channel dye. A warning will be displayed if two targets contain the same channel dye. Optionally you can provide information regarding the quencher dyes used, but the information is not required by the software to execute the run successfully.
	Add multiple probes for the same target if required for Allelic Discrimination, by ticking the Contains Alleles check box.	A maximum of four probes can be selected. Select the reporter dyes and quenchers used. This will set the channels required for acquisition. Probes with the same acquisition channel will not be allowed.
Dual Hybridisation Probes	Enter the name and sequence information for each probe.	Select the reporter/donor dye for the 3' position in the first probe, which will set the channel required for acquisition, and the 5' quencher/acceptor in the second probe. The second probe will also be labelled with a phosphate group at the 3' end, which prevents the probe from initiating polymerase extension.
LUX Primers	Select the reporter dye used in either the forward or reverse primer sequence.	This will set the channel required for acquisition.

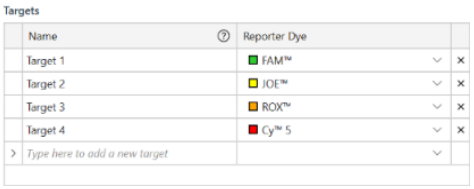
Next, select whether the assay type is an Assay, which contains multiple targets within a single tube, or a Panel, which consists of multiple tubes, each containing one or more targets.



NOTE	Panel assays are only compatible with **qPCR Runs** and cannot be used with **Reaction-Driven qPCR Setup** runs.

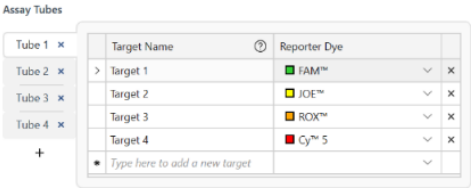
Enter the names of each of your Targets and select respective Reporter Dye(s).

If using multiple targets for the one Assay (multiplexing), enter the new target name in the row below. Unticking the Show mic compatibility warnings box will allow more than four Reporter Dye(s) to be used.



If your dye is not shown in the drop-down menu simply select the dye closest to yours (see Appendix H – Compatible Dyes for Mic qPCR Cycler and Myra+ for more information about compatible dyes).

To set up a Panel assay in which multiple tubes contain one or more targets, use the '+' button under New Assay to define each tube individually. Within each table, add targets by entering them in separate rows, and assign the appropriate reporter dye using the drop-down menu.



Next, enter the Name and Sequence information of each oligonucleotide. (optional).

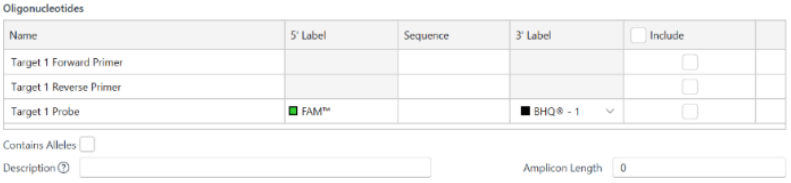
By default, the name entered as the Target will be displayed as the name of each primer or probe followed by the oligonucleotide type (e.g., Forward Primer). You can overwrite the default name by entering a new name in the cell for the oligonucleotide name.

The name entered here will also automatically appear in the reagent component list for each oligonucleotide.

You have the option to enter the sequences in a 5' to 3' direction along with any labels in addition to the reporter dye (e.g., quencher molecules).

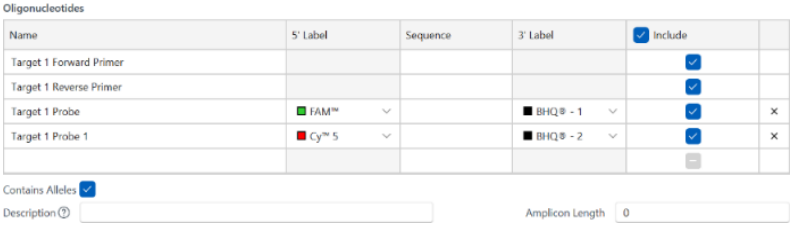
Untick the Include option if you do not wish to use it in the reagent component list.

This might be the case if you have combined the oligonucleotides or you are using lyophilised material or a pre-made master mix (e.g., if using a kit).



For Allelic Discrimination, it is required to add multiple probes for the same target.

Simply tick the **Contains Alleles** option and more than one probe will be available to use per target. A total of four probes can be used for the Mic.



There are additional options to enter a **Description** of each target and the **Amplicon Length**. Both items will be captured in a report.

Next, complete the Reaction Setup table. (optional for Mic qPCR and necessary for Myra systems)

Enter the name for each component required for the reaction.
Oligonucleotides are automatically populated into the table unless unticked in the oligonucleotide table.

Tick if the solution is Viscous.
Pipetting speeds will be slowed down when handling viscous reagents to improve pipetting accuracy. Use this option for reagents such as enzyme mixes that contain a high amount of glycerol.

Tick if the solution is Bubbly.
The frequency of level sensing will be reduced to decrease the number of bubbles created and push the tip a little deeper into the liquid to avoid bubbles that have formed on the surface. Use this option for reagents that contain high concentrations of surfactants such as lysis buffers.

Tick Mix Before Use to mix the solution prior to taking an aliquot.
This avoids concentration gradients forming and affecting final concentrations in the reaction mix.

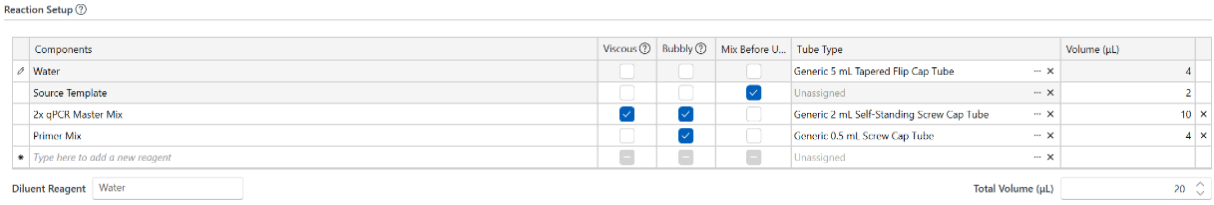
Select the type of tube used to store the component (e.g., 2.0 mL Self-Standing Screw Cap Tube).
This selection helps the software correctly allocate components on the deck layout and ensures accurate pipetting by applying the correct tube profile during the run.

Enter the volume required for each component.
The default template volume is 1 µL. If you wish to add the template to the master mix, enter the Source Template as zero and add your template to the components list. A warning will be displayed telling you that no template will be included in the reaction. This can be safely ignored.

Enter the total reaction volume.
The default total volume is 25 µL. For a Mic or Myra+ Cycler run, this volume will be populated in the Profile editor. Untick the Show mic compatibility warnings if you wish to use reaction volumes greater than 30 µL.

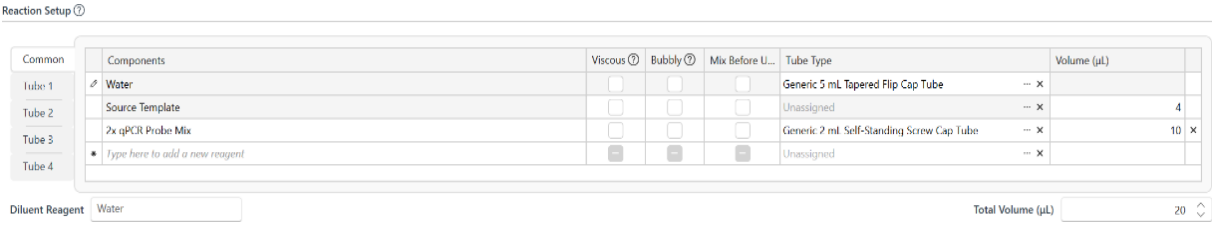
NOTE	If using a pre-made master mix, set the water volume to 0 µL. The pre-made mix will be dispensed directly into the reaction tubes, eliminating the need to generate an intermediate master mix.

You may choose to use a reagent other than water as the diluent for constructing standard curves. If the diluent name is changed from 'Water', the software will treat it as a separate reagent and assign it to a distinct tube during setup.



For **Panel** assays, each tube is configured with its own individual reaction setup table. In addition, a *Common* table is available for components shared across all tubes, such as Source Template or Master Mix. Any component

added to the *Common* table will automatically appear in the reaction setup table for each individual tube, helping streamline configuration and avoid duplication.



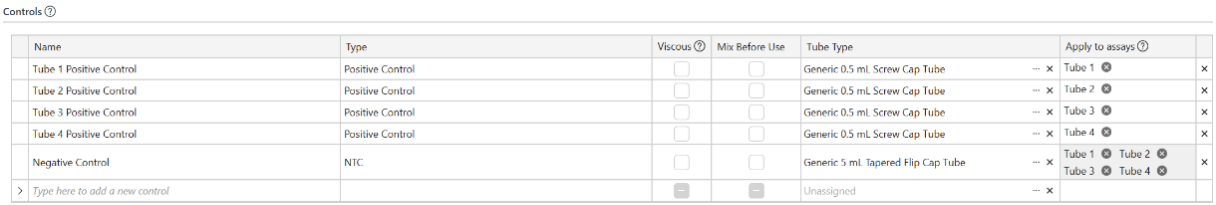
Next, complete the Controls table. (optional for both Mic qPCR and Myra systems)

Enter the name for each control required for the assay and select what type of control they are.
The controls listed in this table will be included in each run setup when using the **qPCR Setup** option, serving as quality control measures to ensure run integrity. It is considered best practice to include controls to confirm the assay is performing as expected. Choices include **Positive Control**, **Negative Control**, **No Template Control (NTC)**, **Extraction Control**, or **No Reverse Transcriptase (NRT)**.

Tick Viscous or Mix Before Use if required.
As above, when ticking Viscous, the pipetting speed will be slowed down. When ticking Mix Before Use, the component will be mixed prior to taking an aliquot.

Select the type of tube used to store the component.
This selection helps the software correctly allocate components on the deck layout and ensures accurate pipetting by applying the correct tube profile during the run.

For **Panel** assays, controls can be assigned to one or more of the individual tubes within the assay. Use the drop-down menu under **Apply to assays** to select which tubes each control should be applied to.



Assay Profile (For Mic and Myra+ Cycler)

Modify the profile as needed to suit the assay.
A generic assay profile is provided by default. The displayed profile will vary depending on the selected chemistry and reporter dye in the Information section.

Click Revert to Default at any time to restore the profile to its original default settings.

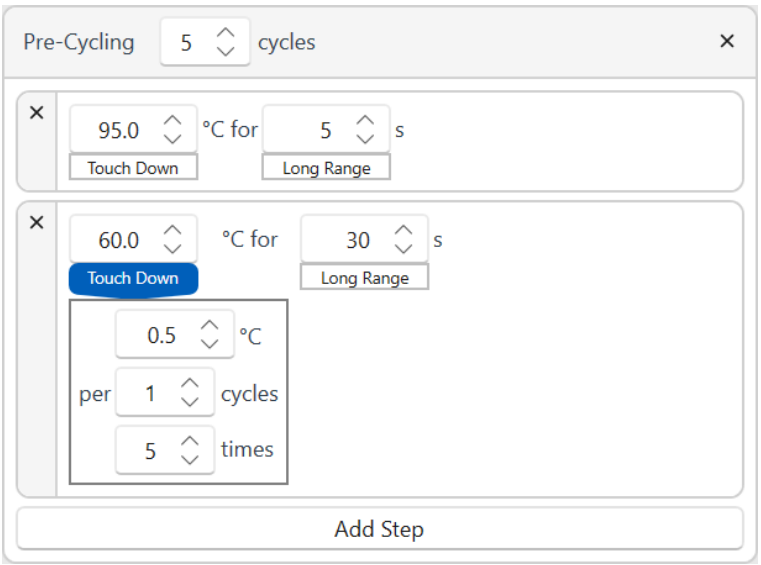
Enter as many hold steps as required under Add Hold.
Hold steps may be required for reverse transcription, UDG activation, activation of hot start polymerase or denaturation of DNA template.

Enter the hold temperature in °C and the hold time in minutes and seconds. The minimum temperature that can be set during an initial hold is 35°C. It is not recommended that temperatures below 40°C be set after holding at a temperature > 80°C.

You can name each of the holds in the title bar by clicking on the name.

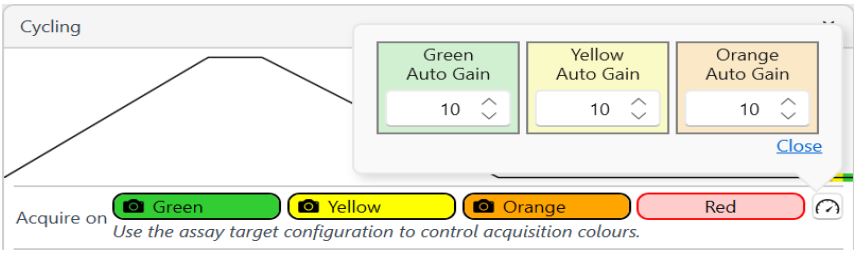
NOTE	Genomic DNA may require longer hold times to ensure complete dissociation of the complementary strands and unravelling of tertiary structures. Failure to fully denature the genomic DNA may result in a significant drop in baseline fluorescence at the start of the run, late amplification, and poor reaction efficiencies.

Add an initial cycling that does not require acquisition under Add Pre-Cycling.
This feature is useful in applications where the early stages of PCR influence signal behaviour. For example, in touchdown protocols, fluorescence is inversely proportional to temperature, leading to an artificial linear increase in signal. While the Ignore Cycles option can be applied during analysis, it may be beneficial to hide this signal shift in the raw data. Pre-cycling can also allow for a faster run due to the lack of acquisition data and does not adversely affect the impact of PCR as amplification is not typically recorded within the first 5 to 10 cycles. Importantly, these cycles will not be added to Cq values in analysis output.



Adjust the gain settings for each channel by selecting the Adjust gain settings icon under Cycling.

A pop-up window with all the channels selected to acquire on will appear that allows you to select the appropriate background level to begin the run. This is called auto gain and defines the baseline setting.



When using chemistries that start with a high background and reduce in signal during cycling, such as quenched FRET dual hybridisation probes or Plexor®, set the baseline to a high level. The default option for such chemistries is 70 units. The instrument will adjust the gain of the selected optical channel such that the baseline is around 70 units or until the maximum gain and scale are reached.

For all other chemistries that begin with a low baseline and increase in signal during cycling, select a low baseline. The default option for intercalating dye chemistry is 3 units, and for all other chemistries it is 10 units. The instrument will adjust the gain of the selected optical channel such that the baseline is around 3 or 10 units or until minimum or maximum gain is reached. The tube with the highest fluorescence is used to achieve optimisation. Specific tubes can be selected to run the gain optimisation on (see Run Profile in qPCR Run).

ATTENTION 	Ensure tubes from a previous run are not left over into a new run. These tubes have significantly higher fluorescence values compared with new reactions and will result in the old tubes being used to optimise autogain, resulting in lower fluorescence values for new reaction tubes. See Appendix G – Removing old reaction tubes from Mic qPCR Cycler rotor for more information.

Enter the number of Cycles required.

Program the cycling parameters by either adding or removing Steps.
Use the **Add Step** button to provide an additional step or the **Delete** button (x) to remove a current step.

Enter a temperature and a hold time for each step. The minimum allowable time is 1 second and the maximum allowable time is 120 seconds.

The allowable temperature range is from 40°C to 99°C.

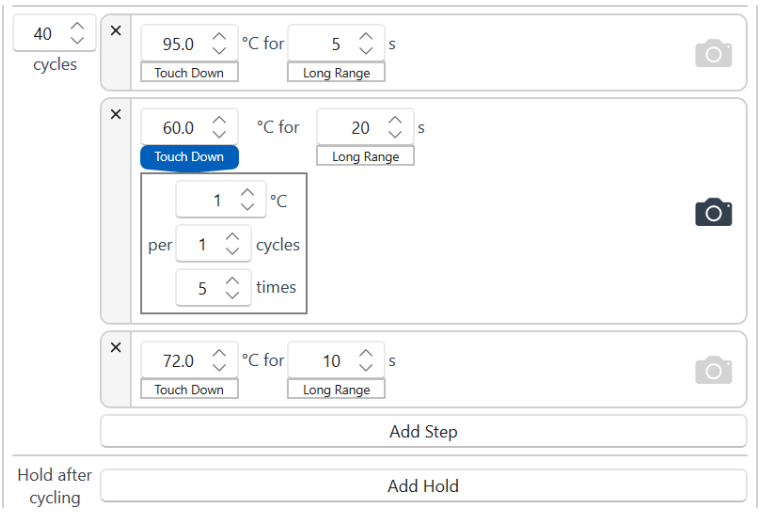
There are two additional options available during cycling:

- **Touchdown:** Used to decrease or increase the temperature during the initial cycles. Touchdown will increase the specificity of primer binding at the beginning of cycling by applying a more stringent temperature for annealing and then increase amplicon yield later by reducing the stringency of primer annealing. For a standard touchdown, select 1°C decrement at 1 cycle each for 10 cycles. This will decrease the temperature 1°C each cycle for the first 10 cycles. Alternatively, you could set a one-off increase in temperature of 10°C at cycle 20. To use Touchdown:
    - Set the decrement in temperature required using a positive number and increment using a negative number.
    - Enter the number of cycles per decrement.
    - Indicate the number of times you wish to apply the decrement.
- **Long Range:** Used to increment the hold time of the selected step. The concept of long range is like touchdown, utilising the annealing hold time rather than temperature. To use Long Range:

Set the number of cycles, the increment in time per cycle and the number of instances required.

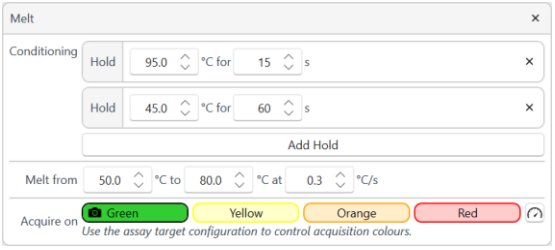
Select the step to acquire on by selecting the camera icon.
Acquisition should primarily occur following the annealing or extension steps and only one step can be chosen for acquisition.

Add a hold after cycling if required.
A hold after cycling could be applied to ensure complete extension of amplicon prior to post PCR analyses such as gel electrophoresis or sequencing.

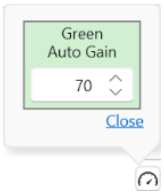


Modify or remove a Melt from the run.
Melts will only be added for specific chemistries compatible with melt analysis; i.e., a melt cannot be added for Hydrolysis Probes. Only one channel will be acquired per melt. A second melt is applied for a second channel, where up to 4 melts can be applied.

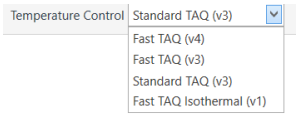
- Add conditioning hold steps to improve the melt performance
- Enter the start and end temperatures.
- Enter the ramp rate required. The minimum allowed ramp rate is 0.025°C/s. Ramp rates greater than 1°C/s will generate data that may not be substantial enough to provide meaningful information.



Adjust the gain settings for the melt acquisition.
Adjust the gain setting by selecting an appropriate baseline to start the melt with. For typical melts that start with a high background the default baseline is set to 70 units. For chemistries that have an increase in signal during the melt, the default baseline is set to 30 units.



Select the desired temperature control setting. Available options include:



- **Fast TAQ (v4)** (default): This profile is designed for rapid cycling and is pre-programmed to ensure heating of the tubes prior to commencing the run, for optimal reaction conditions.
- **Fast TAQ (v3):** This profile does not include heating of the tubes prior to commencing the run. On the Myra+ Cycler, Fast TAQ (v4) is the recommended and default profile.

ATTENTION 	Assay performance on the Myra+ Cycler cannot be guaranteed if Fast TAQ (v4) is not utilised. BMS Workbench automatically prompts users to utilise this polymerase option if using the Myra+ Cycler.

- **Standard TAQ (v3):** This profile is useful in situations when the manufacturer has not specified compatibility with fast cycling, where the rate of heating will be slowed down to allow for sufficient time to complete extension at the optimum temperature range for most Taq polymerases (70 – 80°C).
    - The advantage of using Standard TAQ (v3) is that you can avoid using longer annealing steps, which can reduce analytical specificity; and running a third step at 72°C, thereby saving time.
    - It is recommended to use this profile when using a standard Taq polymerase with two step cycling and short annealing times (<20 s).

NOTE	Due to fast temperature ramping speeds possible on the Mic and Myra+ Cycler, failure to apply a slow rate of heating for standard Taq polymerases without using longer hold times at anneal (>20 s) or three step cycling may result in poor amplification, with reduced analytical sensitivity and amplification efficiencies.

- **Fast TAQ Isothermal:** This profile supports minimum hold, cycling, and melt temperatures as low as 30°C, and accommodates up to 210 cycles.

ATTENTION 	When using the Fast Taq Isothermal (v1) profile, it is strongly recommended to first run water tubes or a small number of samples to assess performance. Importantly, the ambient temperature must be at least 5°C lower than the programmed cycling temperature. If the Mic has recently completed a qPCR run, allow the instrument to cool for a minimum of 15 minutes before initiating any isothermal tests.

Select the Reaction Volume to be used.

To ensure optimal cycling performance, it is important to select the correct volume. The volume selected will determine the appropriate thermal model the instrument should use for heating and cooling.

ATTENTION 	To achieve optimum temperature uniformity, it is important to load tubes pre-filled with water into unused wells of the rotor using the same volume as reaction tubes.

The time to complete the run is also displayed next to the reaction volume and is updated if there are any modifications made to the profile.

Assay Analysis – Mic and Myra+ Cycler

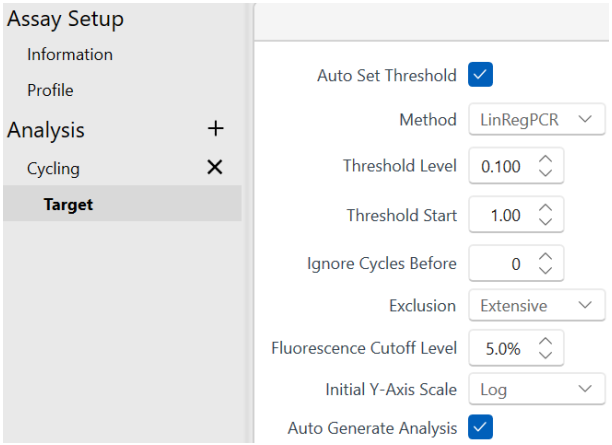
This optional time-saving feature allows you to store all relevant analysis parameters within the assay, eliminating the need to manually adjust settings for each new qPCR run and subsequent analysis. It also provides the option to automatically generate selected analyses using the predefined parameters upon run completion by selecting the Auto Generate Analysis checkbox within any of the analysis types—except for Relative Quantification, which requires multiple assay groupings to complete analysis. This feature should only be enabled once the analysis parameters have been fully optimised for the specific assay conditions, to ensure accuracy and consistency across runs.

To predefine analysis parameters, select the required Analysis for the assay by using the + button.
Analysis options include: Cycling, Melt, High Resolution Melt, Absolute Quantification, Allelic Discrimination, Identifier and Relative Quantification. For a comprehensive explanation of each analysis type and associated parameter settings, refer to Analysis under qPCR Run.

Cycling

Uncheck the Auto Set Threshold box if you want to manually set the cycle Threshold Level and Threshold Start.
For some data, it may be important to start the cycle threshold after a region of baseline interference.

Select an appropriate Method for baseline correction.
There are four options to select from the Methods drop down list. The default method selected will depend on the chemistry type selected for the assay (e.g., Hydrolysis probes will default to Dynamic method).



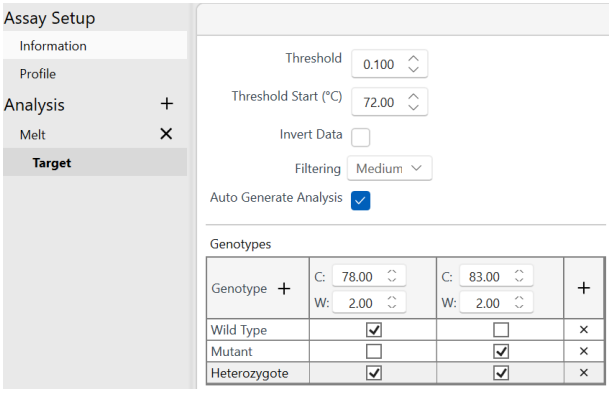
Set the number of initial cycles to ignore from analysis under Ignore First Cycles.
It is possible to ignore initial cycles to remove any significant baseline fluctuations that may influence the results, which is commonly observed when using Dynamic or Fixed-length baseline correction.

Select the level of Exclusion.

Set the Fluorescence Cut-off Level.
Any sample with a fluorescence signal change below a user defined percentage of the sample with the maximum signal change will be excluded from analysis.

Choose the Initial y-axis Scale.
The default scale is logarithmic, with an option to display a normalised curve instead.

Melt



Set the temperature Threshold and Threshold Start for the melt.

Select whether to invert the data.
Select Invert Data for chemistries such as quenched FRET dual hybridisation probes or Plexor®.

Set the Filtering level required.
Options range from none too high. The default is medium.

Set the Tm bins for each expected allele if doing genotyping.
The bins are the expected temperature peak values for each allele.

Also enter the expected range for each bin. The range should consider the melt reproducibility and repeatability of the assay.

It is recommended that the bins be set following assay optimisation.

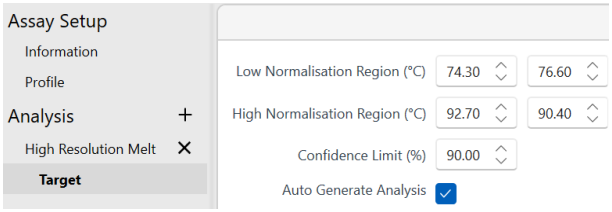
You can add more temperature bins by using the + button on the top right corner of the Genotypes table.

Organise genotypes.
Add genotypes and link them to a Tm Bin. Heterozygotes will use both bins.

You can add more genotypes by using the + button next to Genotype.

Genotypes can be deleted using the x button in the last column of the Genotypes table.

High Resolution Melt (HRM) (Mic only)



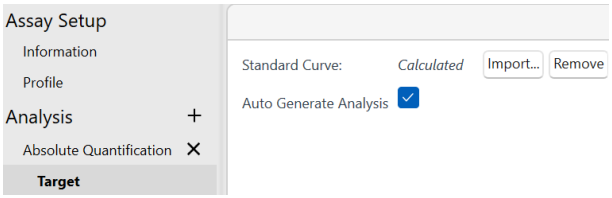
Enter the normalisation regions for the start (Low Normalisation Region (°C)) and end (High Normalisation Region (°C)) of the melt curves.
These regions will be used to normalise the melt curves for HRM.

Enter a Confidence Limit percentage threshold.
Samples below this value will not be reported with a genotype but rather will be reported as unknowns.

NOTE	To use High Resolution Melt (HRM) analysis on the Mic, the purchase of an additional license is necessary. HRM is not currently available on the Myra+ Cycler.

Absolute Quantification

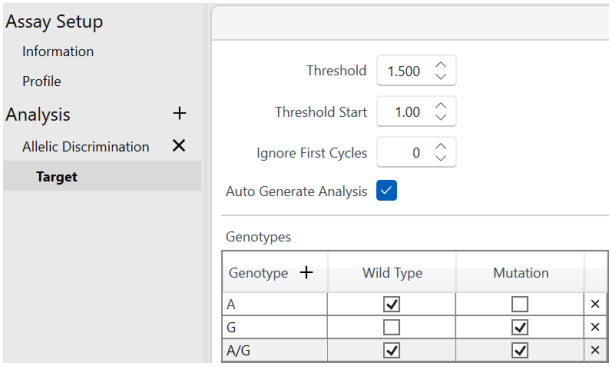
Import a standard curve.
Select the required standard curve file (*.micsc) from the file location it was previously exported to.



Allelic Discrimination

Enter a Threshold level that allows sufficient differentiation of each allele on the normalised real-time curve.
For some assays, low level amplification signals might be detected for a probe in the other channels. This may occur due to cross reactivity between probes for the target, cross talk between dyes or self-hydrolysis of the probes.

Change the Threshold Start or Ignore First Cycles options that are used for normalisation.



Enter the Genotype names required into the Genotypes table.

Link each Genotype to one of the alleles (probes) by ticking the box.
For heterozygotes select both alleles (probes). Up to four probes can be used per target.

The name for the probes provided in the Oligonucleotides table in Information will be displayed in the Genotypes table.

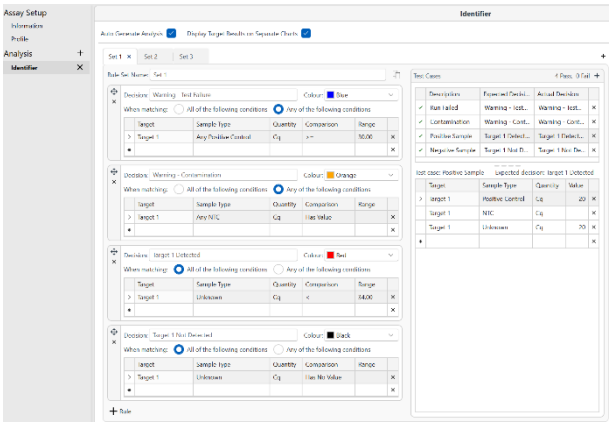
Identifier Analysis

Setup the required number of Rule Sets and provide a Rule Set Name for each one.
Use the + icon at the top right corner to add another target set of rules.

The copy icon next to the Rule Set Name can be used to create a clone of a selected Rule Set.

Create your decisions.
The initial rules applied should use various controls within a run to qualify the test.

Use the + Rule button to add more decisions to the Rule Set.



Select Display Target Results on Separate Charts to view the Cycling Analysis results for each target individually.
If this option is not selected, results for all targets will be displayed within a single combined chart.

Add a test case by selecting the + icon at the top right corner of the Test Cases table.
You can also choose to evaluate your rules by using Test Cases. You can test the rule you have created, or you can create a typical set of conditions for all scenarios you wish to capture and then create and tweak Identifier rules until results match what was defined in the Test Cases.

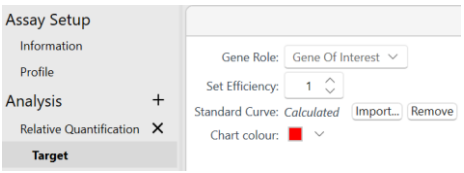
Provide a simple Description of the case, and the Expected Decision.

Below the test case, enter the values for a Target(s) to evaluate the test case.
For example, enter the Cq value for the target of interest to ensure you can observe a positive call in the Actual Decision. Ensure that you nominate the correct Sample Type. A blank value indicates no calculable Cq or efficiency (i.e., a match for the "Has No Value" test).

Each test case has its own evaluation table.

Create multiple test cases to evaluate each decision using various conditions for each rule set.
The number of test cases that Pass or Fail are reported at the top along with a tick or cross next to the test case.

Relative Quantification



Select the Gene role for the target as either Gene of Interest or Reference Gene.

Enter a Set Efficiency value for the target rather than using individually calculated efficiencies for each sample or using a standard curve in each run (optional).
The Set Efficiency value can be derived from previous experiments or use the default value of 1 (behaviour is like ΔΔCt method and not recommended).

Import a Standard Curve to run the efficiency calculation with.

Select a Chart colour to represent the gene in the analysis chart.

Saving a qPCR Assay

Once all parameters have been entered, the Assay can be saved by clicking on the Save As button in the tool bar menu.
Assays will automatically be saved into the following directory of your PC: Libraries/Documents/Bio Molecular Systems/micPCR/Assays.

You can create and store assays in subfolders of the main assay library.

Alternatively, you can save the assays into another personal or shared directory including network drives or external storage drives such as a USB Flash drive.

It is important to have all the assays stored together so that finding them and linking them to a sample in a run will be easy.

qPCR Setup

qPCR Setup provides a streamlined method for creating qPCR reactions on Myra systems. Workbench automatically populates reactions based on sample inputs and any controls defined within the assay. Sources and reactions plates and/or tubes are automatically allocated onto the deck, requiring only the manual addition of reagent components.

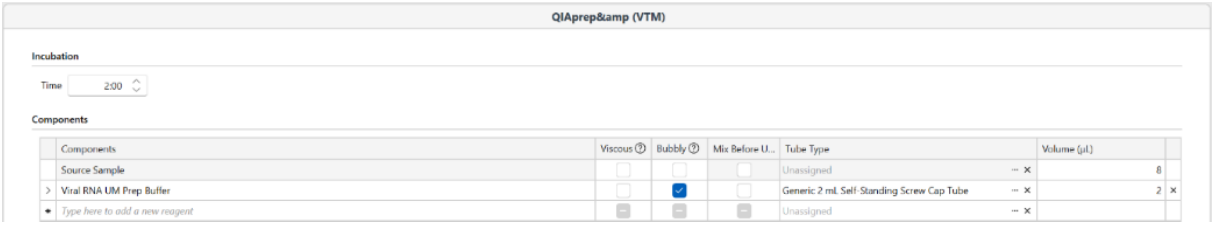
An optional step within **qPCR Setup** allows for raw sample preparation at the start of the run. Currently, this supports QIAGEN's QIAprep& Viral RNA UM Kit, with additional liquid-based sample preparation protocols configurable via the Custom Simple Prep and Independent Prep options.

On the Myra+, an additional advantage of using qPCR Setup is the ability to automatically trigger Myra+ Cycler runs upon completion of the setup, enabling a fully integrated workflow from reaction preparation through to thermal cycling. For a guide on how to setup qPCR reaction on Myra, refer **to Workbench Application Note 1: Sample Prep and qPCR Setup using Myra and Mic**, found under the Quick Links section on the Start Page.

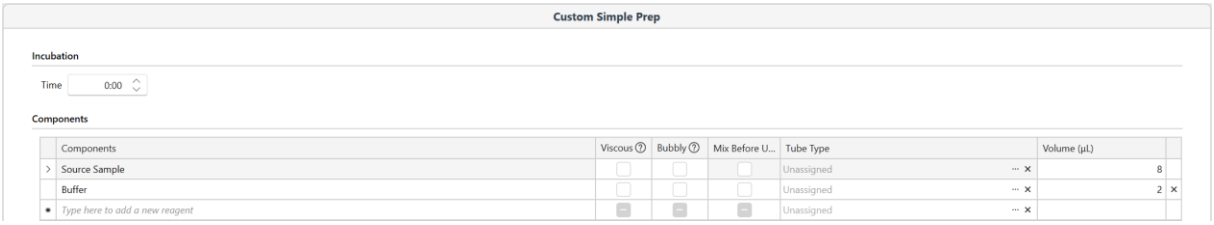
Sample Prep

Select the Sample Prep method of choice using the '...' icon. The following options are available:

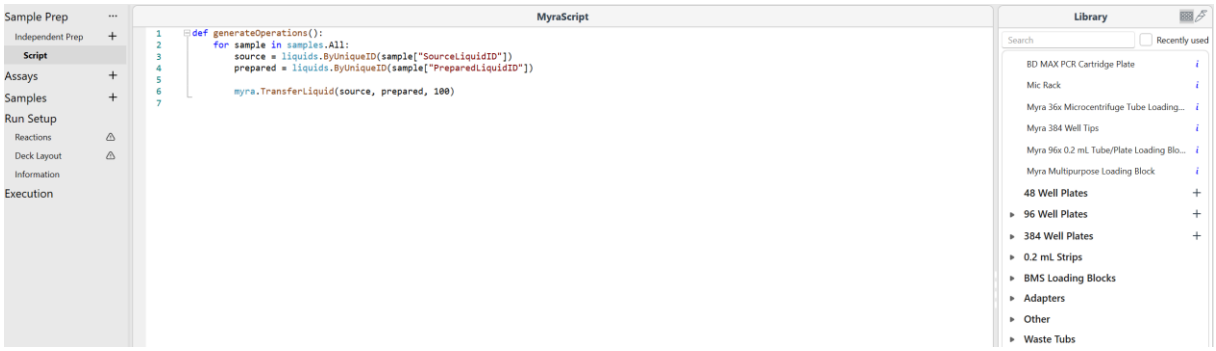
- **QIAprep&amp (VTM):** Performs QIAGEN's QIAprep&amp Viral RNA UM Kit sample preparation prior to qPCR setup. It utilises multi-aspiration, drawing all components, including the sample, into a single tip before dispensing the complete mixture into the reaction plate.



- **Custom Simple Prep:** Performs simple liquid-based sample preparation by multi-aspirating reagents and sample into a single tip before dispensing the complete mixture to the reaction plate. An optional incubation step can follow if required. Incubation times, components, and their respective volumes, are fully configurable. The aspiration sequence is determined by the order of components listed in the **Components** table.



- **Independent Prep:** Offers full flexibility for creating custom sample preparation workflows using Python-based MyraScript. It processes source material into fresh destination wells and allows each component and step to be defined independently, including volumes, speeds, mix types, tip reuse, incubation, and reagent additions. This mode is ideal for advanced workflows, such as magnetic bead-based extraction, or when samples must be handled in isolation or require intermediate processing before qPCR setup. For detailed guidance on configuring and scripting an Independent Prep workflow, please refer to the **MyraScript Manual**, available via the **Help** icon on the Start Page toolbar.



- **None:** No sample preparation will be performed.

Assays

The most essential function for **qPCR Setup** is to link each run to an Assay or to multiple Assays. An Assay encompasses all components necessary to setup qPCR reactions. For more information on how to create Assays, refer to **qPCR Assay**.

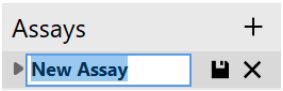
Select the Assay(s) required for the run by selecting the '+' button next to Assays.
By default, assays saved to the micPCR Assays folder will be displayed.

Alternatively, you can select an Assay from any directory using the folder icon labelled 'Browse….'
Use the file explorer to locate the assay you require from any location including network drives or external hard drives such as a USB Flash drive.

You can create an alternative source location for your assays by selecting the '+' button next to Personal Shortcuts and browsing to the directory you wish to assign as a new Personal Shortcut assay library.

If multiple users are sharing a PC or network drive, you can create a shared shortcut assay library by selecting the '+' button next to Shared Shortcuts and browsing to the desired directory location.

You can create a new assay directly within the run file by selecting the '+' button next to New Assay and entering a name for the assay in the field provided in the Navigator Bar.
The assay name can be changed at any time by selecting the assay in the Navigator Bar and clicking on its name to edit. For more information on how to set up an assay, refer to **qPCR Assay**.



You can edit and save any changes made to an Assay by using the Save button next to the Assay name.

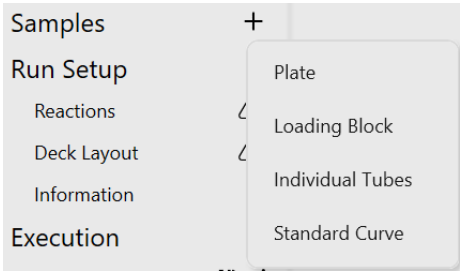
The Information, Assay Profile and Analysis Settings can be viewed and edited once the assay is selected.

Delete any Assays by using the × button next to the Assay name.

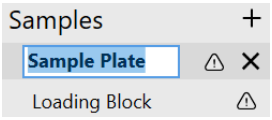
Samples

First, select if your samples are loaded in a Plate, Loading Block containing the same tube type or Individual Tubes.

The software can be configured to accommodate multiple types of sample sources:



- **Plate**: Suitable for samples contained in plates or strip tubes. Selecting this option will load the Plate Library, which includes a range of formats such as 96-well and 384-well plates.
- **Loading Block**: Designed for loading primary samples in tubes. For example, a 48-position microcentrifuge tube block is well suited for processing oropharyngeal swabs suspended in VTM within 2 mL tubes. Note that only one tube type can be used per loading block.
- **Individual Tubes**: Appropriate when a mixture of tube types or volumes is required, allowing for more flexible sample input.

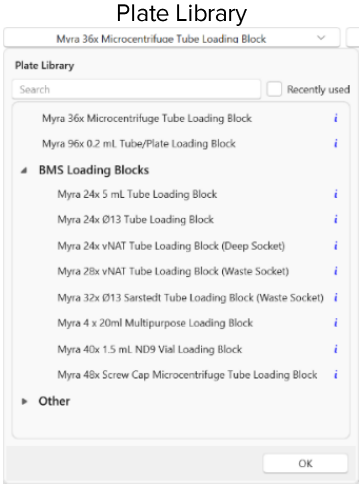


You can rename the selected plates by clicking on the heading in the Navigator Bar.

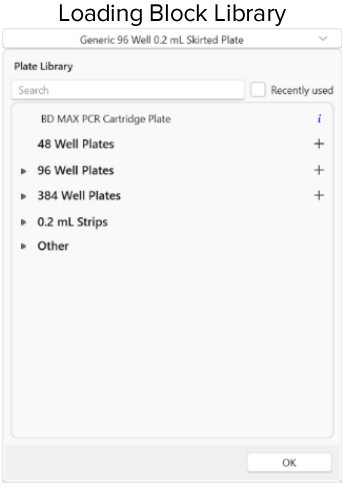
If using Plates or Loading Blocks, select the type of plate being used or the loading block that contains your sample tubes.

BMS Workbench is pre-loaded with a Plate Library. To define new plates, refer to Plate Editor.

Plate Library



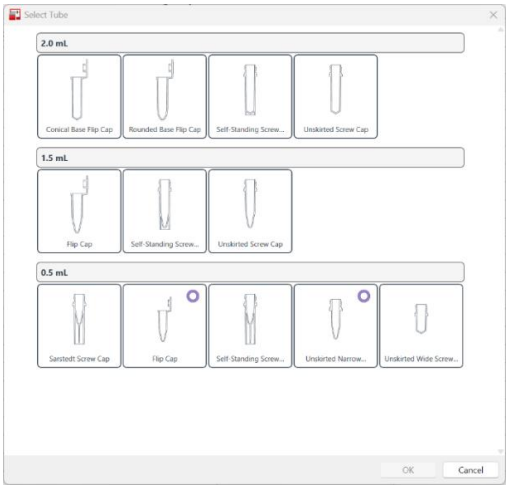
Loading Block Library



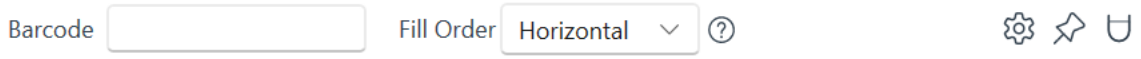
When using Loading Blocks, also select the tube type for your samples.

Only tubes compatible with the selected loading block will be displayed.

Hovering over each option supplies additional information about the height, base shape, capacity, and dead volume for each tube type.



Use the following options to customise the layout and behaviour of the sample plate:



- **Barcode:** A unique barcode associated with the plate or loading block can be entered for identification and tracking purposes. A barcode scanner/reader can be used to simplify this process. (Optional for Plates and Loading Blocks)
- **Fill Order:** For Plates and Loading Blocks, determines how wells are arranged in the sample plate table and can either across rows (**Horizontal**) or down columns (**Vertical**).
- **Liquid Sensing** (Accessed via the Gear icon): Determines how sample volumes are estimated during aspiration from Plates or Loading Blocks. Options include:
    - **Define Fixed Sample Volume:** Used to estimate liquid height based on the plate/tube profile. Best used when all sample volumes are consistent. Enter a uniform sample volume (in µL)
    - **Use Automatic Liquid Sensing:** Liquid level is sensed individually for each well or tube to determine actual sample volume. Recommended when sample volumes vary or when precise aspiration is critical.
- **Sealing Method** (Accessed via the Pin icon): Specifies whether wells or tubes are sealed or uncovered. Options include:
    - **No Foil**: Sample is aspirated directly from the well.
    - **Pierce Foil (Replace Tip):** Tip is ejected after piercing foil to avoid contamination.
    - **Pierce Foil (Keep Tip):** Same tip is used to pierce and aspirate the sample.
      When piercing foil, the tip makes multiple perforations across the well opening before sliding back to create a clean and consistent opening.
- **Swab Type** (Accessed via the Well icon): For Loading Blocks only, swabs can be in primary samples. Options include:
    - **None:** Aliquot is taken directly from the centre as normal.
    - **Rhinoswab:** Aliquot is taken from the centre to avoid the swab, which typically sits low and off-centre.
    - **Flocked:** Aliquot is taken from the side of the tube to avoid the swab, which generally protrudes centrally.
      When a swab is present, the pipette head oscillates and moves slowly to detect and avoid collisions. If a collision is detected, the tip is automatically repositioned to a clear area in the tube before aspirating. Additional time and tips are required for level sensing when swabs are present.

You can now fill in the Samples table.
The Samples editor is displayed in a table format and allows you to annotate your samples. Failure to properly annotate samples can affect the run setup and subsequent qPCR analysis.

The same toolbar section also offers options to import samples. Refer to Importing Data for more information on how to import samples.

A visual Well Filter tool is provided on the top right-hand corner of the sample editor.

Select the Colour you want for each sample. (optional)
Chose any colour from the colour pallet or generate your own colours using the colour chart.

To create a gradient, select the first colour and highlight all the way down to the last colour required, and then click the Auto fill icon. Use the Copy down icon to apply the same colour to multiple samples.

The colour of each sample can be used as a display on the Deck Layout.

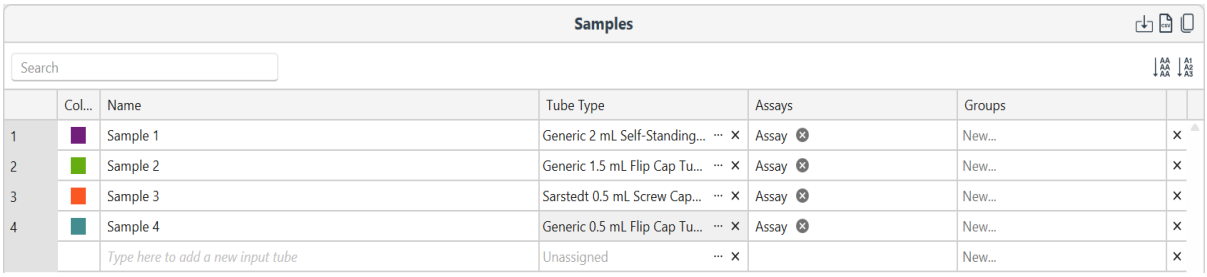
Enter the Name of each sample.
Each sample Name will be used as a template by the Myra. Samples with the same name will have a warning appear, prompting the user that samples have duplicate names as replicates are created on the Reactions page.

To annotate sequential characters (e.g. sample 1, sample 2, sample 3…) use the Auto fill icon.

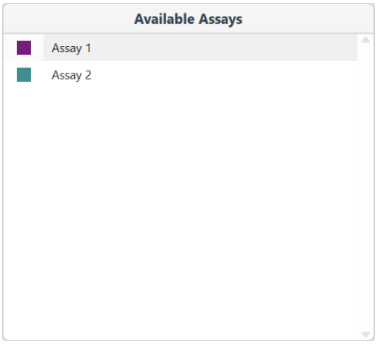
To delete data from table cells, you will need to deactivate the editor by selecting the escape key. Once deactivated, you can use the delete key to clear the contents of the selected cells.

You can use a barcode reader/scanner to simplify the sample editing process.

For Individual Tubes, select the Tube Type for each sample.
Individual Tubes samples can be added to any empty well on the deck in the Deck Layout screen, which allows for different tube types to be selected for each sample. The tube type can be selected in the Deck Layout if preferred. This option is ideal if there are a few additional samples required for a run that do not fit on the deck in a separate loading block. They can, for example, be added to the block containing assay reagents.



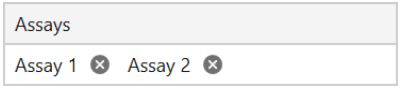
Link an Assay to a sample by selecting the required samples in the Name or Assays column and drag and drop the required assay(s) from the Available Assays window into the highlighted cells.
Ctrl + Click can be used to highlight non-adjacent samples. Alternatively, click on the selected cells in the Assays column and use the drop-down list to select the required assay(s).



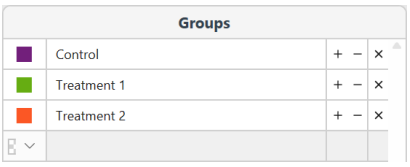
If using only one assay in the run, the software will automatically add the assay to any well that has a name entered.

The colour of the assay can be edited as well and used as display on the reaction plate in the *Deck Layout*.

To remove an assay from a sample, use the delete key of your keyboard or click on the x button next to the assay in the cell.



Allocate one or more groups to your samples, begin by entering the required group names in the Groups table, then assign groups to your samples. (optional)
Use sample groups to allow you to calculate statistics for a collection of samples that are not replicates (e.g., Treatment or Control). You will be required to create and allocate groups when using Relative Quantification analysis.



To allocate a group, click on the Groups column cell of the relevant sample and select the desired group from the drop-down list. If you wish to assign a group to multiple samples, you can highlight multiple cells in either the Groups or Name column. To select non-adjacent samples, hold Ctrl while clicking. Select the + button next to the group name under the Groups table on the bottom left corner to allocate the desired group/s.

Alternatively, the name of the group can be typed directly into the Groups column. If the group exists, a list of options beginning with the first set of characters will appear. If the group does not exist, it will be captured in the group list after you have completed entering the name and exited out of the cell.

You can remove groups assigned to Samples by using the Delete button on your keyboard or the × button next to the group name in the Groups column of the Samples Editor. Alternatively, you can use the – button next to the group name in the Groups table.

The colour for each group can be edited in the Groups table and can be used to display on the reaction plate on the Deck Layout.

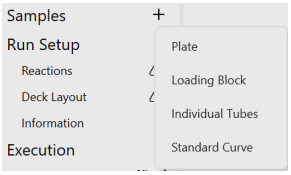
Various warnings will be displayed if annotations have not been completed correctly. Some examples include:

- Duplicate sample names.
    - To generate replicates using the **qPCR Setup** option, users should use the Replicates feature in the Reactions editor.
- No reactions have been configured.
- There are no assays included in the run.
- Some samples have no assay configured. No reactions will be generated for these samples.
- Assay is incompatible with the prep protocol; ensure that the assay has been configured correctly to accept the volume of the prepared sample defined by the selected prep protocol.
- Assay reaction volumes are not consistent.
- Fixed volume is less than the volume required for the run to execute; ensure that the fixed volume setting is greater than the dead volume for the selected plate or tube type.

Creating a Standard Curve

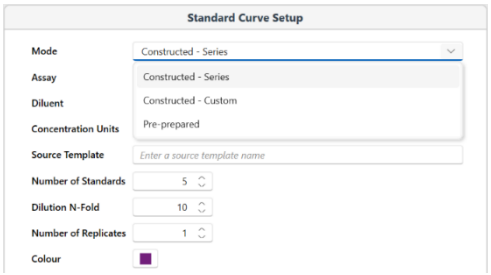
The **qPCR Setup** option simplifies the process of generating a standard curve. For a guide on how to setup a dilution series, with an example, refer to **Workbench Application Note 7 – Serial Dilutions under** the Quick Links section on the Start Page.

First, select Standard Curve from the + button next to Samples and name your standard curve.
This name will be used as a prefix to label each standard within the curve. Each constructed standard will follow the naming convention: Standard Curve Name – Standard #. Samples in the Reactions output will be automatically generated using the same naming convention.



First, select the mode in which the standard curve is constructed. There are three modes available:

- **Constructed – Series**: Provides a quick and easy way to prepare a simple dilution series when only one dilution factor is required.
- **Constructed – Custom**: Allows for the concentration of each standard in the dilution series to be individually defined.
- **Pre-prepared**: For when a dilution series has already been prepared in advance.



Next, select the Assay to be used for the standard curve. The Assay will auto-fill if only one Assay is linked to the run.

Click on the Targets button to select the target(s) to which this standard curve will apply to.
Where the standard curve applies to an assay with multiple targets, select the target/s that apply to the curve. If each target has a separate standard stock, create multiple standard curve items, selecting the required targets for each. If the standard stock contains multiple targets, select all that apply to that stock. For constructed standards, the concentration for each target in the stock can be different, but the dilution factor applies to each dilution is the same for all target/s.

Check that the correct Diluent is being used.
If one Assay is linked to the run, the Diluent will be auto-filled with the Diluent Reagent specified in the Reaction Setup table in the Assay.

Define the Concentration Units to be used.
A drop-down list will contain the concentration units available. You can also choose to define your own concentration unit by typing in the box.

Determine how many standards should be prepared using the Number of Standards option.

Define the Number of Replicates required for each standard and select the Colour used to visually identify the standard curve.

Next, follow the below steps depending on the mode being used:

Constructed – Series

Name the Source Template to be used to create the standard curve.
The name input here will be reflected in Deck Layout. Users can also define Source Templates within the chosen sample loading option for Plates, Loading Blocks, or Individual Tubes.

Decide the Dilution N-Fold.
This represents the factor by which the original concentration will be diluted when preparing subsequent solutions in the series.

Fill the Constructed Standards table by specifying the concentrations for both the stock solution and the first standard.
If the first standard concentration differs from the stock concentration, only the defined first standard concentration will be used when generating the reactions.

In the following example, 4 standards were prepared using a 10-fold dilution factor, each in triplicate.

Constructed Standards

Name	Target 1	Dilution N-Fold	Replicates
Stock Concentration	10000		
Standard Curve 1 - Standard 1	10000	1	3
Standard Curve 1 - Standard 2	1000	10	3
Standard Curve 1 - Standard 3	100	10	3
Standard Curve 1 - Standard 4	10	10	3

Constructed – Custom

Name the Source Template to be used to create the standard curve.
The name input here will be reflected in Deck Layout. Users can also define Source Templates within the chosen sample loading option for Plates, Loading Blocks, or Individual Tubes.

Fill the Constructed Standards table by defining the concentrations for each standard.
The toolbar also the fill-down and auto-fill functions to make constructing standards easier.

In the following example, 5 standards were prepared using user-defined concentrations, allowing for variable dilution factors, including both 2-fold and 10-fold steps.

Constructed Standards

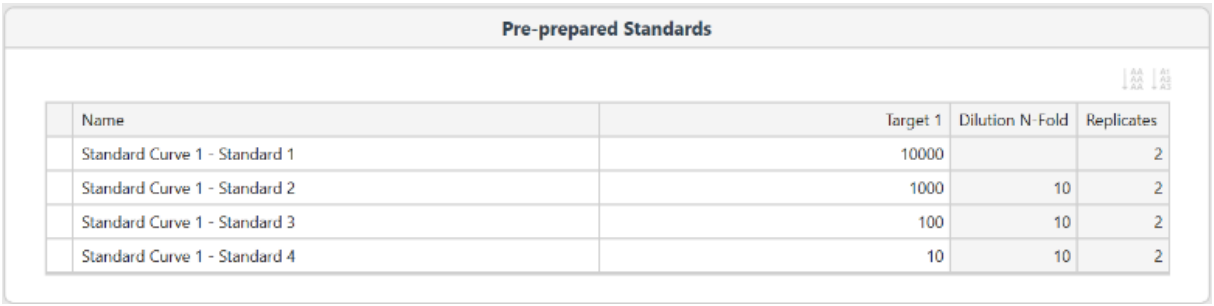
Name	Target 1	Dilution N-Fold	Replicates
Stock Concentration	1E+06		
Standard Curve - Standard 1	1E+06	1	2
Standard Curve - Standard 2	5E+05	2	2
Standard Curve - Standard 3	50000	10	2
Standard Curve - Standard 4	5000	10	2
Standard Curve - Standard 5	2500	2	2

Pre-prepared

Fill the Pre-prepared Standards table by defining the name and concentrations for each standard.
There is no restriction on the concentration of each target in each standard. Enter the concentrations to match the supplied or manually constructed standards.

The toolbar also the fill-down and auto-fill functions to make constructing standards easier.

In the following example, the standards were created by entering the known concentrations of the pre-made standards: 10,000, 1,000, 100, and 10.



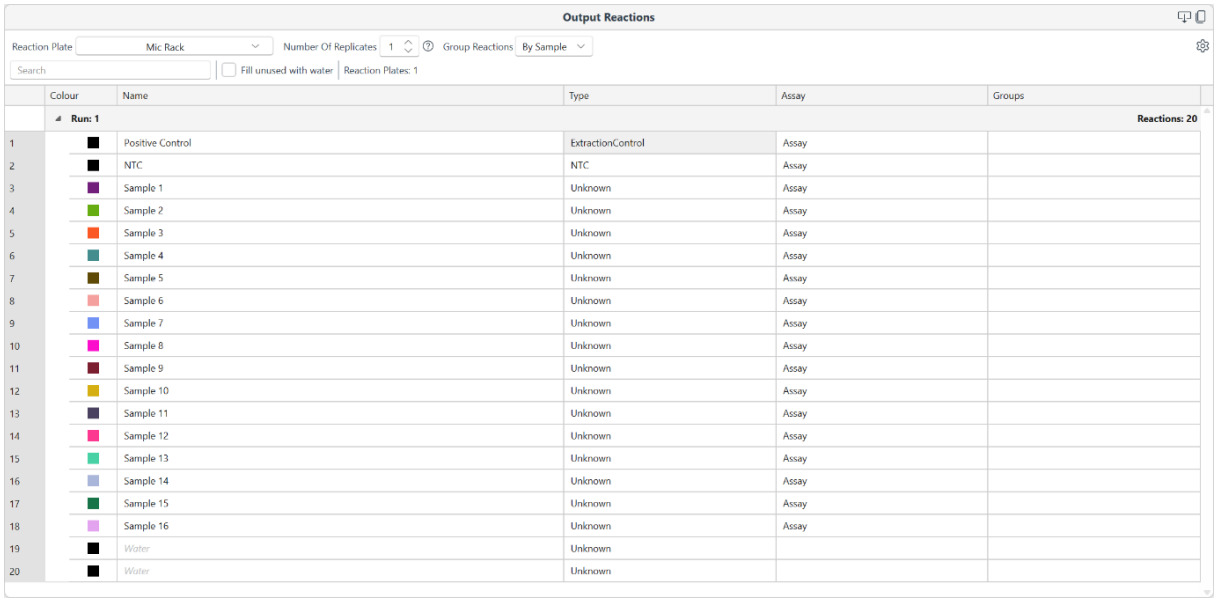
Pre-prepared Standards

Name	Target 1	Dilution N-Fold	Replicates
Standard Curve 1 - Standard 1	10000		2
Standard Curve 1 - Standard 2	1000	10	2
Standard Curve 1 - Standard 3	100	10	2
Standard Curve 1 - Standard 4	10	10	2

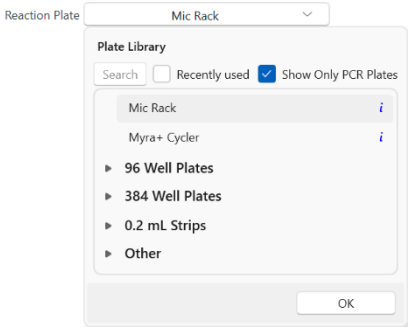
Run Setup

Reactions

Reactions are automatically generated by BMS Workbench based on the order samples were input. If the assay assigned to a sample contains any controls, these will always be placed in the order they are defined in the Controls table in Assay Information. This is an advantage offered by BMS Workbench, where reactions are auto-populated to minimise user error.



Select the Reaction Plate output based on your desired qPCR cycler.
BMS Workbench is pre-loaded with a Plate Library based on the most common cyclers on the market.



NOTE	If the Deck Template (accessed for the Gear icon) is not already set to Myra+, or you have not connected to the Myra+, the following warning will appear when you attempt to use the Myra+ Cycler option. Select Yes to continue with setup using Myra+.

Use the following options to customise the layout and behaviour of the reaction plate:

- **Number of Replicates:** The number of replicates to make of each sample.
- **Group Reactions:** When using multiple assays, reactions can be grouped the following ways:
    - **By Sample:** All reactions belonging to the same sample are grouped together on the same plate.
    - **By Assay:** All reactions using the same assay are grouped together on the same plate. Different assays are separated onto different plates.
    - **By Panel:** All assays within a panel are grouped together for each sample. Different panels are separated onto different plates.
    - **By qPCR Profile + Assay:** Reactions are grouped by assay but separated onto different plates if associated with different qPCR profiles.
    - **By qPCR Profile + Panel:** Reactions are grouped by panel but separated onto different plates if associated with different qPCR profiles.
- **Run Layout:** Available for all Group Reactions options except By Sample. Options include:
    - **Default**: Reactions are added sequentially to plates and will spill over to the next plate once full.
    - **One Reaction Group Per Run**: Each reaction group is placed on a separate plate.
    - **Avoid Splitting Reaction Group**: Ensures that all reactions within a group are kept together on the same plate.
- **Fill Order:** Determines the layout of the reaction plate and can be either across rows (**Horizontal**) or down columns (**Vertical**).
- **Exclude Outside Wells:** Allows selected outer wells to be excluded from reactions to minimise edge effects commonly observed with plate cyclers.
- **Controls Order** (Accessed via the Gear icon): The position of controls relative to the reaction group can be determined as either before (**Controls Before Reaction Group**) or after (**Controls After Reaction Group**).
- **Fill Unused with Water:** To ensure temperature uniformity on the Mic or Myra+ Cycler, all tubes within the rotor should contain the equal liquid volumes. This can be achieved by enabling **Fill unused with water**, which loads any empty tube with equal volume of water. This option is selected by default for Myra+ Cycler reactions and cannot be changed.

The deck is now ready to be configured.

Deck Layout

Refer to the Deck Layout section above for more information on how to setup your deck.

Information (Additional Settings)

Refer to the Information (Additional Settings) section above for more information on how to access Advanced Settings, LIMS Settings, and Checklist Settings.

The run can now be commenced. If you wish to define additional settings, you can do so in Information.

Reaction Driven qPCR Setup

qPCR reactions can be setup in two different ways, depending on your unique workflow requirements. **Reaction Driven qPCR Setup** allows users to annotate information for pre-prepared samples and configure the output qPCR reaction order. In this run type, users define the location and tube type(s) for all reagents and samples, including controls.

NOTE	To utilise the Myra+ for qPCR reaction setup for the Myra+ Cycler, please refer to the qPCR Setup section.

Assays

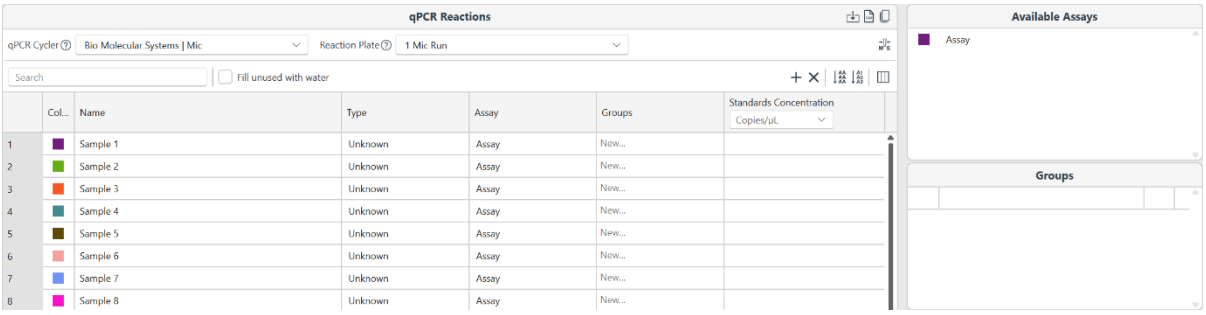
The most essential function for **Reaction Driven qPCR Setup** is to link each run to an Assay or to multiple Assays. An Assay encompasses all components necessary to setup qPCR reactions. For more information on how to create Assays, refer to **qPCR Assay**.

Select the Assay(s) required for the run by selecting the '+' button next to Assays.
For more information on how to add assays, refer to the **Assays** section in **qPCR Setup**.

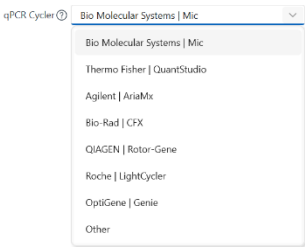
Run Setup

Reactions Editor

All reactions, including controls have to be manually configured to be included in the setup of output reactions.

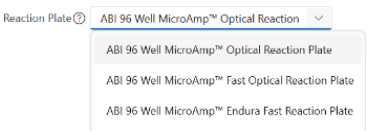


Select the Reaction Plate output based on your desired qPCR cycler.
BMS Workbench is pre-loaded with a Plate Library based on the most common cyclers on the market.

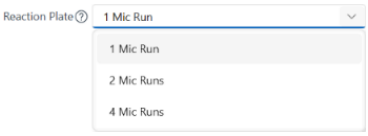


First, select the qPCR Cycler using the drop-down menu.
We have provided a list of the most common cyclers on the market. Choose the Other option if your cycler is not listed.

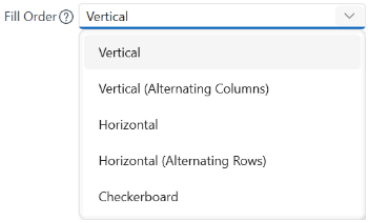
Define your Reaction Plate using the drop-down menu.
Displayed reaction plates will be based on the options available for the chosen qPCR cycler. We have selected several recommended plates for each of the platforms available. If your plate is not within the list, simply select the Other option for the qPCR cycler and then choose one of the generic options that best matches your plate type.



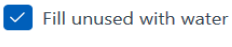
The Bio Molecular System | Mic option will allow you to select multiple tube racks for simultaneous setup of multiple Mic qPCR Cycler runs.



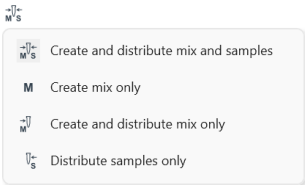
For non-Mic plates, select the Fill Order to determine how wells are arranged in the sample plate table.
The fill order can be either Vertical, Horizontal, Vertical (Alternative Columns), Horizontal (Alternating Rows) or in a Checkerboard pattern.



Select whether to fill any unused wells with water or not.
To ensure temperature uniformity on the Mic qPCR Cycler, all tubes within the rotor should contain the equal liquid volumes. This can be achieved by enabling **Fill unused with water**, which loads any empty tube with equal volume of water.

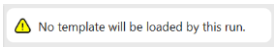


Select how you want to create and distribute components.
The default setting is to Create and distribute mix and samples. There are additional options to Create mix only, Create and distribute mix only and Distribute samples only, depending on the needs of your lab.



Some labs prefer to separate the creation of mix and samples to avoid contamination.

A warning will be displayed indicating that either no template or mix will be used depending on the option selected.



You can now fill in the Samples table.
The Reactions editor is displayed in a table format and allows you to annotate your samples. Failure to properly annotate samples can affect the run setup and subsequent qPCR analysis.

The same toolbar section also offers options to import samples. Refer to Importing Data for more information on how to import samples.

Additional commonly used qPCR cyclers now also have the option of exporting into their software. For more information on how to export reaction information.

A visual Well Filter tool is provided on the top right-hand corner of the sample editor.

Select the Colour you want for each sample. (optional)
Chose any colour from the colour pallet or generate your own colours using the colour chart.

To create a gradient, select the first colour and highlight all the way down to the last colour required, and then click the Auto fill icon. Use the Copy down icon to apply the same colour to multiple samples.

The colour of each sample can be used as a display on the Deck Layout.

Enter the Name of each sample.
Each sample Name will be used as a template by the Myra system. Samples with the same name will have a warning appear, prompting the user that samples have duplicate names as replicates are created on the Reactions page.

To annotate sequential characters (e.g. sample 1, sample 2, sample 3…) use the Auto fill icon.

To delete data from table cells, you will need to deactivate the editor by selecting the escape key. Once deactivated, you can use the delete key to clear the contents of the selected cells.

Alternatively, the + button can be used to insert a new reaction at selected row and the x button can be used to remove selected reactions.

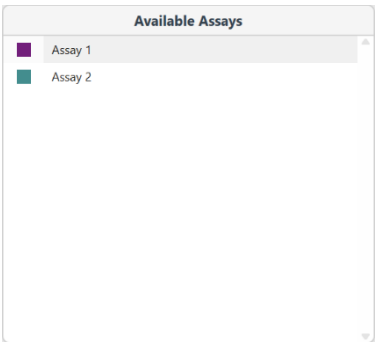
You can use a barcode reader/scanner to simplify the sample editing process.

Select the reaction Type.
There are six options to choose from. The type chosen will determine the way in which the reaction is utilised during Mic qPCR analysis.

To change multiple cells at once, highlight the cells, use the F2 key on your keyboard, and then select from the following options:

- **Unknown** (default): Any sample that is under investigation.
- **Standard**: A reaction of known quantity, used to generate a standard curve from which an unknown reaction template quantity can be calculated, or used to determine amplification efficiency. The standard can be defined as a template or part of a dilution series.
- **Positive Control**: The reaction is known to contain the target of interest. A positive control is used to confirm that the assay is working and helps prevent false negatives.
- **Negative Control**: The reaction is known not to contain the target of interest. A negative control is used to monitor for contamination of the assay and is helpful in preventing false positives. Unlike the NTC, the negative control can contain an internal amplification control template to ensure the PCR is working.
- **NTC**: A reaction that contains no template. NTCs are used to monitor for amplicon contamination in the reaction and for the amplification of non-specific amplicons (primer dimers) when using intercalating dye chemistries. If no reaction name is entered the Myra will automatically name it water and use the water reagent as the template.
- **NRT**: The reaction has not undergone reverse transcription. The NRT control is used to monitor for genomic DNA amplification during RT-qPCR. Only cDNA, derived from mRNA, should be amplified during RT-qPCR when used to determine gene expression values. If an assay is positive for genomic DNA, consider changing the primer design to target intron exon boundaries. If none exist then improve the extraction of RNA and the removal of genomic DNA from the sample, such as using a DNase.

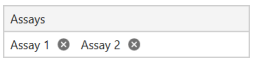
Link an Assay to a sample by selecting the required samples in the Name or Assays column and drag and drop the required assay(s) from the Available Assays window into the highlighted cells.
Ctrl + Click can be used to highlight non-adjacent samples. Alternatively, click on the selected cells in the Assays column and use the drop-down list to select the required assay(s).



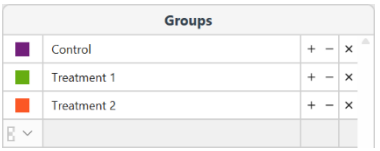
If using only one assay in the run, the software will automatically add the assay to any well that has a name entered.

The colour of the assay can be edited as well and used as display on the reaction plate in the Deck Layout.

To remove an assay from a sample, use the delete key of your keyboard or click on the x button next to the assay in the cell.



Allocate one or more groups to your samples, begin by entering the required group names in the Groups table, then assign groups to your samples. (optional)



Use sample groups to allow you to calculate statistics for a collection of samples that are not replicates (e.g., Treatment or Control). You will be required to create and allocate groups when using Relative Quantification analysis.

To allocate a group, click on the Groups column cell of the relevant sample and select the desired group from the drop-down list. If you wish to assign a group to multiple samples, you can highlight multiple cells in either the Groups or Name column. To select non-adjacent samples, hold Ctrl while clicking. Select the + button next to the group name under the Groups table on the bottom left corner to allocate the desired group/s.

Alternatively, the name of the group can be typed directly into the Groups column. If the group exists, a list of options beginning with the first set of characters will appear. If the group does not exist, it will be captured in the group list after you have completed entering the name and exited out of the cell.

You can remove groups assigned to Samples by using the Delete button on your keyboard or the × button next to the group name in the Groups column of the Samples Editor. Alternatively, you can use the – button next to the group name in the Groups table.

The colour for each group can be edited in the Groups table and can be used to display on the reaction plate on the Deck Layout.

Columns can be removed from the Reactions editor and additional ones can be added using the Select visible sample data columns icon. (optional)

The following columns can be added or removed from the table:

- **Standards Concentration** (default): When using a dilution series, opt to input in the amount of template present. The dropdown menu presents options on the concentration used, from Copies/µL to pg/µL.
- **Sample Description**: Utilise this option for a sample description.
- **Input DNA concentration**: Note the starting amount of DNA per reaction.
- **Input RNA concentration**: Note the starting amount of RNA per reaction (RT-qPCR)
- **RIN**: Provide an RNA integrity number for each input RNA.
- **Groups** (default): Easily group your samples together.

Various warnings will be displayed if annotations have not been completed correctly.

These include:

- No reactions have been configured.
- There are no assays included in the run.
- Some samples have no assay configured. No reactions will be generated for these samples.
- Some samples have no assay configured. No reactions will be generated for these samples.
- Assay reaction volumes are not consistent.

Creating a Dilution Series

In **Reaction Driven qPCR Setup,** a standard curve can be created with different dilutions. For a guide on how to setup a dilution series, with an example, refer to **Workbench Application Note 7 – Serial Dilutions** under the Quick Links section on the Start Page.

A dilution series will be created if the same sample Name is entered with different Standards Concentrations. The first standard will be considered the stock solution, with subsequent standards being prepared using an intermediate mix dilution series. If the concentration of the stock solution is higher than the desired first standard concentration, it is recommended to use **qPCR Setup** to create your standard curve.

There is also an option to use pre-diluted standards by inputting different names for each dilution and specifying the concentration of each.

To prevent confusion, Workbench recognises different concentrations for analysis of a standard curve and in a Mic qPCR Cycler run, populates each Sample Name with concentration next to it (e.g., Standard 1000 Copies/µL).

Name all reactions the same for a dilution series to be created.
Name reactions in alphanumeric order to designate a pre-made standard curve.

Define Type as Standard.
If reactions are named 'Standard,' the reaction Type will automatically be assigned as Standard.

Select the units you are using under the Standards Concentration table heading.
You can also enter your own units in the provided text box.

Enter Standards Concentrations for each standard.
When using standards, it is a requirement to provide a value for each. The value can be quantifiable or arbitrary. Scientific notation is also acceptable (1E03 = 1 x 10^3= 1000).

Enter the values one at a time or use the Auto fill option to quickly add a serial dilution with replicates.

In the following example, 6 standards were prepared using a 10-fold dilution factor, each in duplicate.

	Col...	Name	Type	Assay	Groups	Standards Concentration Copies/µL
1		Standard	Standard	Assay	New...	1000000
2		Standard	Standard	Assay	New...	1000000
3		Standard	Standard	Assay	New...	100000
4		Standard	Standard	Assay	New...	100000
5		Standard	Standard	Assay	New...	10000
6		Standard	Standard	Assay	New...	10000
7		Standard	Standard	Assay	New...	1000
8		Standard	Standard	Assay	New...	1000
9		Standard	Standard	Assay	New...	100
10		Standard	Standard	Assay	New...	100
11		Standard	Standard	Assay	New...	10
12		Standard	Standard	Assay	New...	10

Deck Layout

Refer to the Deck Layout section above for more information on how to setup your deck.

Information (Additional Settings)

Refer to the Information (Additional Settings) section above for more information on how to access Advanced Settings, LIMS Settings, and Checklist Settings.

The run can now be commenced.

qPCR Run

qPCR Run is used for setting up and running qPCR reactions on the Mic qPCR cycler or Myra+ Cycler. This feature also allows you to subsequently analyse the fluorescence data produced, which include Cycling analysis, Melt analysis, Absolute Quantification analysis, Allelic Discrimination analysis, Identifier analysis and Relative Quantification analysis. There is also an option to purchase a High-Resolution Melt (HRM) analysis, exclusive to the Mic qPCR Cycler.

With an intuitive software layout and run setup, analysis become simple, with many quality control features to provide confidence in the result. Most aspects of the software were designed to meet the MIQE4 guidelines (Bustin et al., 2009) and proper thought has been used to apply the most up to date methods for qPCR analysis.

NOTE	Relative Quantification and Allelic Discrimination analysis will not work without pre-defined assays. To use High Resolution Melt (HRM) analysis, an additional license will need to be purchased on the Mic, and cannot be purchased for the Myra+ Cycler

Adding Assays

The most essential function for a **qPCR Run** is to link each run to an Assay or to multiple Assays. An Assay encompasses all components necessary to perform a run and analysis on the Mic. This includes target information, cycling conditions and pre-set analysis settings. A run can be started without any defined assays; however, it is not recommended. For more information on how to create Assays, refer to **qPCR Assay**.

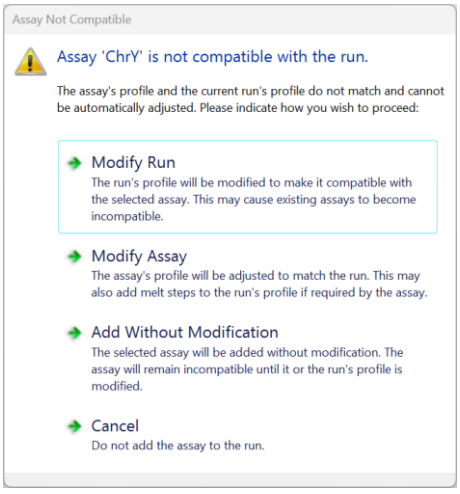
Select the Assay(s) required for the run by selecting the '+' button next to Assays.
For more information on how to add assays, refer to the **Assays** section in **qPCR Setup**.

Assay Profile Compatibility

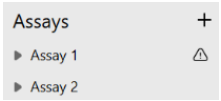
The Run Profile is based on the first Assay that is selected. Any following Assay selected that does not match the Run Profile temperatures and hold times, number of cycling, temperature control, or reaction volume will be determined as non-compatible. If this case, the software will bring up the following warning message:

Select from the following options:

- **Modify Run**: The run's profile will be modified to make it compatible with the selected assay. This may cause existing assays to become incompatible.
- **Modify Assay**: The assay's profile will be adjusted to match the run. This may also add melt steps to the run's profile if required by the assay.
- **Add Without Modification**: The selected assay will be added without modification. The assay will remain incompatible until it or the run's profile is modified.
- **Cancel**: Do not add the assay to the run.



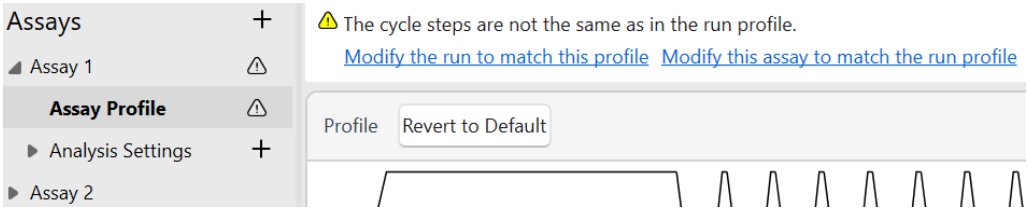
Non-compatible assays are reported with a caution symbol.



Specific reasons for the non-compatibility can be seen if you hover over the warning symbol in the Navigator Bar. You can also enter the Assay Profile of the incompatible assay to view the warnings for incompatibility to the run profile.

In the warning area, you have the choice to:

- **Modify the run to match this profile**: The run's profile will be modified to make it compatible with the selected assay. This may cause existing assays to become incompatible.
- **Modify this assay to match the run profile**: The assay's profile will be adjusted to match the run. This may also add melt steps to the run's profile if required by the assay.



NOTE	It is strongly recommended to validate any modified profiles using inexpensive reagents or water and initially test on a small subset of samples. This approach ensures the changes are suitable before proceeding with a full-scale experiment. Failure to do so may result in suboptimal qPCR performance, compromising data quality and potentially leading to significant loss of valuable samples and reagents.

Run Setup

Run Profile

Modify the Run Profile if required. (optional)
For more information on how to set up the various parameters in the run profile, refer to the Assay Setup in qPCR Assay.

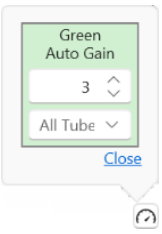
The following options can be further configured in the run profile settings:

Selecting channels to Acquire on.
Multiple channels can be selected.

Channels will be preselected if the reporter dyes have been selected for individual targets in the Information section of the Assay Setup.

If running a conventional PCR then no acquisition channel is required. A warning will be displayed if no channels are selected. Acknowledge the warning if you wish to proceed with no acquisition.

Adjusting gain settings for each channel by selecting the Adjust gain settings icon.
To perform gain adjustment, you have the option to use All Tubes (sample with maximum signal intensity is used) or select a specific sample from the drop-down list. This option is only available in the Run Profile editor.



Adding/removing Melt.
Multiple Melts can be selected.

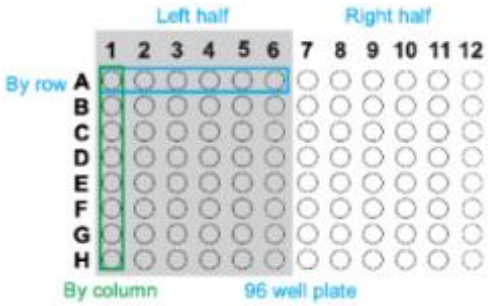
Channels will be preselected if the Reporter Dyes have been selected for individual targets in the Information section of the Assay setup.

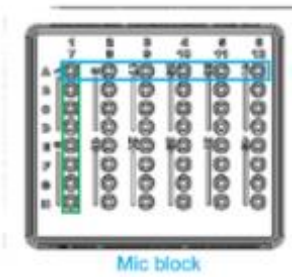
Samples Editor

The Samples editor is displayed in a table format and allows you to annotate your samples. Samples can be annotated before, during or after a run. Failure to properly annotate samples can affect analysis.

The same toolbar section also offers options to import samples. Refer to Importing Data for more information on how to import samples.

Select your desired well orientation. (optional)
The well layout can accommodate loading from a 96 well plate using a multichannel pipette, where the orientation switches from columns to rows.





To ensure the software displays this change, select from the options found on the top right corner of the table (from left to right):

- Display well number in numeric style 1 – 48.
- Display well number in alpha-numeric style from left hand of 96-well plate A1-H6.
- Display well number in alpha numeric style from right hand of 96-well plate A7-H12.
- Display wells sorted by columns.
- Display wells sorted by rows.

Select the Colour you want for each sample. (optional)
Chose any colour from the colour pallet or generate your own colours using the colour chart.

To create a gradient, select the first colour and highlight all the way down to the last colour required, and then click the Auto fill icon. Use the Copy down icon to apply the same colour to multiple samples.

Enter the Name of each sample.
Samples with the same characters and assay will be treated as replicates and will be reported with a mean ($\bar{x}$) and standard deviation ($x\sigma_{n-1}$) in most analyses.

Samples with the same characters but different assays will be linked based on the type of analysis chosen. For example, in Relative Quantification a sample with the same characters will be used for both the gene of interest and reference gene. Cycling Data from the two independent assays will be linked together to calculate the gene expression value.

You can highlight multiple cells within a column and enter the same characters to annotate replicates. Alternatively, enter the name in one cell, highlight that cell and other cells that will be part of the replicates (use Ctrl + Click to highlight non-adjacent cells), and then select the Fill down icon to give all the selected cells the same name. This is also applicable to other columns in the sample editor.

To annotate sequential characters (e.g. sample 1, sample 2, sample 3...) use the Auto fill icon.

To delete data from table cells, you will need to deactivate the editor by selecting the escape key. Once deactivated, you can use the delete key to clear the contents of the selected cells.

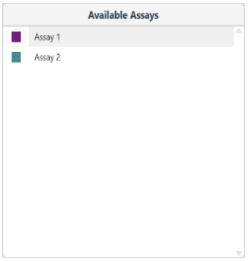
You can use a barcode reader/scanner to simplify the sample editing process.

Select the sample Type.
There are six options to choose from. The type chosen will determine the way in which the reaction is utilised during Mic qPCR analysis.

To change multiple cells at once, highlight the cells, use the F2 key on your keyboard, and then select from the following options:

- **Unknown** (default): Any sample that is under investigation.
- **Standard**: A sample of known quantity, used to generate a standard curve from which an unknown sample quantity can be calculated, or used to determine amplification efficiency.
- **Positive Control**: The sample is known to contain the target of interest. A positive control is used to confirm that the assay is working and helps prevent false negatives.
- **Negative Control**: The sample is known not to contain the target of interest. A negative control is used to monitor for contamination of the assay and is helpful in preventing false positives. Unlike the NTC, the negative control can contain an internal amplification control template to ensure the PCR is working.
- **NTC:** A sample that contains no template. NTCs are used to monitor for amplicon contamination in the reaction and for the amplification of non-specific amplicons (primer dimers) when using intercalating dye chemistries.
- **NRT**: The sample has not undergone reverse transcription. The NRT control is used to monitor for genomic DNA amplification during RT-qPCR. Only cDNA, derived from mRNA, should be amplified during RT-qPCR when used to determine gene expression values. If an assay is positive for genomic DNA, consider changing the primer design to target intron exon boundaries. If none exist then improve the extraction of RNA and the removal of genomic DNA from the sample, such as using a DNase.

Link an Assay to a sample by selecting the required samples in the Name or Assays column and drag and drop the required assay from the Available Assays window into the highlighted cells.



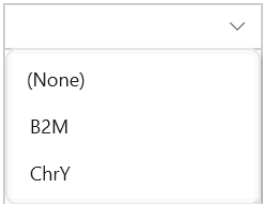
An *Assay* must be linked to a sample to allow the software to recognise and properly analyse the sample. Failure to allocate an *Assay* to a sample will result in the sample not being analysed. If the run only contains one *Assay*, that *Assay* will automatically be linked to each sample that has been input in the table.

Ctrl + Click can be used to highlight non-adjacent samples. Alternatively, click on the selected cells in the *Assays* column and use the drop-down list to select the required assay.

If using only one assay in the run, the software will automatically add the assay to any well that has a name entered.

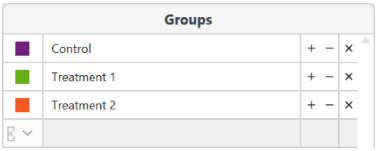
The colour of the assay can be edited as well.

To remove an assay from a sample, select (None) from the drop-down menu, or use the delete key of your keyboard to delete an assay from multiple samples.



Allocate one or more groups to your samples, begin by entering the required group names in the Groups table, then assign groups to your samples. (optional)

Use sample groups to allow you to calculate statistics for a collection of samples that are not replicates (e.g., Treatment or Control). You will be required to create and allocate groups when using Relative Quantification analysis.



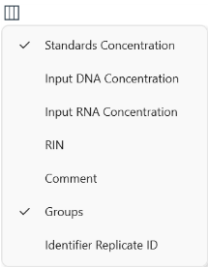
To allocate a group, click on the Groups column cell of the relevant sample and select the desired group from the drop-down list. If you wish to assign a group to multiple samples, you can highlight multiple cells in either the Groups or Name column. To select non-adjacent samples, hold Ctrl while clicking. Select the + button next to the group name under the Groups table on the bottom left corner to allocate the desired group/s.

Alternatively, the name of the group can be typed directly into the Groups column. If the group exists, a list of options beginning with the first set of characters will appear. If the group does not exist, it will be captured in the group list after you have completed entering the name and exited out of the cell.

You can remove groups assigned to Samples by using the Delete button on your keyboard or the × button next to the group name in the Groups column of the Samples Editor. Alternatively, you can use the – button next to the group name in the Groups table.

The colour for each group can be edited in the Groups table and can be used to display on the reaction plate on the Deck Layout.

Additional columns can be added to the Samples editor using the Select visible sample data columns icon. (optional)
The following columns can be added or removed from the table:



- **Standards Concentration** (default): concentrations are used for Standard curve analysis.
- **Input DNA concentration**: note the starting amount of DNA per reaction.
- **Input RNA concentration**: note the starting amount of RNA per reaction (RT-qPCR)
- **RIN**: provide an RNA integrity number for each input RNA.
- **Comment**: provide additional information about the samples.
- **Groups** (default): allocate groups for analyses such as Relative Quantification.
- **Identifier Replicate ID**: assign replicates a number for identifier analysis.

All the fields can be locked down to prevent accidental changes occurring. Once locked, none of the fields are annotatable until unlocked again.

Various warnings will be displayed if annotations have not been completed correctly. Some examples include:

- If standards have been selected as Type but no values have been entered into the Standards Concentration field.
- Assays have not been linked to an edited Sample row.

Creating a Dilution Series

Enter your Standard Concentration for each of your Standards.
Select the units you are using under the Standards Concentration table heading.

When using standards, it is a requirement to provide a value for each. The value can be quantifiable or arbitrary. Scientific notation is also acceptable (1E03 = 1 x 10^3= 1000).

Enter the values one at a time or use the Auto fill option to quickly add a serial dilution with replicates by doing the following:

Select the Toggle between single and multiplex standards icon to use the option of entering standard concentrations for each channel individually. 
You must ensure the Assay has been setup as a multiplex.

Switching between single and multiplex is possible, however, only one type can be viewed and applied during analysis.

Green Channel Standard... Copies/µL	Yellow Channel Standar... Copies/µL	Orange Channel Standa... Copies/µL	Red Channel Standards... Copies/µL
100000	5000000	250000	300000
10000	500000	25000	30000
1000	50000	2500	3000
100	5000	250	300
10	500	25	30
1	50	2.5	3
0.1	5	0.25	0.30000000000000004

In the following example, 6 standards were prepared using a 10-fold dilution factor, each in duplicate.

	Colour	Name	Type	Groups	Assay	Standards Concentration Copies/µL
1		Standard 1	Standard		Assay	1000000
2		Standard 1	Standard		Assay	1000000
3		Standard 2	Standard		Assay	100000
4		Standard 2	Standard		Assay	100000
5		Standard 3	Standard		Assay	10000
6		Standard 3	Standard		Assay	10000
7		Standard 4	Standard		Assay	1000
8		Standard 4	Standard		Assay	1000
9		Standard 5	Standard		Assay	100
10		Standard 5	Standard		Assay	100
11		Standard 6	Standard		Assay	10
12		Standard 6	Standard		Assay	10

Information (Additional Settings)

Refer to the Information (Additional Settings) section above for more information on how to access Advanced Settings, LIMS Settings, and Checklist Settings.

The run can now be commenced.

Analysis

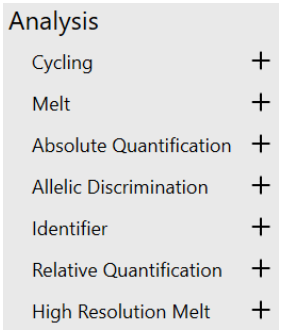
There are several analysis types available in the software, including Cycling, Melt, Standard Curve, Absolute Quantification, and Relative Quantification. The software also includes three different methods for achieving genotyping analysis, which are dependent on the type of chemistry used: Melt, HRM or Allelic Discrimination. Analysis specific parameters are provided for editing, and report tables are displayed along with various graphs depending on the analysis type.

We have taken care to provide the most robust up-to-date methods for qPCR analysis to ensure confidence in the result. Details regarding some of the analyses are described in the literature and we have provided references to certain publications that best describe these methods.

If the Assay(s) added to the run have been setup to automatically generate analysis, those analysis types along with any pre-set parameters will be automatically applied upon run completion.

To start a new analysis after a run has been completed, select the + button next to the analysis type under the Analysis heading in the Navigator Bar.

Select from the following options:

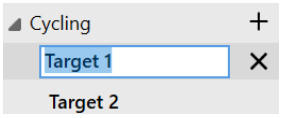


1. Cycling
2. Melt (includes Genotyping)
3. Absolute Quantification (includes Standard Curve)
4. Allelic Discrimination
5. Identifier
6. Relative Quantification
7. High Resolution Melt (optional, requires license and upgrade)

Then select the target to analyse from the list of options.

The targets listed will be based on the assays chosen before starting the run. For multiplex assays, each target will be shown as part of the Assay. For Allelic Discrimination assays, the added probe names will also be shown along with the target name.

The target name selected will be displayed in the Navigator Bar below the analysis type. You may edit the name by double-clicking on it.

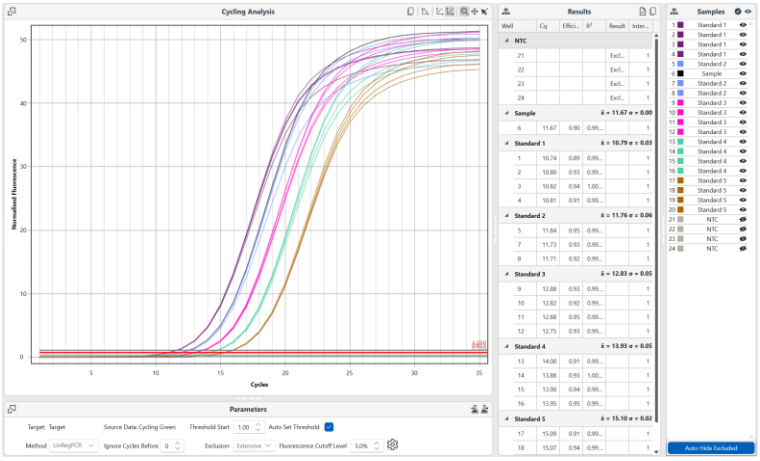


Multiple analyses are possible for each run, with the analysis being viewed highlighted blue on the Navigator Bar.

Delete any analysis by selecting the × button next to the target name.

Cycling Analysis

Cycling analysis allows you to determine the quantification cycle (Cq) and reaction efficiency of each sample in your data set. Cycling analysis is always provided with any other analysis type that uses cycling data (Absolute Quantification, Identifier and Relative Quantification), allowing you to adjust parameters associated with generating Cq and efficiency values. It will appear beneath the activated analysis type in the Navigator Bar. Use child page of Cycling analysis to make modifications to Cycling analysis parameters so that it will be applied to the paired analysis type.

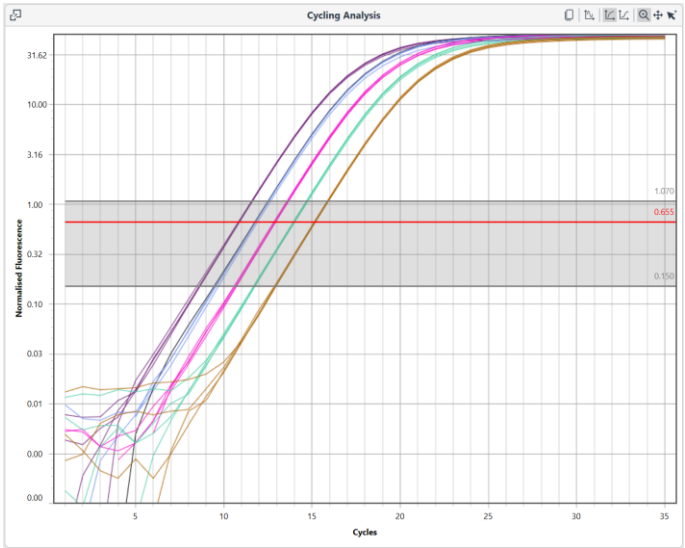


Cycling Graph Display

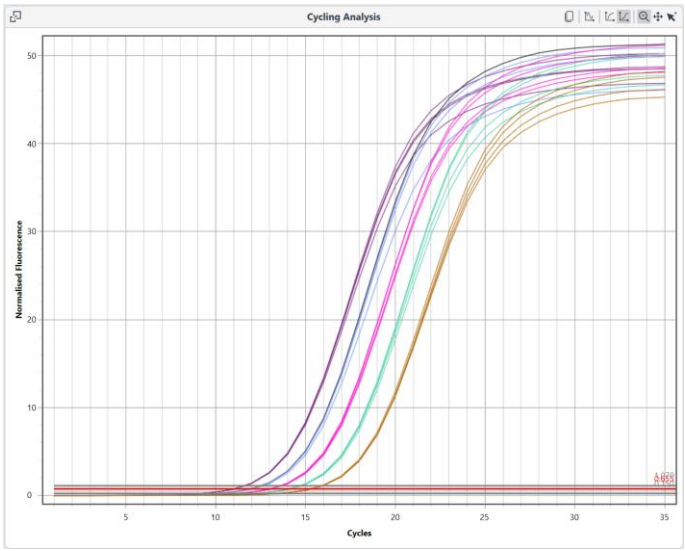
The software will, by default, plot baseline-corrected curves as **Normalised Fluorescence** (y-axis) against **Cycles** (x-axis) for the target that was chosen.

To change the display or your normalised fluorescence data, toggle between Derivative on left, Logarithmic in middle or Linear on right found on the top right corner of the graph toolbar.

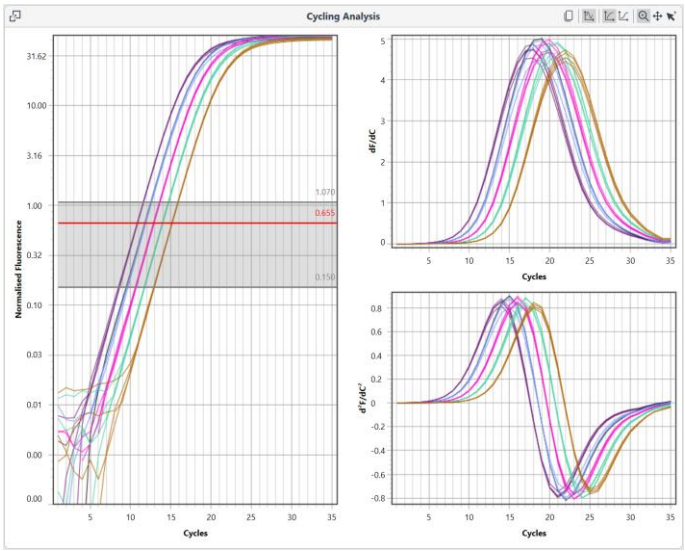
- **Log y-axis** (default): Displaying the data in the logarithmic view allows you to better visualise the exponential region of the amplification curve and is therefore the default option. You can revert to the logarithmic scale by selecting the Log y-axis icon.



- **Linear y-axis**: displays the baseline corrected cycling data with the y-axis in linear scale by selecting the Linear y-axis icon.



- **Derivatives**: The first (displayed as $\frac{dF}{dC}$ on y-axis and Cycles on x-axis) and second derivative (displayed as $\frac{d2F}{dC2}$ on y-axis and Cycles on x-axis) curves for the selected data can be displayed in parallel with baseline corrected amplification curves. The derivative curves can be used as a reference to set an appropriate threshold or to gauge amplification quality. Either log or linear y-axis can be displayed with this option.



Cycling Analysis Parameters

A default set of parameters will be applied to the assay data, providing automatic calculation of Cq and efficiency values, which are reported in the results table. You can change the following parameters:

- **Method**: The type of baseline correction method that has been applied to the raw fluorescence data to determine the quantification cycle (Cq) and reaction efficiency of each sample. There are four options available, in which the default method is based on the chemistry type selected in the assay.
    - LinRegPCR (default): uses a baseline estimation algorithm that iteratively adjusts the baseline to ensure a constant slope is achieved between the upper half of the log linear portion of the amplification curve and the bottom half of the curve for all samples in the assay data set. In effect, this reconstructs the exponential phase below the baseline noise. It assumes that amplification efficiency is constant from the very first cycle onward. This method minimises the propagation of errors in quantification due to errors in efficiency calculation caused by either over or underestimation of the baseline values (Ruijter et al., 2009).

NOTE	Due to the accumulative nature of signal for hydrolysis probes, LinRegPCR might not be a viable option. The preferred method would be *Dynamic*.

    - Dynamic: determines the average baseline value measured prior to the detection of specific amplification (take-off), subtracts the average value from the measured values, then considers any slope in the baseline curve, to baseline correct the sample. The take-off is calculated by using the second derivative maximum as a starting point. The same algorithm applied in the LinRegPCR method is used to find a W-o-L from which a cycle threshold can be set.
    - Fixed Length: the baseline average of the first five cycles, after the ignored cycles (see ignore first cycles below), is subtracted from the measured baseline values to baseline correct the sample. This is the most basic method of baseline correction. For some data sets this method may not be compatible with the setting of a W-o-L.
    - None: no baseline correction. This method allows you to view the raw data in Cycling Analysis. A W-o-L is not possible without any baseline correction.
- **Ignore Cycles Before**: This setting can be used if there is a significant deviation in the baseline at the start of the run. These changes can occur due to many factors including too much template or insufficient denaturation of double stranded DNA. Applying this may improve analysis using Dynamic baseline correction but will have minimal if any effect when using LinRegPCR.
- **Exclusion***:* If the sample is excluded, its Cq value is not determined and it is not included in the W-o-L calculation.
    - **None**: no samples will be excluded.
    - **Simple**: samples will be excluded if they display no amplification or normalisation fails.
    - **Extensive**: samples will be excluded if they display no amplification, if normalisation fails or they fail the fluorescence cut-off. A sample may be excluded for one or more of the following reasons:
        - **No amplification**: The filtered fluorescence trace for the sample does not show sufficient amplification. The change in fluorescence for the sample must increase by 7 times. To be included the sample fluorescence must satisfy the following condition:

$$f_{first} - f_{min} \geq 7 \times (f_{max} - f_{min})$$

Where:
f_{min} is the smallest filtered fluorescence value;
f_{first} is the first filtered fluorescence value different to f_{min};
f_{max} is the maximum filtered fluorescence value.

- **Fluorescence Cutoff**: The largest value for the filtered fluorescence trace for the sample does not exceed the fluorescence cut-off level and fluorescence cut-off is enabled.
- **Normalisation failure**: The cause of the normalisation failure depends on the type of normalisation being performed:
    - **LinRegPCR**: The iteration limit is exceeded trying to find a satisfactory base line.
    - **Dynamic**: An initial linear region is unable to be found.
    - **Fixed Length**: There are no failure conditions.

If a sample is excluded, its Result column entry of the Cycling analysis Result table will be set to Excluded. If a non-excluded sample was excluded from the window of linearity calculation, then it will not have an efficiency or R^2 value calculation and its Result column entry of the Cycling Analysis Results table will be set to Excluded from WOL.

NOTE	Any sample which is excluded will not be included in the W-o-L calculation. In addition, samples may also be excluded from the W-o-L calculation if: the second derivative maxima occur at the extreme of the data range; the logarithm of the normalised fluorescence trace is not defined near the second derivative maxima.

- **Fluorescence Cutoff Level**: This parameter will exclude samples that fall below a user defined percentage of the maximum fluorescence change. Small changes in fluorescence can interfere with the determination of Cq values by changing the W-o-L. Some of these small changes can be due to probe self-hydrolysis and cross talk between dyes. The default level is set to 5%.
    - **None**: no samples will be excluded.
    - **Simple**: samples will be excluded if they display no amplification or normalisation fails.
    - **Extensive**: samples will be excluded if they display no amplification, if normalisation fails or they fail the fluorescence cut off.
- **Threshold Start**: You can avoid interfering parts at the start of the baseline-corrected real time curve by moving the threshold start position. This can be achieved by either entering the value in the Threshold start text box or moving your mouse left or right clicked on the bubble at the start of the red threshold line.
- **Cycle threshold**: The cycle threshold is used to determine the Cq value of each selected sample and can be set automatically by the software or manually by the user with the following options:
    - **Auto Set Threshold** (ticked by default): Uses the W-o-L to set the cycle threshold.
      For some data sets the W-o-L may not be determined and the threshold will be set to a default value of 0.01. If this occurs, try using another Method or set the threshold manually.

      Both the W-o-L and threshold will change if samples are deselected or reinstated into the analysis, as the W-o-L is determined from the assay data set. If you wish to view specific samples without affecting the analysis, then use the View function in the Samples Selector to remove curves just from the graph rather than the analysis.

NOTE	For some data sets with poor amplification, a W-o-L may not be determined, resulting in an inability to set the threshold automatically. Under such circumstances a warning is displayed next to the Auto set threshold field and on top of the graph.

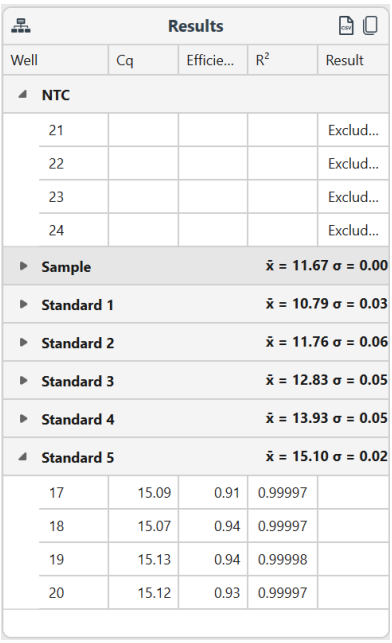
- **Manual Threshold**: You must deselect the Auto set threshold to manually set the threshold. Enter the value in the Threshold level text box or drag the red threshold line on the graph using your mouse.

NOTE	Use the derivative curves and cross hairs to set an appropriate threshold. With the cross hairs positioned on the second derivative maximum, reference the same point on the baseline-corrected curves. Set the threshold just below this point to ensure you are within the region of log-linear amplification.

Cycling Analysis Results Table

The results table is organised to display the mean ($\bar{x}$) and standard deviation ($x\sigma_{n-1}$) of the Cq values for sample replicates; and the individual sample results, which are organised just below the replicate row, into the following columns:

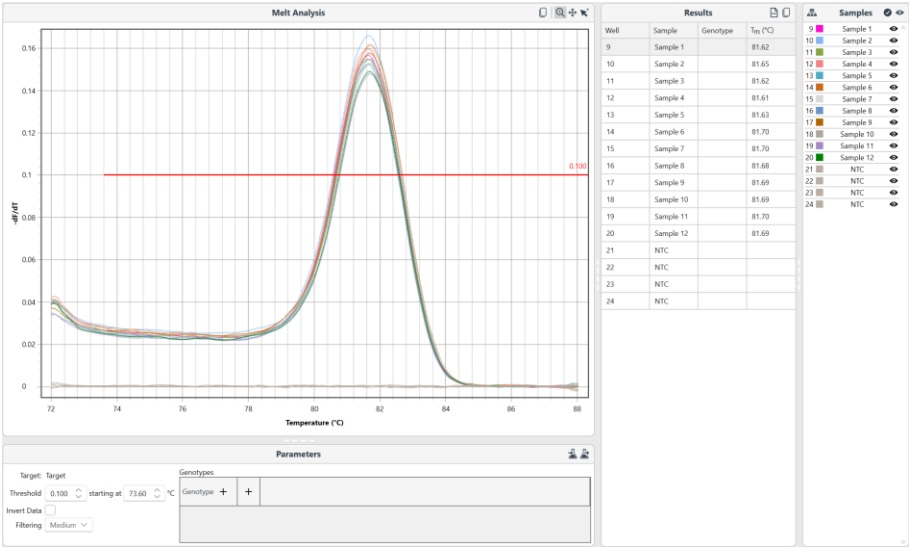
- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Cq**: The quantification cycle value for each sample, which is dependent on cycle threshold set.
- **Efficiency**: The amplification efficiency is calculated for each sample using the LinRegPCR algorithm described by Ramakers et al., 2003. Using the slope of the linear regression line from the calculated W-o-L, efficiency is calculated as: $\mathrm{E} = 10^{\mathrm{slope}} - 1$. Alternatively, a single efficiency value can be determined for a selected assay using the Standard Curve Analysis method. This can be found in Absolute Quantification.
- **R^2**: The R-squared value is a quality measure of the linear regression used to calculate amplification efficiency (values > 0.98 are acceptable).
- **Result**: Any issue related to the quality of the sample is reported in the results column. For example, if a Cq value cannot be determined.



Melt Analysis

Melt analysis allows you to determine the peak dissociation temperature (T_m) of a sample from the melt data. Basic melt analysis can be typically used as a measure of analytical specificity for an assay, especially when using intercalating dyes, by detecting any non-specific amplicons such as primer dimers. Melt analysis can also be applied for the determination of genotypes using chemistries such as dual hybridisation probes.

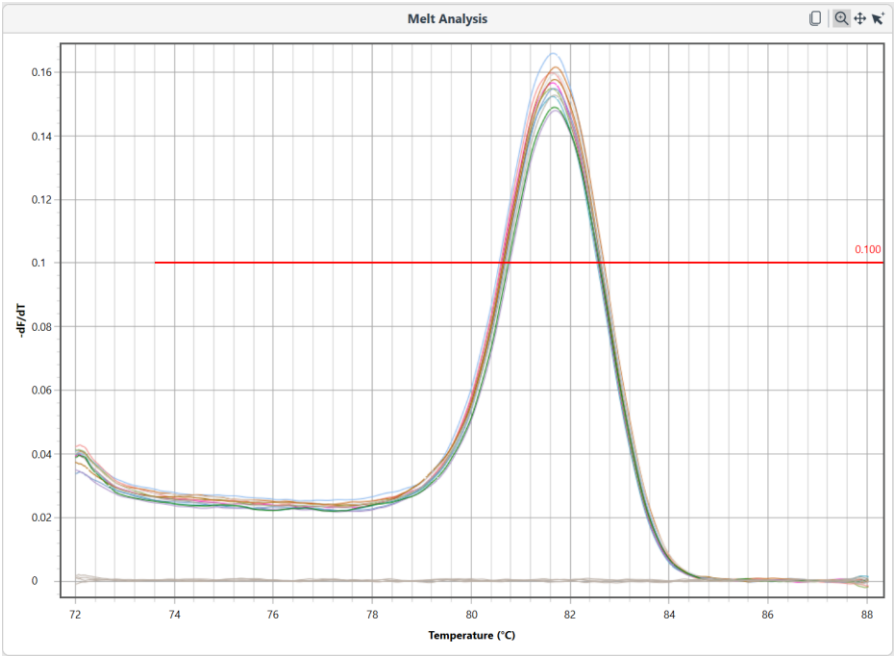
For a guide on how to use Melt analysis, including an example, refer to **Workbench Application Note 11 – Melt Genotype Analysis**, found under the Quick Links section on the Start Page.



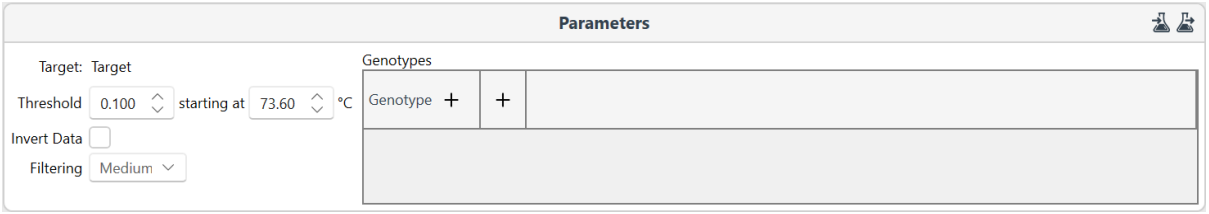
Melt Graph Display

Upon selecting the Melt analysis option, a graph will be displayed showing the **first derivative** curve plotted as $-\frac{dF}{dT}$ (*y*-axis) against **Temperature** (°C, *x*-axis), for the target that was chosen.

The Melt curve threshold can be set to any value, along with various other melting parameters available for genotyping.



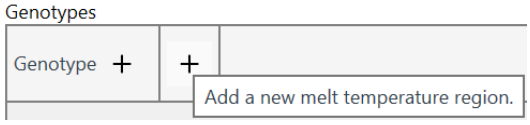
Melt Analysis Parameters



The following parameters can be changed:

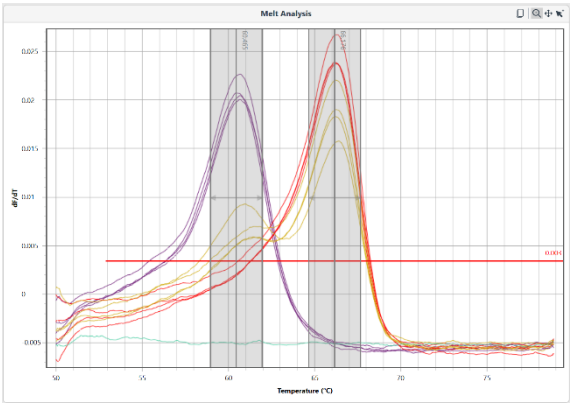
- **Threshold**: The threshold level can be changed by either sliding the red line up or down on the graph; or by entering a numerical value in the Threshold text box. Only peaks above the threshold line will be reported.
- **Threshold Temperature Start**: Earlier peaks can be ignored, by entering the starting temperature (°C) after starting at text box or clicking on the bubble at the start of the red threshold line and moving your mouse left or right.
- **Invert Data**: The first derivative melt curves can be inverted to allow for analysis of data generated using chemistries such as quenched FRET dual hybridisation probes or Plexor®. Tick the Invert box to invert the melt curves.
- **Filtering**: The appropriate level of filtering required for the melt data can be selected. Options are Medium or Strong. The default option is Medium and will be appropriate for most assays. For some chemistries such as dual hybridisation probes, a Strong filtering option might help improve with melt peak definition.
- **Genotypes**: Samples can be classified into known genotypes by using the specific Tm values associated with each genotype.

  Create Tm Bins for each allele by selecting the + button.

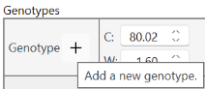


Select the Peak T_m for each bin by either moving the centre of the bin left or right, or entering a value in the Peak text box provided in the Genotyping table.

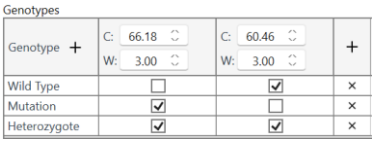
Select the Width of the bin by moving the outer perimeter inwards or outwards, or entering a value in the Width text box provided in the Genotyping table. The width allows for variations in T_m between samples. Ensure that the bin widths do no overlap with other bins.



Enter a *Genotype* by selecting the + button and providing the name in the column provided.



Link the *Genotype* to a *Bin* by using the tick boxes under each bin column within the row for each genotype.
If a genotype shares both bins (heterozygote), tick both boxes.



Melt Analysis Results Table

The results table is organised to display the peak dissociation temperature (Tm) of samples and their reported genotype (if applicable) into the following columns:

- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Sample**: The name of each sample in well order.
- **Genotype**: Reported genotype for individual samples if the Genotypes table in Parameters has been edited.
- **Tm (°C)**: The value/s of the melt temperature peaks (Tm) for each sample is reported. Only peaks above the threshold line will be reported. Samples with peaks below the threshold line will be reported as 'No melt temperature.'

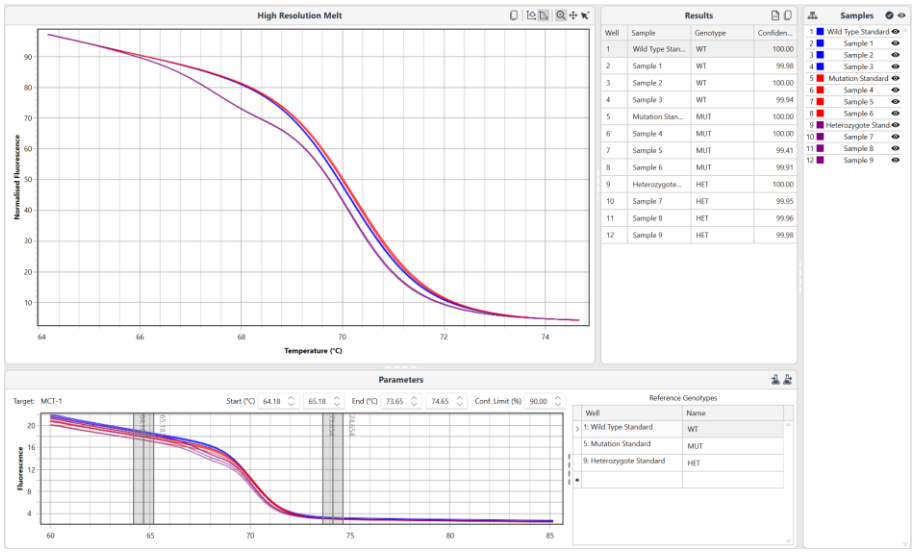
Results

Well	Sample	Genotype	T_m (°C)
1	Sample 1	Wild Type	60.53
2	Sample 2	Wild Type	60.64
5	Sample 3	Mutation	66.18
6	Sample 4	Mutation	66.20
9	Sample 5	Heterozygote	60.94, 66.35
10	Sample 6	Heterozygote	61.58, 66.26
13	NTC		

High Resolution Melt Analysis (Mic only)

High Resolution Melt (HRM) analysis will allow you to identify DNA sequence variants, including single base changes, insertion-deletions, and base pair substitutions by analysing DNA melt curves. The software characterises DNA samples according to their dissociation behaviour as they transition from double stranded DNA to single stranded DNA with increasing temperature.

HRM analysis is provided as an additional module that will need to be activated using a key, provided upon sale of the upgrade (see Upgrading to HRM).

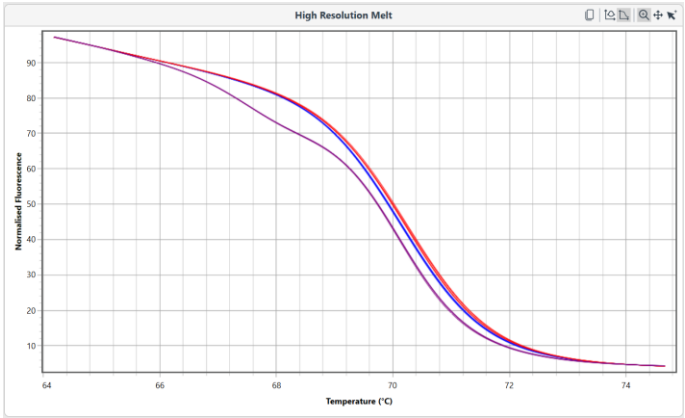


HRM Graph Display

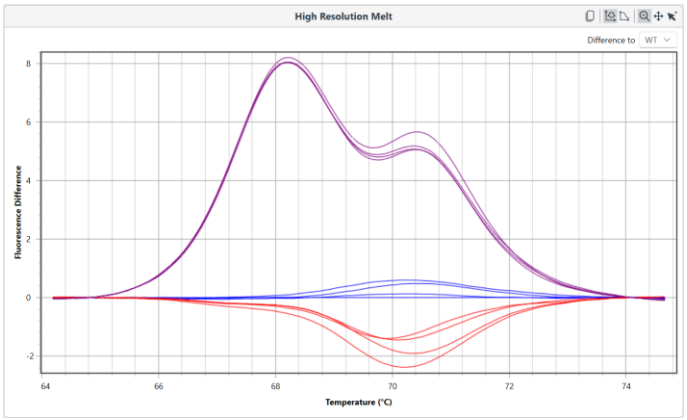
Selecting the High-Resolution Melt analysis option will bring up a graph with the normalised melt curves for all samples selected for the chosen target. Normalisation is dependent on the regions set in the parameters. The determination of genotypes can only be achieved once the reference genotypes have been edited in the parameters section.

To change the display of your normalised fluorescence data, toggle between left for Fluorescence difference and right for normalised fluorescence found on the top right corner of the graph toolbar.

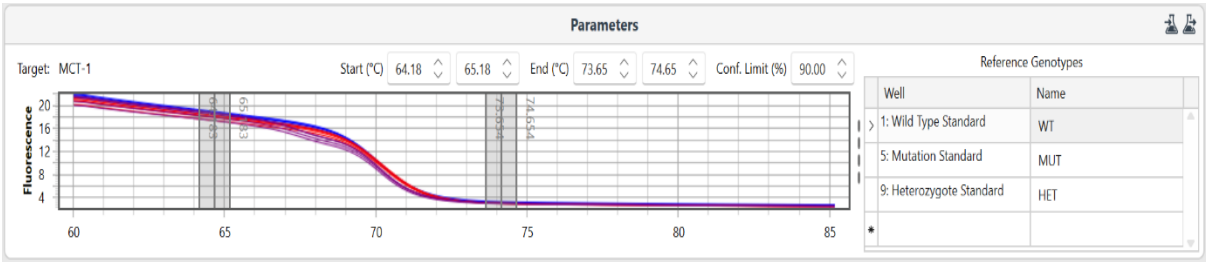
- **Normalised fluorescence** (default): Displays the **Normalised Fluorescence** (y-axis) against **Temperature** (°C, x-axis) for the melt data. Normalisation is dependent on the regions set in the parameters section.



- **Fluorescence difference**: Once the reference genotypes have been set in the Parameters section, the Difference Plot option can be used to display the differences for all analysed curves to a selected reference genotype (this is typically the wild type but can be any of the reference genotypes set). This graphical plot displays the **Fluorescence Difference** (y-axis) against **Temperature** (°C, x-axis) for the normalised fluorescence data which allows for a better visualisation of curve character differences between genotypes. The reference genotype to compare from can be selected from the Difference to drop-down list on the top right-hand corner of the graph display.



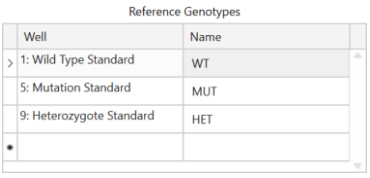
HRM Analysis Parameters



The following parameters can be changed:

- **Normalisation Regions**: The Raw melt curve graph can be used to set the two normalisation regions by dragging the midpoint line left or right for each region. Move the ends to widen or narrow the normalisation regions. Alternatively, enter the values for the Start (°C) and End (°C) regions in the text boxes provided. The software will prevent the Start (°C) and End (°C) regions from crossing over. Data outside of the regions is ignored in the normalised fluorescence and fluorescence difference plots.
- **Conf. Limit (%):** A percentage Confidence Limit (°C) can be used as an integrity check of the auto-called results. The confidence limit ensures samples that do not match closely to one of the reference genotypes can be flagged in the analysis.
- **Reference Genotypes**: Reference genotypes can be used to automatically classify each unknown sample into a specified genotype by comparing and matching the unknown curve to the nearest curve of each of the reference genotype curves.

  Create as many reference genotypes required for the analysis by editing the Reference Genotypes table.
  Select the sample you wish to use as the genotype reference by using the drop-down list in the *Well* column. Then enter the *Name* of the reference genotype in the second column.

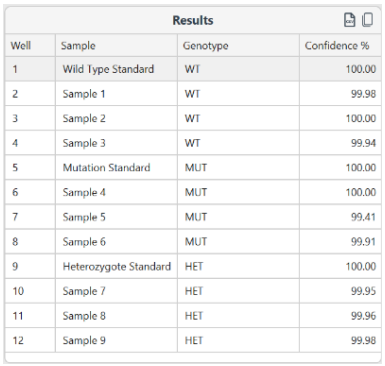


  You can delete any reference genotype by using the delete key on your keyboard.

HRM Analysis Results Table

The results table is organised to display the reported genotype along with a confidence percentage into the following columns:

- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Sample**: The name of each sample in well order.
- **Genotype**: Reported genotype for individual samples if the Reference Genotypes table has been edited.
- **Confidence %**: The level of confidence that the detected variation is accurate. The closer the confidence percentage is to 1, the closer the match of the unknown to the reference genotype.



Results

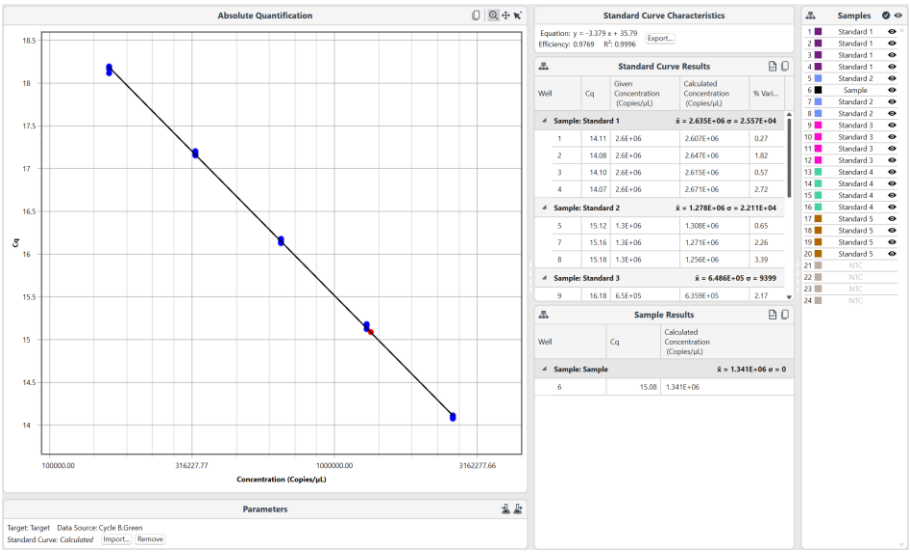
Well	Sample	Genotype	Confidence %
1	Wild Type Standard	WT	100.00
2	Sample 1	WT	99.98
3	Sample 2	WT	100.00
4	Sample 3	WT	99.94
5	Mutation Standard	MUT	100.00
6	Sample 4	MUT	100.00
7	Sample 5	MUT	99.41
8	Sample 6	MUT	99.91
9	Heterozygote Standard	HET	100.00
10	Sample 7	HET	99.95
11	Sample 8	HET	99.96
12	Sample 9	HET	99.98

Samples that fall below the confidence limit defined in the parameters are reported as an Unknown genotype. Samples with low percentages could be due to poor amplification and should be re-analysed, or investigated further for the potential of additional variations using sequencing methods.

Absolute Quantification Analysis

Absolute Quantification analysis allows you to quantify the unknown concentration of a sample using a standard curve generated with samples of a known quantity. The method has been applied to applications such as the determination of viral load in patients. This feature can also be simply used for standard curve analysis which allows you to determine the efficiency of an assay by using a dilution series of known sample. This method can be used as an alternative to the LinRegPCR method in calculating reaction efficiency.

For a guide on how to use Absolute Quantification analysis, including an example, refer to **Workbench Application Note 8 – Absolute Quantification Analysis**, found under the Quick Links section on the Start Page.

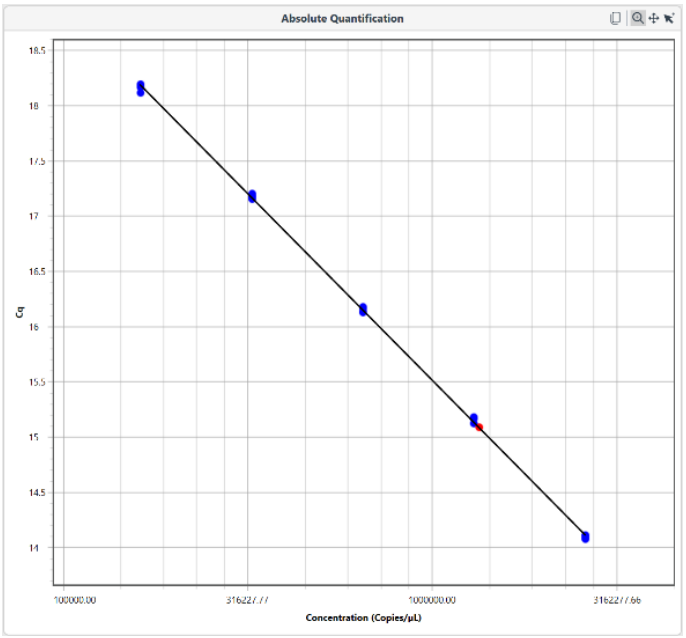


Absolute Quantification Graph Display

The method utilises the Cycling Analysis feature to determine **Cq values** and plots them on the y-axis against the log of the given **Concentration** on the x-axis for each standard annotated in the Samples editor. A line of best fit is generated for the data plot from which the slope of the line is determined.

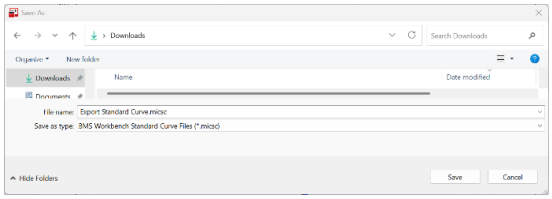
In the graph for the standard curve, standards are shown as **blue** dots and unknown samples as **red** dots.

Unknown samples that fit outside the limits of the standard curve will be displayed on a dashed line. Samples outside the limits of a standard curve should be treated with caution. To avoid having samples outside the limits of the standard curve, ensure that your standard curve contains enough points to encompass all your potential unknowns. This may require the determination of the linear dynamic range and/or limit of detection (LoD) for the assay.



The data generated for the standard curve can be found in the Standard Curve Characteristics which contains the following information:

- **Equation**: Is the slope-intercept form of the equation of a straight line. In the equation y = mx + b, m is the slope of the line (representing amplification efficiency) and b is the y-intercept (representing the baseline Ct value). x and y represent the distance of the line from the x-axis and y-axis, respectively.
- **Efficiency**: From the slope of the line the efficiency is calculated using the equation $10^{\frac{-1}{\text{Slope}}} - 1$, and is reported in a summary window as a value from 0 to 1 along with the r-squared (R^2) value, Equation of the line, which includes the gradient of the line (M), and y-intercept. You can also enter the efficiency value for the assay into the Relative Quantification analysis option in the Assay setup. This value will be used in future relative quantification analyses as the default efficiency.
- **R^2**: The R-squared Value (R^2) is a measure of the percentage of data that matches the hypothesis that the given standards form a standard curve. In other words, if the R^2 value is low then the given standards do not aggregate to the line of best fit very well, and therefore, the calculated efficiency may not be reliable. A value > 0.98 is typically a good R^2 value. However, a good R^2 value can still be achieved for a poor standard curve if not enough standards have been used. It is recommended that the standards extend to at least 5 $\log_{10}$ concentrations.
- **Export...**: The standard curve can be saved into a file location as a *.micsc file to be used in following runs. This file can be imported into any run containing cycling data and be used as part of the Absolute Quantification analysis.



Absolute Quantification Analysis Parameters

The following parameters can be edited:

- **Import...**: A previously exported standard curve file (*.micsc) can be applied to the current run data. The software will apply the standard curve formula to the run data and display the curve on the graph.

  It is required that a standard curve calibrator be used to verify the imported standard curve.
  In the Samples editor annotate the calibrator by selecting it as **Standard** in the **Type** column. Then enter the concentration for the standard calibrator. Several replicates can be run for a calibrator but only one calibrator can be applied per imported curve.

  Failure to apply a standard calibrator will result in a warning being displayed above the standard curve graph.

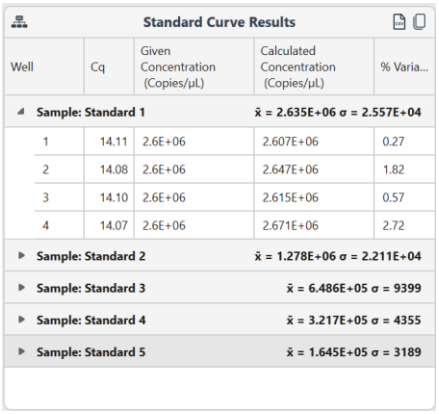
  A warning will also be displayed if the standard calibrator varies in Cq by more than ± 1 cycles.

- **Remove**: This will remove any imported standard curve run file to the standard curve calculation of the current run dataset.

Absolute Quantification Analysis Results Table

There are two results tables in Absolute Quantification analysis, with one for the standard curve generated and the other containing calculated concentrations for unknown samples.

The **Standard Curve Results** table contains the following columns:

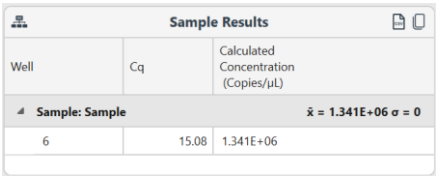


Standard Curve Results

Well	Cq	Given Concentration (Copies/µL)	Calculated Concentration (Copies/µL)	% Varia...
Sample: Standard 1			**x̄ = 2.635E+06 σ = 2.557E+04**	
1	14.11	2.6E+06	2.607E+06	0.27
2	14.08	2.6E+06	2.647E+06	1.82
3	14.10	2.6E+06	2.615E+06	0.57
4	14.07	2.6E+06	2.671E+06	2.72
Sample: Standard 2			**x̄ = 1.278E+06 σ = 2.211E+04**	
Sample: Standard 3			**x̄ = 6.486E+05 σ = 9399**	
Sample: Standard 4			**x̄ = 3.217E+05 σ = 4355**	
Sample: Standard 5			**x̄ = 1.645E+05 σ = 3189**	

- **Well**: The order of the well numbers will depend on the grouping of the standards as the name of the standard order can be alphanumeric (grouped) or numeric (ungrouped).
- **Cq**: The quantification cycle value for an individual standard.
- **Given Concentration (Copies/µL)**: The value annotated in the Samples editor. The reported units are also displayed in the column heading. The unit measure is selected from the list provided in the Samples editor.
- **Calculated Concentration (Copies/µL)**: An adjusted concentration for an individual standard based on the line of best fit. The adjusted concentration is calculated by using the C_q value to interpolate the new concentration from the line of best fit. The mean ($\bar{x}$) and standard deviation (σ) for a set of replicates is also provided in the top row for the set. The reported units are displayed under the Calculated Concentration title and are chosen from a list in the Samples editor.
- **% Variation**: The percentage difference between the given and calculated concentrations.

The **Sample Results** table contains the following columns:



Sample Results

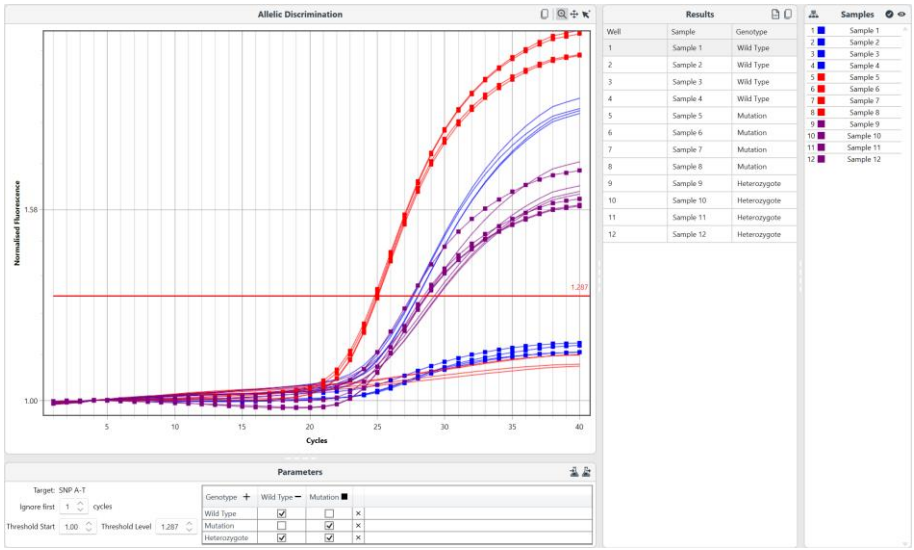
Well	Cq	Calculated Concentration (Copies/µL)
Sample: Sample		**x̄ = 1.341E+06 σ = 0**
6	15.08	1.341E+06

- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Cq**: The quantification cycle value for an individual sample.
- **Calculated Concentration (Copies/µL)**: Reported calculated concentration for an individual samples based on the line of best fit produced by the standard curve. The calculated concentration is calculated by using the Cq value produced by the sample. The mean ($\bar{x}$) and standard deviation (σ) for a set of replicates is also provided in the top row for the set.

Allelic Discrimination Analysis

Allelic discrimination analysis allows for the determination of genotypes using real time kinetic data obtained from multiplexed assays using hydrolysis probe chemistry. A differently fluorescent labelled probe is targeted toward a specific allele, such as a single nucleotide polymorphism (SNP), for a given target. The presence of an allele is indicated by a real-time amplification curve within the specific channel corresponding to the probe designed. The presence of both alleles will indicate heterozygosity.

The alleles need to be defined when setting up the assay. Each allele will correspond to a specific fluorescently labelled probe, and therefore channel. Using the assays setup, each allele is defined in the **Assay Information** page (see Assay Setup for more information on how to setup up required alleles). Probe names entered in the Oligonucleotides Table will be displayed in the Genotypes Table in the Parameters section.

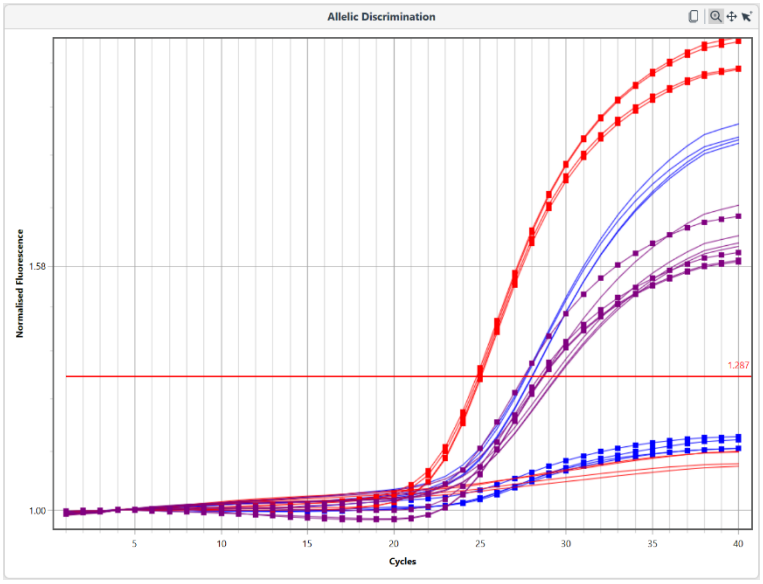


Allelic Discrimination Graph Display

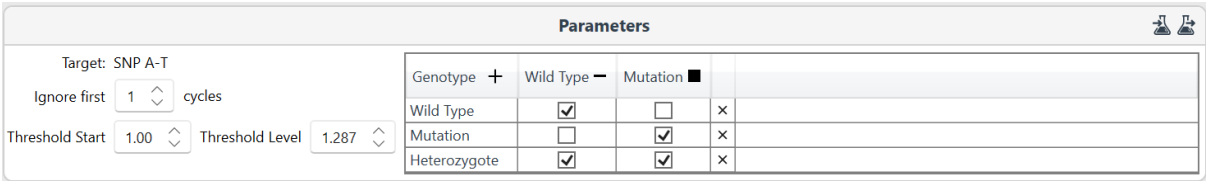
Using the real time kinetic data from each channel, the software combines the data and normalises it to display all the curves on one graph as **Normalised Fluorescence** (y-axis) vs. **Cycles** (x-axis).

Each allele (probe) is represented in the graph with a specific type of point symbol. The type of symbol associated with the allele is shown in the Genotypes table found in the Parameters section.

A threshold is set and samples with curves for each channel that cross the threshold are called positive for the allele they correspond to.



Allelic Discrimination Analysis Parameters



The following parameters can be changed:

- **Ignore First Cycles**: The option to ignore the first number of cycles is available.
- Threshold Start: You can avoid interfering parts at the start of the baseline-corrected real time curve by moving the threshold start position. This can be achieved by either entering the value in the Threshold start text box or moving your mouse left or right and clicking on the bubble at the start of the red threshold line.
- **Threshold Level**: For some assays, minor cross reactivity between probes, cross talk between dyes, or probe self-hydrolysis may result in low levels of kinetic signal for samples that are negative to the allele. Set the threshold above the level of these signals.
- **Genotypes**: Samples can be classified into known genotypes by assigning them specific alleles displayed in the Genotype Table.

  Add and name the required number of Genotypes for the target.

  Link each Genotype to one of the alleles displayed in the genotype table.

  The allele names displayed are derived from the assay setup.

Genotype +	Wild Type −	Mutation ■	
Wild Type	☑	☐	×
Mutation	☐	☑	×
Heterozygote	☑	☑	×

Allelic Discrimination Analysis Results Table

The results table is organised to display the reported genotype into the following columns:

- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Sample**: The name of each sample in well order.
- **Genotype**: Reported genotype for individual samples once genotype names have been linked to each allele and the threshold is set.

Results

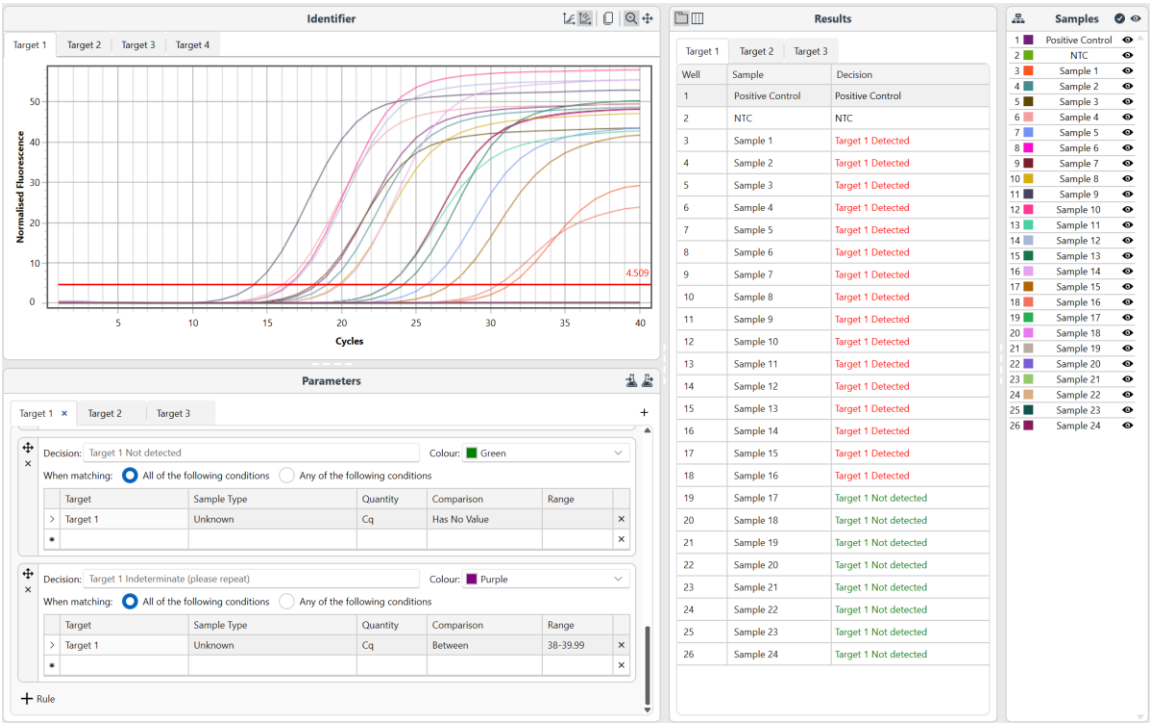
Well	Sample	Genotype
1	Sample 1	Wild Type
2	Sample 2	Wild Type
5	Sample 3	Mutation
6	Sample 4	Mutation
9	Sample 5	Heterozygote
10	Sample 6	Heterozygote

Identifier Analysis

The Identifier analysis uses a set of user-defined rules to auto-call presence or absence of a target(s) in an unknown sample. Controls are also utilised to make appropriate decisions regarding the result of each sample and the run as a whole. These rules can be pre-defined for each target used in the assay individually or as a whole group of targets.

The logic engine will use the data from a run to determine a decision for each sample. If the conditions are met for the rule, then that decision will be called for the sample/s. It will call the first rule matched according to the order in the list. If the conditions are not met, then the software will continue to the next rule until all the samples are called.

For a guide on how to use Identifier analysis, including an example, refer to **Workbench Application Note 10 – Identifier Analysis**, found under the Quick Links section on the Start Page.

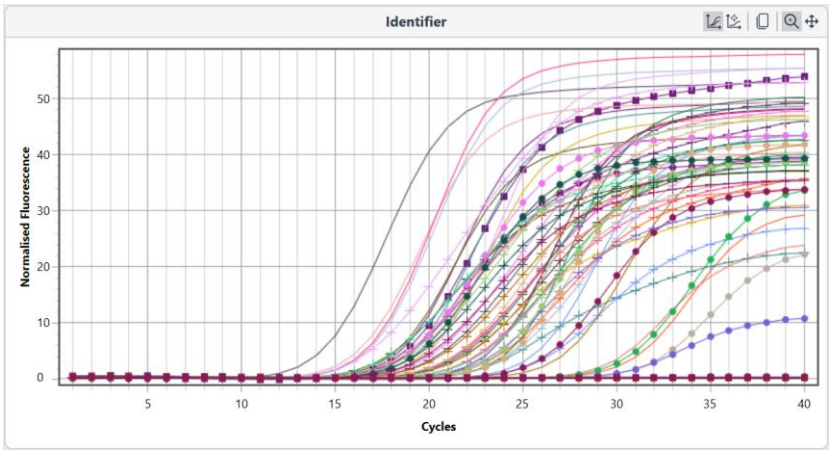


Identifier Graph Display

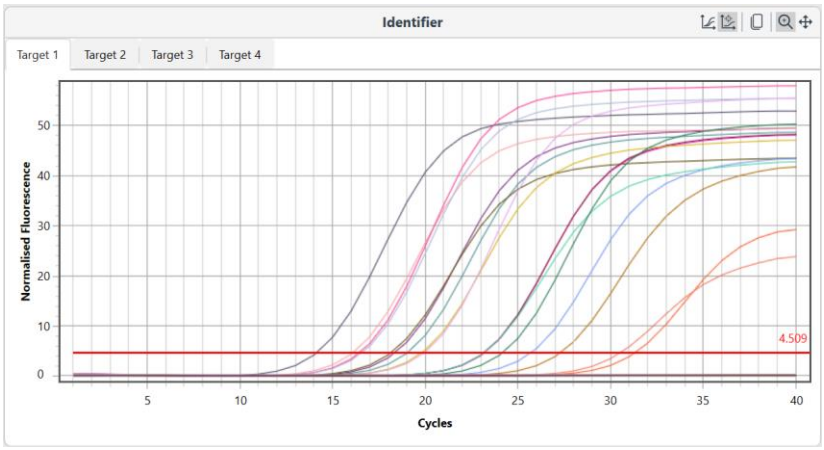
Normalised real-time PCR curves are shown for each target in the analysis graph. Curves are displayed in linear format with *Normalised Fluorescence* along the *y*-axis and *Cycles* on the *x*-axis.

To change the display of your normalised fluorescence data, toggle between Display Results From All Targets On One Chart on the left or Display Results For Each Target On A Separate Chart on the right found on the top right corner of the graph toolbar.

- **Display Results From All Targets On One Chart** (default): All targets will be presented on the same graph. A different type of symbol is used for each target displayed for easy differentiation, on each curve in the graph.

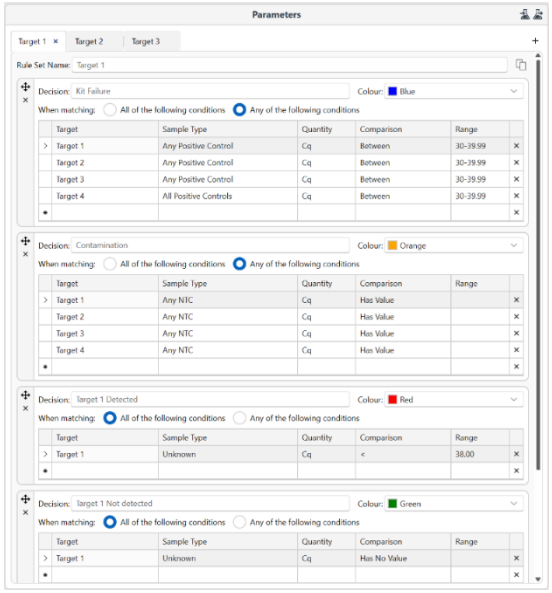


- **Display Results For Each Target On A Separate Chart**: When the targets are displayed individually, selection of the required target is achieved using the chart tabs.



Identifier Analysis Parameters

The parameters section contains the rulesets that are defined for the Identifier logic engine for the assay which is used to help automate the identification of a target. The logic engine is a set of rules defined by the user that enables the software to make the identification. The analysis works through the rules from the top of the list until it finds a match. Rules after a match is found will be ignored. Therefore, the initial rules applied should use various controls within a run to qualify the test.



The optimum order for specifying rules is:

1. Check assay is working as expected by ensuring controls work successfully.
2. Define positive decisions as the detection of a target.
3. Define negative decisions as the lack of detection of a target.
4. Define when to repeat a sample; i.e., an internal control fails to amplify.

Start by selecting the number of required target rulesets if more than one target is to be used for the Identifier analysis.
Use the + icon at the top to add another target set of rules. Give the ruleset a name. Each target ruleset is displayed as a tab at the top of the rule sets.

Create the first decision.

Enter a name that will best describe the decision. This name will be displayed in the results table if the rule is met.

When matching the conditions to the data, determine if the rule must meet All of the following conditions or Any of the following conditions.
This option is only valid if using more than one target to decide. For example, if either the target or internal amplification control (IAC), for the positive control, fail to amplify, then use the Any option to decide that the run has failed. Since we expect both to amplify in the positive control to pass the test, failure of either one should trigger a failure.

Select the colour of the text for the decision.

Use colours to better differentiate important results. For example, use a red colour to show samples are positive or green colour to show samples are negative.

Set the conditions for the decision.

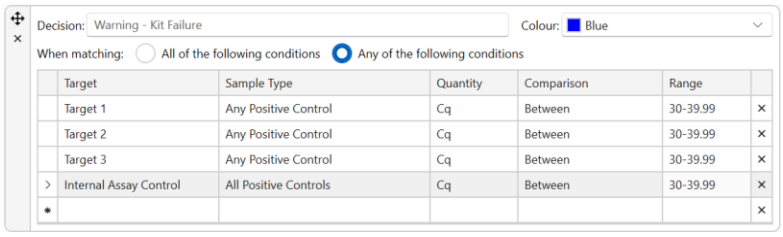
- **Target**: Select the target required for the rule. A drop-down list will display a list of all targets available for the assay.
- **Sample Type**: Includes Unknown sample, Standard, Positive control, Negative control, NTC, or NRT. If using more than one sample in the group type you have the option to select Any or All samples.
- **Quantity**: Select the measured quantity as either C_q or Efficiency.
- **Comparison**: Select from the following options; less than (<), less than or equal to (< =), equal to (=), greater than (>), greater than or equal to (> =), not equal to (! =), Between, Outside, Has Value, or Has No Value.
- **Range**: Enter a range for the quantity for the comparison. For example, C_q is Outside range of 20 – 30.

Create a new decision using the + Rule button at the bottom on the parameter section.
You can create as many rules as required. Rules can also be moved by selecting the cross with arrows and dragging the rule up or down the decision order. Use the × icon to delete a rule.

The following are some simple guidelines to help setup a basic set of rules for a standard presence/absence test.

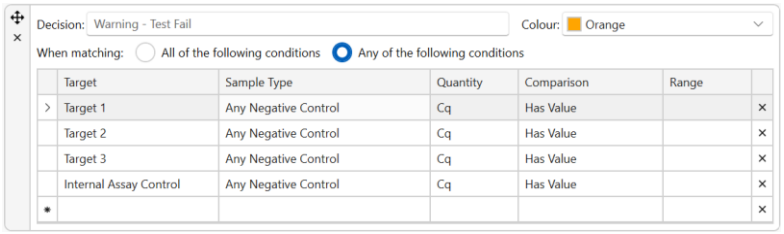
- **Positive Controls:** Use positive controls to qualify the whole run.

  Generally, a positive control should amplify during the run for at least the target of interest in the assay. Therefore, use the C*q* value of the target of interest and select *Has No Value* as the rule to fail the run. For some assays, the positive control might be expected to amplify within a certain range. If the C*q* falls outside this range, the sample should be repeated as the assay has not worked as expected. An example warning might be “Warning - Test Fail.”



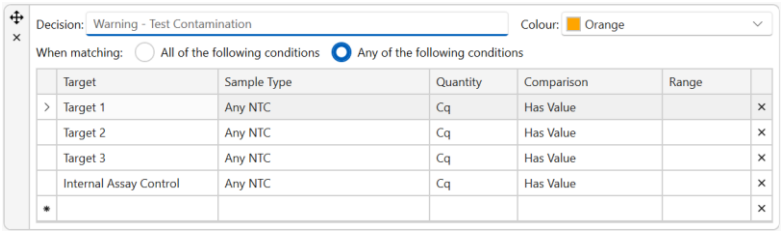
- **Negative Controls:** A negative control should not amplify for the target of interest.

  It differs from the NTC in that the IAC is expected to amplify. You can apply a condition for the Cq value of the target of interest to **Has Value**, and apply a condition that sets the IAC to **Has No Value**. If either of the conditions are met, the test fails, therefore the Any option is more appropriate.



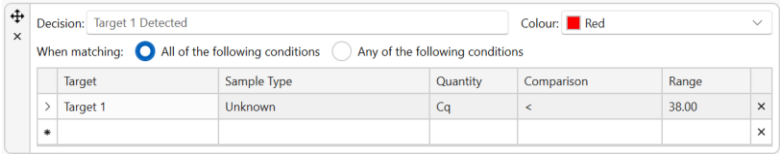
- **NTC:** A no template control is typically used as measure of contamination during PCR setup.

  The NTC for any target should not amplify, therefore, the set rule would to see if NTC Has Value. For some assays, the NTC might contain primer dimer creating a provision for an acceptable level, hence the condition for Cq less than a set value. An example decision might be “Warning – Test Contamination.”



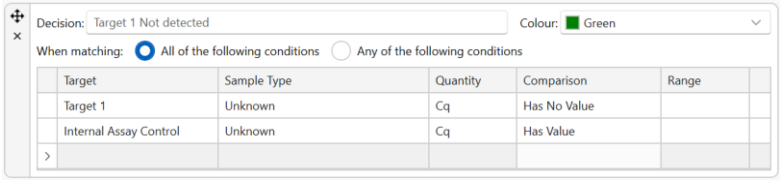
- **Positive Sample:** For a typical assay, a sample will be called positive for a target of interest if the Cq Has Value.

  You might choose to use a distinctive colour such as red to highlight such samples.

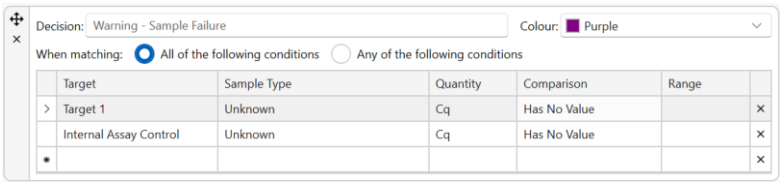


- **Negative Sample:** Samples that do not meet any of the above rules would be assumed to be absent for the target if an internal control Has Value.

  A positive amplification for the internal control indicates that the absence of the target of interest was not due to a sample amplification failure, eliminating doubts such as lack of proper nucleic acid purification.



- **Sample Failure:** A final decision might incorporate a rule that looks for samples where the IAC Has No Value.
  For such results the decision could be “Warning - Sample Failure.”

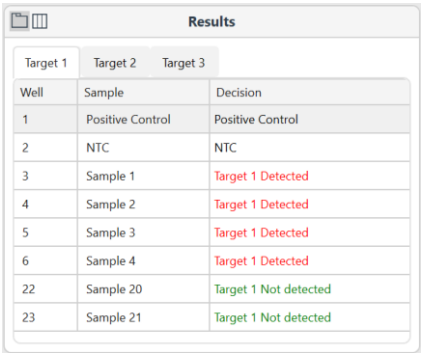


If you are using an assay with more than one target, you can use the *Create a clone of this rule set* option for a set of rules identical to the ones already setup.

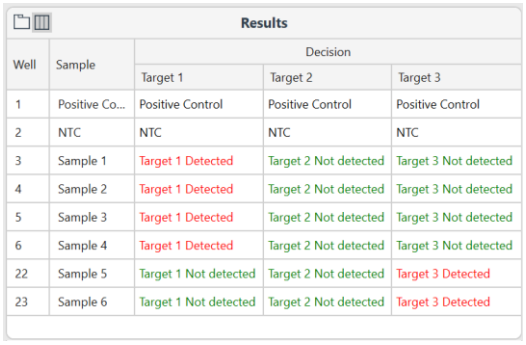
Identifier Analysis Results Table

The identifier results table can be organised to **Display tabs in the results** table or **Display columns in the results table** using the Folder/Grid button on the top left corner.

Display tabs in the results

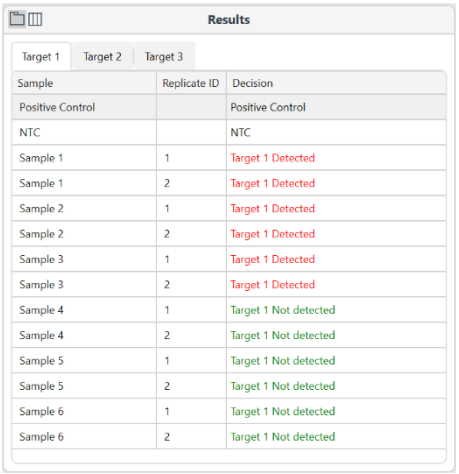


Display columns in the results table



The results table is organised to display the reported decision of each sample into the following columns:

- **Well**: The order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).
- **Sample**: The name of each sample in well order.
- **Replicate ID**: If Identifier Replicate ID was assigned to replicates in the Samples Editor, it will allow for each of the replicates to be presented consecutively. This is applicable for Panel Assays only.



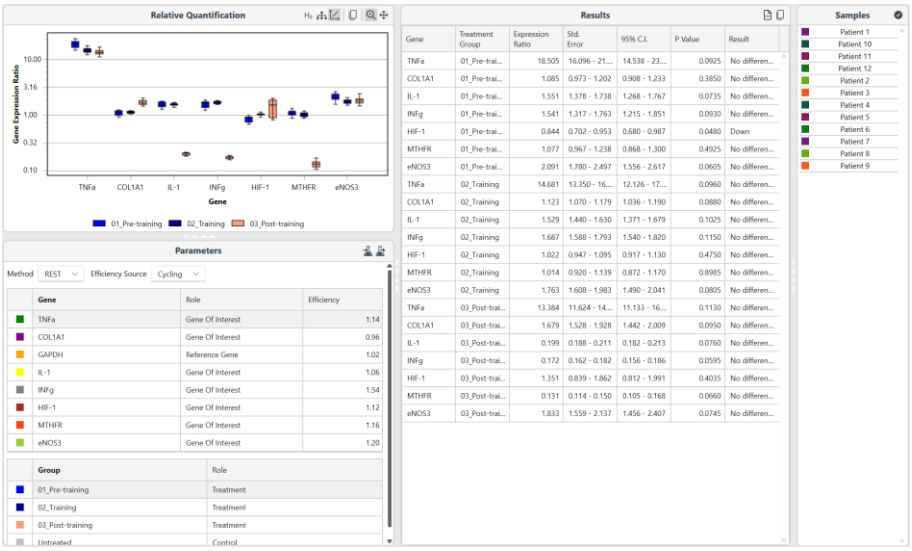
Decision: The decision for each sample being analysed will be displayed in the results table in the text of the chosen colour of the decision. If multiple rules sets are used these will be displayed in a tab format. Samples will be displayed in well order and each of the controls clearly labelled as type in the Decision column. Samples that have been deselected by the user will be reported as such in the decision column.

Relative Quantification Analysis

Relative Quantification analysis allows you to analyse differences in gene expression for a given sample group relative to a control group; for example, measuring gene expression in response to a drug. To achieve most Relative Quantification analyses, an endogenous reference gene must be run in parallel with the gene of interest to normalise for variations in sample loading. According to the MIQE guidelines (Bustin et al., 2009), two or more reference genes are required to ensure validity in the normalisation. Furthermore, the efficiency of each gene must be taken into consideration when calculating expression values.

To achieve Relative Quantification, the Samples editor must be annotated such that each sample is linked to an assay Target (Gene) and Group.

For an example on how to use Relative Quantification analysis, including an example, refer to **Workbench Application Note 9: Gene Expression**, found under the Quick Links section on the Start Page.

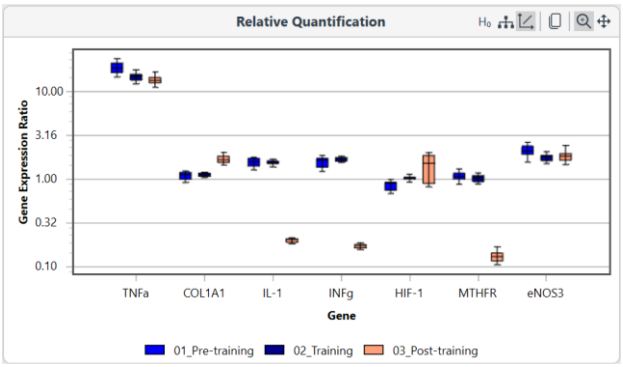


ATTENTION	Runs using the qPCR Setup on the Myra+ using the Myra+ Cycler cannot be added into a qPCR Project. If you intend on using Relative Quantification, you may need to limit the number of groups or number of samples in a group to utilise this analysis.

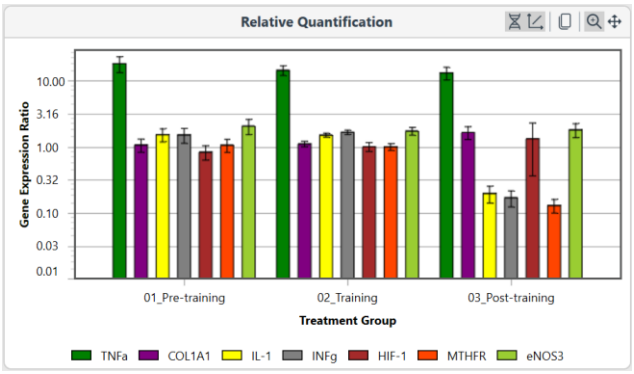
Relative Quantification Graph Display

Gene expression ratios are graphed differently depending on the method chosen, with the **log of the given gene expression** on the y-axis against the **gene or treatment group** on the x-axis.

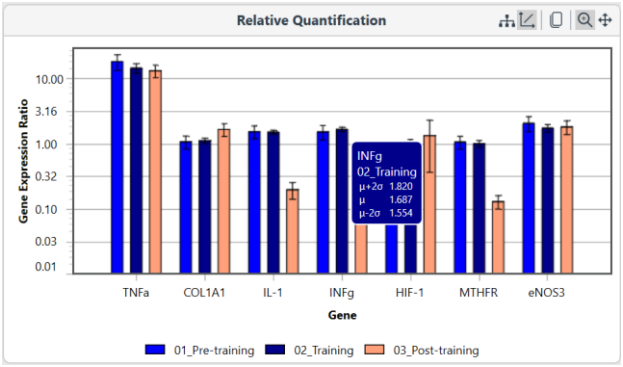
- **Box and Whisker** (default for REST analysis): The box represents the inter-quartile range for the observations, with a median line, and the whiskers are the upper and lower 25% observations from the randomised bootstrapping.



- **Bar Chart** (default for DDCt and DCt analysis): The mean is shown as the bar and 2-standard deviations are drawn as the error bars.

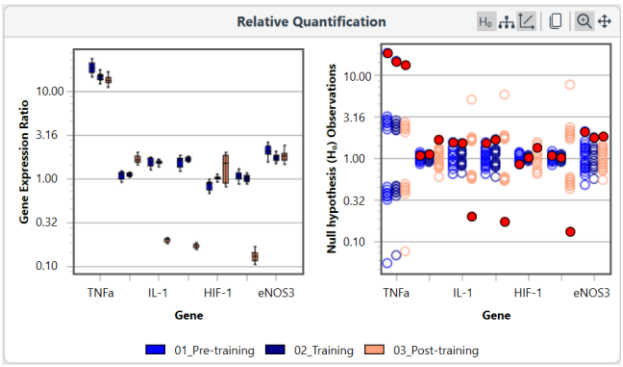


If you hover over the graphs, a tool tip will appear with the statistical values for any of the chart types. Different genes and groups are presented on the same graph with the default minimum range being 0 to 2-fold.

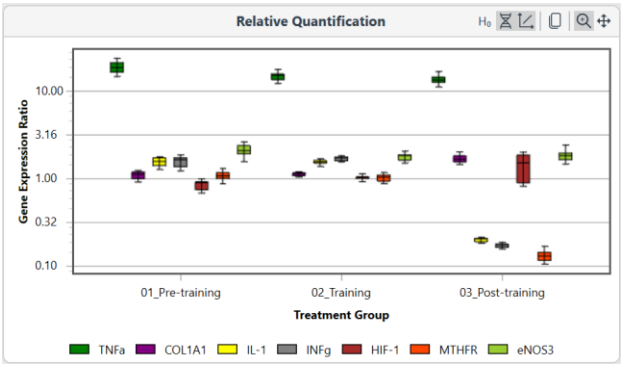


To change the display, toggle between the Display the null hypotheses observation (REST analysis only) on the left or Group chart by gene or treatment group in the middle or Display chart with a logarithmic or linear y-axis on the right options found on the top right corner of the graph toolbar.

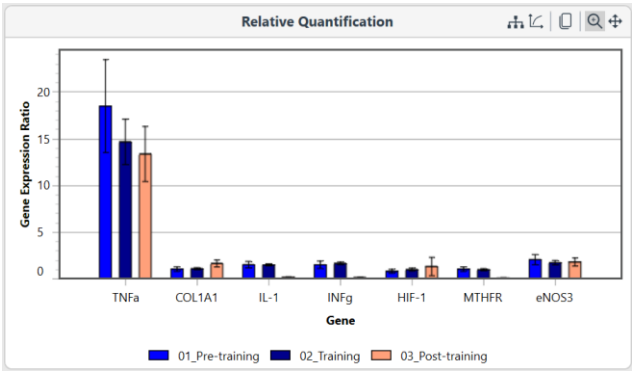
- **Null Hypothesis Observations**: Displays the observations for the random permutations used in calculating the P value for the REST method. Each observation is displayed as an open circle. The calculated mean is shown as a red dot. For a sample to be statistically significant, in relative gene expression, the mean must be toward the outer 5% of observations either up or down. Or to put it another way, there is only 5% or less chance the value is due to randomness.



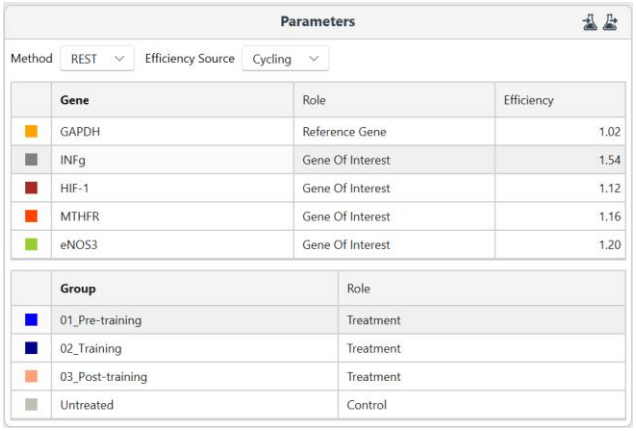
- **Grouping Function**: The graphs can be displayed with either the genes (see Box and Whisker image above) or treatment (image below) groups being labelled on the x-axis.



- **y-axis Scale**: There is an option to display the gene expression ratio in log (see Bar Chart image above) or linear (image below) scale to allow for better visualisation of bigger differences between groups.



Relative Quantification Analysis Parameters



The following parameters can be changed:

- **Method:** There are three methods that can be applied to the analysis:
    - **REST:** The REST method calculates gene expression ratios between two groups (Pfaffl et al., 2002). The method also provides statistical analysis of the calculated expression values. REST uses a mathematical model for calculating gene expression ratios that takes the individual efficiency of each target (gene of interest and reference gene) into consideration. The following formula is used to calculate a relative expression value between two groups:

$$\text{ratio} = \frac{(\text{E}_{\text{gene of ineterest}})^{\Delta \text{Cq}_{\text{gene of interest}}(\text{control}-\text{treatment})}}{(\text{E}_{\text{Reference}})^{\Delta \text{Cq}_{\text{reference}}(\text{control}-\text{treatment})}}$$

      where **E** = **reaction efficiency**, C_q = **cycle threshold value**; control and treatment are the two groups. A modification to the formula allows the use of two reference genes for normalisation.

      Cq values are determined using cycling analysis, with LinRegPCR being the preferred method when using intercalating dyes. Efficiency values can be calculated using the same method or using a standard curve.

      By using randomisation and bootstrapping techniques, REST allows for a statistical evaluation of the calculated expression ratios. Unlike other statistical methods such as a student t-test, the techniques used in REST do not assume normal distribution in the data, which cannot be expected every time when using ratios.

    - **ΔΔCt:** The delta-delta Ct method assumes an efficiency value of 1 for each gene used (Livak & Schmittgen, 2001). However, the software does allow the use of individual gene efficiencies in the calculation of relative expression values. In this instance, the same formula described above is used.
      Unlike the REST method, the ΔΔCt method uses a parametric method for its statistical test. A two-tailed student t-test is applied, with the method assuming that the data is normally distributed. It is important that you determine this is true for your data sets. If you are unsure, then simply use the REST method described above, which does not make such an assumption.
    - **ΔCt**: The delta-Ct method does not apply normalisation of the relative quantification data using a reference gene. It should only be used when the experimental sets are known to be equivalent in starting material.
- **Efficiency Source**: The method that is used to calculate the efficiency of each gene. There are three options:
    - **Standard Curve**: Uses a Standard Curve analysis to determine the reaction efficiency. If this option is selected then the software will use Absolute Quantification analysis to determine the efficiency. Use Absolute Quantification to view and change parameters associated with generating the standard curve.

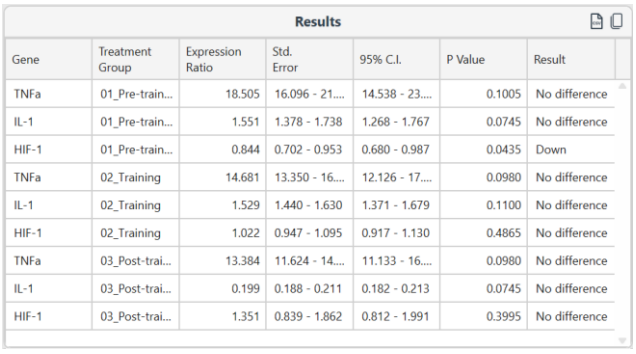
    - **Cycling**: The mean efficiency is determined using the W-o-L calculated efficiencies for each sample of a target.
    - **Set Value**: The efficiency value for each target can be entered manually. This value can be stored for each target in the Assay setup.
- **Gene Colour**: The colour each gene can be edited and it will be drawn in on the graph.
- **Gene Role**: Each Assay Target used in the run must be selected as a Gene of Interest, a Reference Gene, or None (not used in the analysis). The roles can be defined and stored in the Assay. The efficiency being used is displayed next to the gene role. Multiple genes can be used per analysis.
- **Group Colour**: The colour of the group can be edited, which can be drawn in on the graph.
- **Group Roles**: Each group needs to be characterised as a Control, Treatment, or None (not used in calculation). Multiple groups can be used per analysis.

Relative Quantification Results Table

The results table is organised to display the reported decision of each sample into the following columns:

- **Gene:** The gene of interest being investigated.
- **Treatment Group**: Selected treatment group that is being compared with a control group.
- **Expression Ratio**: Difference in gene expression between two groups (i.e., the difference between the selected treatment group and the control group).

If using **REST**:



Results

Gene	Treatment Group	Expression Ratio	Std. Error	95% C.I.	P Value	Result
TNFa	01_Pre-train...	18.505	16.096 - 21....	14.538 - 23....	0.1005	No difference
IL-1	01_Pre-train...	1.551	1.378 - 1.738	1.268 - 1.767	0.0745	No difference
HIF-1	01_Pre-train...	0.844	0.702 - 0.953	0.680 - 0.987	0.0435	Down
TNFa	02_Training	14.681	13.350 - 16....	12.126 - 17....	0.0980	No difference
IL-1	02_Training	1.529	1.440 - 1.630	1.371 - 1.679	0.1100	No difference
HIF-1	02_Training	1.022	0.947 - 1.095	0.917 - 1.130	0.4865	No difference
TNFa	03_Post-trai...	13.384	11.624 - 14....	11.133 - 16....	0.0980	No difference
IL-1	03_Post-trai...	0.199	0.188 - 0.211	0.182 - 0.213	0.0745	No difference
HIF-1	03_Post-trai...	1.351	0.839 - 1.862	0.812 - 1.991	0.3995	No difference

- **Standard Error**: Observation confidence intervals at 68% (equivalent to a single standard error) and 95% (2-standards) calculated using the bootstrapping technique. You must use biological replicates to achieve a meaningful analysis. Technical replicates are considered the same sample and will result in no variation being detected.
- **95% C.I.**: A statistical measure that provides a range within which the true population parameter is likely to fall. This interval indicates the uncertainty associated with the estimated expression level and provides a measure of how precise the estimate is. It is important to note that the 95% confidence interval does not provide information about individual samples or specific experimental conditions. Instead, it gives a range that quantifies the overall uncertainty associated with the estimation of gene expression levels based on the available data.
- **P Value**: The null hypothesis test P(H1) gives the probability that any observed difference between the two groups is due to chance. This is achieved using up to 10,000 random reallocations of control and treatment samples between the groups, and determines the number of times the expression values for the random assigned groups is greater than the sample mean. Expression values that have a P(H1) < 0.05 are statistically significant. The number of biological samples per group must be ≥ 3 to achieve analysis.
- **Result**: Text annotation of gene expression result described as Up, Down or No difference.

If using **ΔΔCt** and **ΔCt**:

- **SEM**: The standard error of the mean is an estimate of how far the sample mean is likely to deviate from the population mean.
- **P Value**: A two-tailed student t-test is applied between the control and treatment group.
- **Result**: Text annotation of gene expression result described as Up, Down or No difference.

Results

Gene	Treatment Group	Expression Ratio	SEM	P Value	Result
TNFa	01_Pre-training	14.017	1.046	0.0000	Up
IL-1	01_Pre-training	1.515	0.097	0.0000	Up
HIF-1	01_Pre-training	0.844	0.058	0.0046	Down
TNFa	02_Training	11.505	0.505	0.0000	Up
IL-1	02_Training	1.502	0.030	0.0000	Up
HIF-1	02_Training	1.018	0.043	0.6610	No difference
TNFa	03_Post-traini...	11.152	0.697	0.0000	Up
IL-1	03_Post-traini...	0.217	0.017	0.0000	Down
HIF-1	03_Post-traini...	1.378	0.268	0.1486	No difference

Error messages: if there are missing (e.g., missing reference gene C*q* values for a sample), the software will report the error along with which sample, group, or target is missing.

qPCR Project

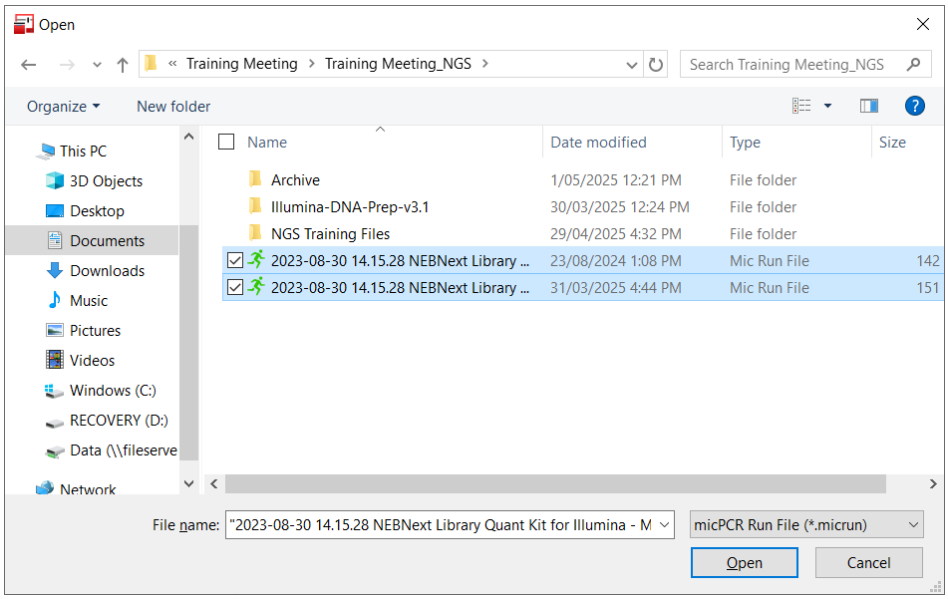
The **qPCR Project** option allows a user to combine multiple Mic qPCR runs for a single analysis. It is recommended to combine a maximum of 10 full runs into a project, to allow for simultaneous analysis of up to 480 samples.

To create a new project, select the qPCR Project application icon on the start screen.
This opens a new document for qPCR Project, with a Navigator Bar.

NOTE	Projects are only available for Cycling, Melt, Absolute Quantification, and Relative Quantification analysis. Myra+ Cycler qPCR runs using qPCR Setup are not currently supported with qPCR Project.

Runs

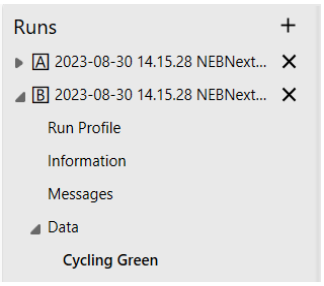
Select the run files you wish to combine and analyse by clicking on the + icon next to Runs.
You can select multiple files at the same time by ticking each run required in the Open file list.



Ensure run files are compatible, with the same run profiles and parameters.

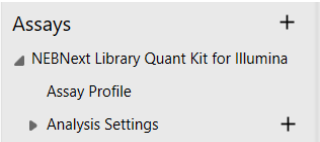
Each added run file will be labelled alphabetically (A, B, C...) in the order they are selected or added into the project. Samples from each run will also be labelled with the run prefix in the results table. At any time, you can remove a run file from the project by using the × button next to the run file name.

Information about each run file, including the Run Profile, Information, Messages, and raw Data, can be accessed by opening the run file tree in the Navigator Bar.



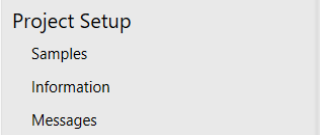
Assays

Each Assay used will appear under the Assay section in the Navigator Bar.
You can modify Assay settings by opening the tree further.



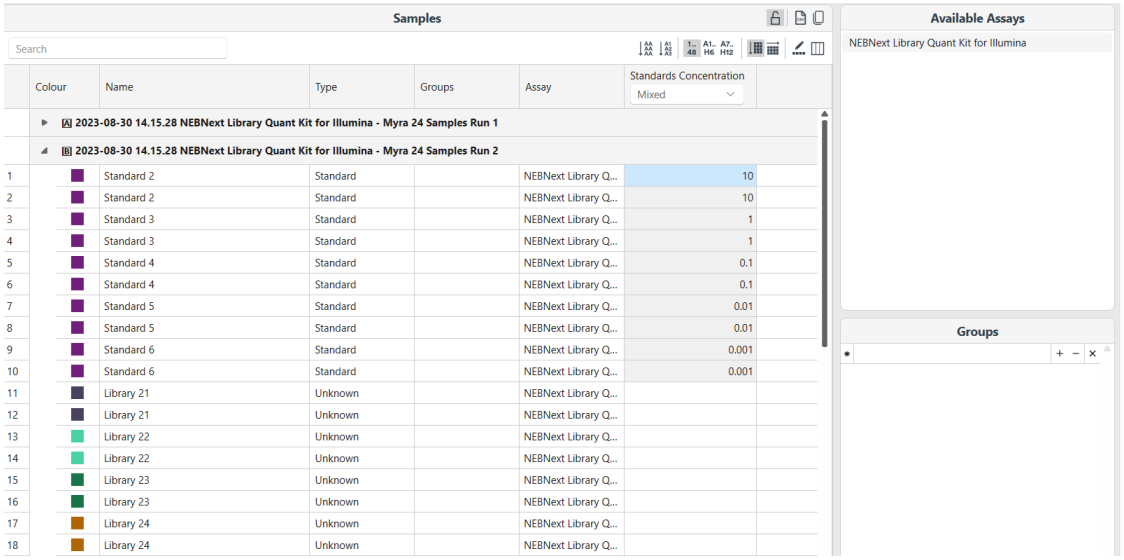
Project Setup

Samples can be edited by using the Samples editor under the Project Setup heading in the Navigator Bar.
Samples are displayed for each run and are listed in the order that the run was selected at the start.



The run name is displayed at the top of the sample list and each run list can be collapsed or expanded using the small triangle icon to the left of the run name.

Well-number styles are retained until you select the format in the project sample editor where all runs are reset to that format. The selected button in the sample editor will be set to the option used by the first run, so if you want to change all runs to match, you must select another option then change back to the one required.

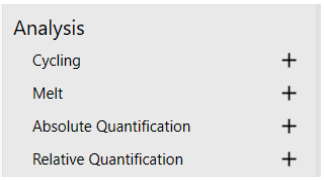


Importantly, the Samples editor toolbar is retained from a qPCR Run. For more information on how to use each of these options, refer to Samples Editor under qPCR Run - Mic Option.

Enter any **Information** about the project in the field provided.

All operations during the analysis of the project will be recorded and displayed in the **Messages** section.

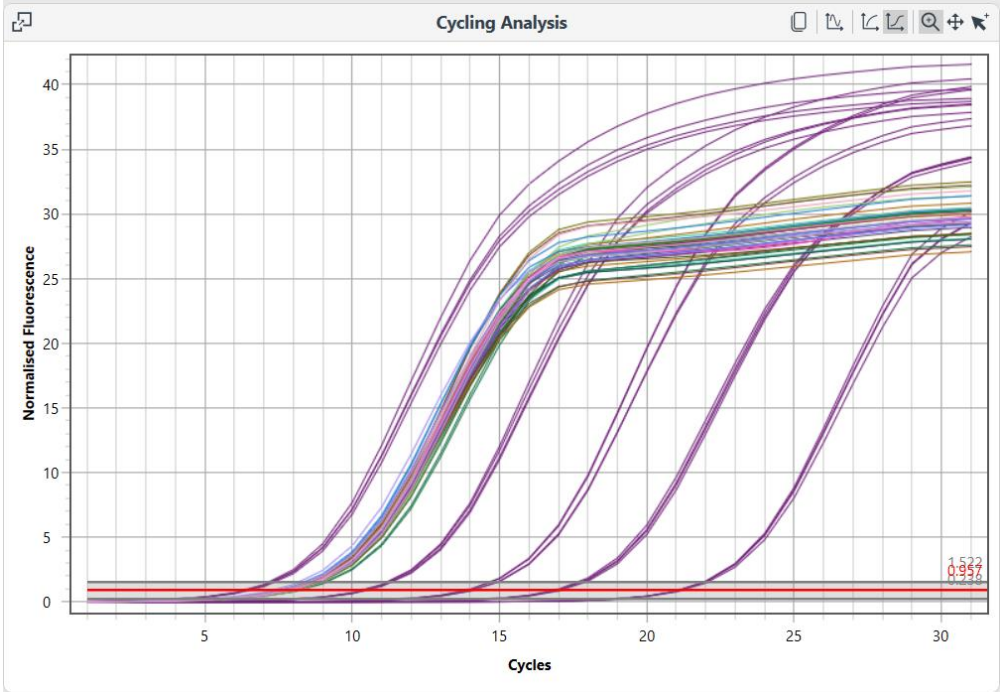
Analysis



Additional **Analysis** can be created at any time using the + icon next to each analysis type.

To start a new analysis, select the + icon next to analysis type required.
Choose the assay-specific target you would like to analyse.
Targets are auto populated in the order that Assays are added to the project file.
If an assay contains data for a target that is unnamed, it will be called "Non-Assay" for clarification.

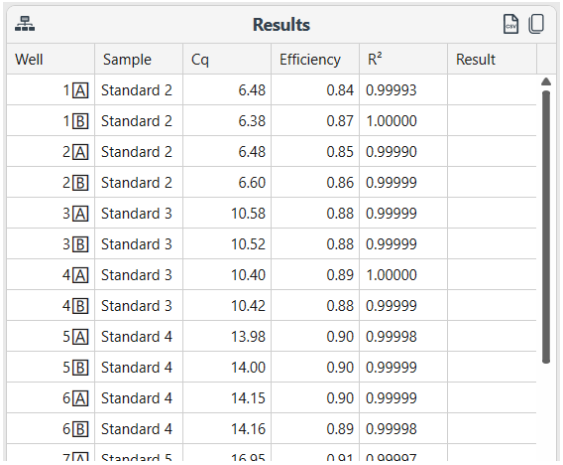
All samples from different runs are displayed on a single graph for a project.



Analysis options are the same across **qPCR Runs** and **qPCR Projects**. For detailed information on each analysis, refer to the respective sections under Analysis in qPCR Run.

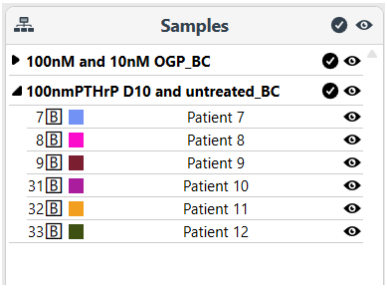
The Results table is also similar, however, in a **qPCR Project**, individual samples are reported with a Run file order postfix following the well number (e.g., 2 A).

The default view for the Samples Selector is divided into runs, with the run name displayed above the list of samples. These can be expanded or collapsed using the triangle icon.



Well	Sample	Cq	Efficiency	R^2	Result
1 A	Standard 2	6.48	0.84	0.99993	
1 B	Standard 2	6.38	0.87	1.00000	
2 A	Standard 2	6.48	0.85	0.99990	
2 B	Standard 2	6.60	0.86	0.99999	
3 A	Standard 3	10.58	0.88	0.99999	
3 B	Standard 3	10.52	0.88	0.99999	
4 A	Standard 3	10.40	0.89	1.00000	
4 B	Standard 3	10.42	0.88	0.99999	
5 A	Standard 4	13.98	0.90	0.99998	
5 B	Standard 4	14.00	0.90	0.99999	
6 A	Standard 4	14.15	0.90	0.99999	
6 B	Standard 4	14.16	0.89	0.99998	
7 A	Standard 5	16.95	0.91	0.99997	

The Samples Selector can also be used to show samples in order of Source Run, Assays, Sample Groups, Sample Name or None; selecting None will display in well order. Groups can also be all expanded or all collapsed.



To correct for variations in signal amplitude for amplified samples between runs and instruments, the software uses an Amplitude Correction algorithm. To ensure amplitude correction is applied during cycling analysis you must ensure that the same sample is added between different runs that will generate a signal with an observable amplitude.

The sample, called an Amplitude Corrector, can be an Unknown, Standard or Positive Control. NTC, NRT and Negative Controls should not be used. Ensure the same name in the Sample editor is used between the different runs. Multiple samples can be used, but a link must exist between each of the different runs being analysed as part of a project. For example: Sample 1 could be used between runs A and B, and Sample 95 could be used between runs B and C.

Amplitude correction works by using the First Derivative Maximum to determine a scaling factor between the different runs using the amplitude corrector samples. The scaling factor is then applied to all samples to correct for amplitude differences observed between runs or instruments.

Reports

Reports can be generated for each project by using the + icon next to the Reports heading in the Navigator Bar.

Normalisation, Pooling, Transfer

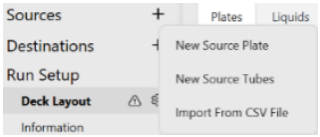
Normalisation, Pooling, Transfer

This application can be used for simple transfers of liquid from one source to another, normalisation, or dilution of samples to a set concentration, or pooling several samples into a single destination.

It is also possible to combine these features, such as for applications that require a pooling step with normalisation.

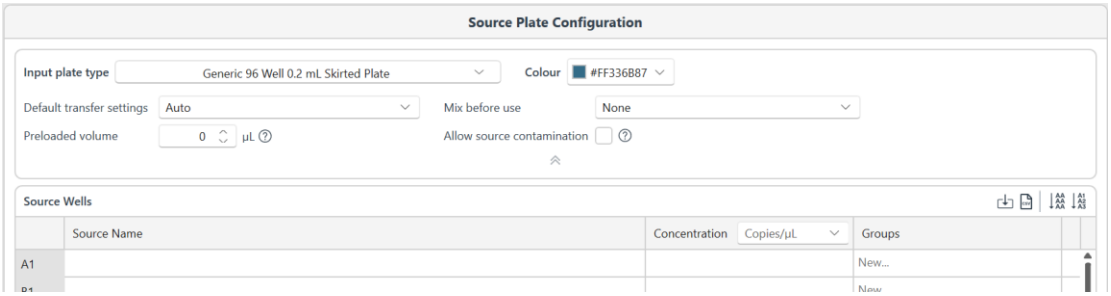
Sources

Add the input samples to transfer from by clicking on + button to the right of Sources.

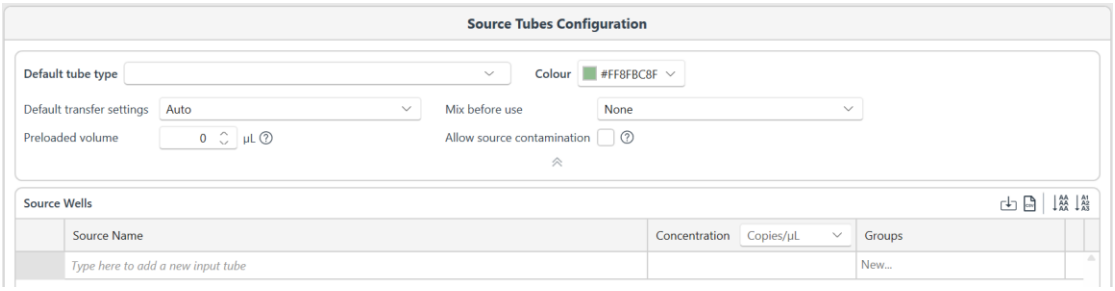


There are three options available to input Sources:

- **New Source Plate**: Choose the Input plate type from the Plate Library in the drop-down menu.



New Source Tubes: Choose a Default tube type from the options provided from the Select Tube window.



- **Import from CSV File:** Refer to Importing Data.

Give each source a name by typing in the space provided in the Navigator Bar. Multiple Sources can be added, but all source and destination items must fit onto the deck for the run to proceed.

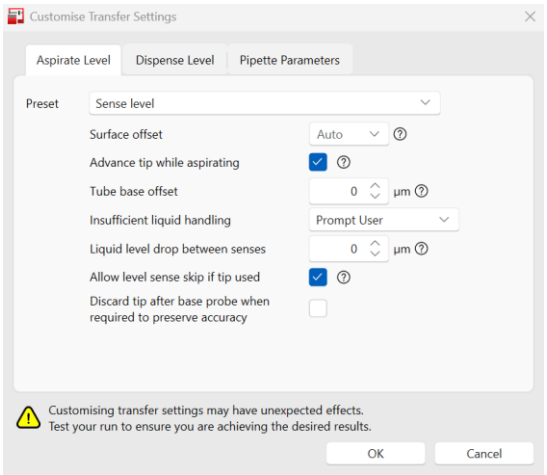
The input section can be expanded to display source configuration options:

- **Colour:** Used to display the source wells in the Deck Layout for improved visual tracking.
- **Preloaded Volume:** Indicates the volume already present in the source wells. If volumes vary, enter the *minimum* volume known to be present in all used wells. Used for optimising tip usage and determining appropriate mixing logic.
- **Allow Source Contamination:** When this option is selected, transfers from sources will reuse the tip regardless of their usage history, which could result in contamination of sources.
- **Mix Before Use:** Enables mixing of source wells prior to first use. Options include:
    - **None**: No mixing performed.
    - **Auto**: Uses optimum mixing type for the volume and tube type to be mixed.
    - **Auto without vortexing**: Same as Auto but excludes vortex mixing.
    - **Vortex**: High-speed circular pipette movements with synchronised Z-axis motion; ideal for rapid and consistent mixing.
    - **Advanced**: Thorough mixing using synchronised pipette and Z-axis motion at multiple heights.
    - **Simple**: Basic aspirate/dispense cycles at top and bottom of well; suitable for small volumes.
    - **Quick**: A shortened version of Simple; used for light mixing of small volumes.
    - **Custom**: User-defined parameters for fully tailored mixing control.
- **Default Transfer Settings:** Transfer settings to apply for the transfer of sources to their destinations. Options include:
    - **Auto**: Uses MyraSense to optimise pipetting based on liquid type and volume.
    - **Default**: Applies standard pipetting and transfer parameters.
    - **Viscous**: Slows pipetting to improve accuracy with viscous liquids.
    - **Bubbly**: Reduces level sensing frequency to prevent foam and bubbles and increases immersion depth to avoid bubbles that may be present in bubbly sources.
    - **Non-contact**: Uses non-contact dispensing to minimise contamination and avoid tip contact.
    - **Ethanol**: Reduces the maximum volume to prevent ethanol wicking up into the filter.

Default Transfer Settings provide full flexibility to optimise pipetting behaviour for different reagents and labware. Parameters are grouped into three categories: **Aspirate Level**, **Dispense Level**, and **Pipette Parameters**. These should be configured carefully, as they directly influence tip positioning, accuracy, and liquid handling performance.

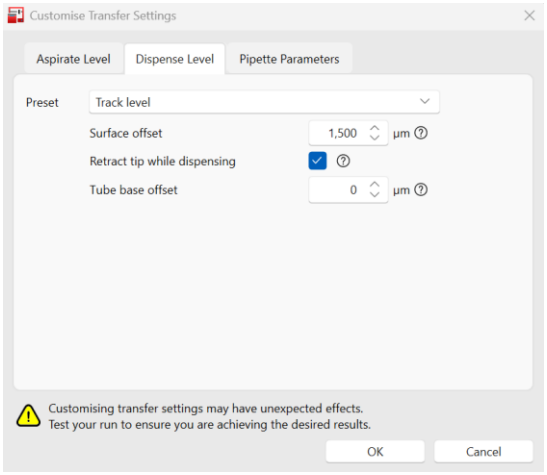
Aspirate Level defines how the pipette tip behaves during aspiration. It has the following parameters available:

- **Preset:** Predefined configurations such as Sense level or Track level for common use cases.
- **Surface Offset:** Defines the vertical offset (in µm) from the sensed liquid surface where aspiration begins. Can be set to Auto or a manual value.
- **Advance Tip While Aspirating:** When enabled, the tip gradually moves deeper during aspiration to track the liquid level in the tube
- **Tube Base Offset:** Offset (in µm) from the bottom of the tube to avoid contact. This is the lowest position in the tube that the tip will be placed during the operation.



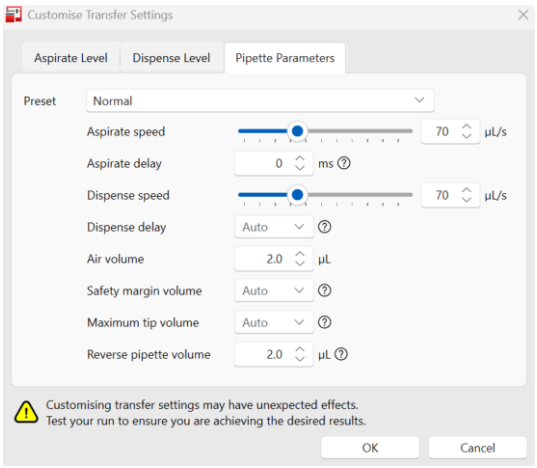
- **Insufficient Liquid Handling:** Defines the behaviour if insufficient liquid is detected. Options include Prompt User, Skip Operation or Retry.
- **Liquid Level Drop Between Senses:** Defines a threshold (in µL) for acceptable drop in detected liquid volume between senses before triggering a warning or action.
- **Allow Level Sense Skip if Tip Used:** When enabled, skips level sensing if the tip is not new, saving time in multi-dispense operations.
- **Discard Tip After Base Probe:** Ensures tip is discarded after probing the base, used to maintain accuracy where precision is critical.

Dispense Level defines tip positioning and motion during dispensing. It has the following parameters available:



- **Preset**: Preset options such as Track level for dynamically following liquid level.
- **Surface Offset**: Distance (in µm) above the sensed liquid surface where dispensing begins. Larger offsets help avoid splashing or overpenetration.
- **Retract Tip While Dispensing**: When enabled, the tip slowly retracts during the dispense action, ideal for avoiding liquid accumulation or bubbles.
- **Tube Base Offset**: Distance (in µm) from the base of the tube at which the dispensing should stop. Helps avoid disturbing settled beads or pelletised material.
- **Maximum Distance Below Top**: Limits how far the tip is allowed to descend below the top edge of the tube.

Pipette Parameters controls flow rate, timing, and volumes. It has the following parameters available:



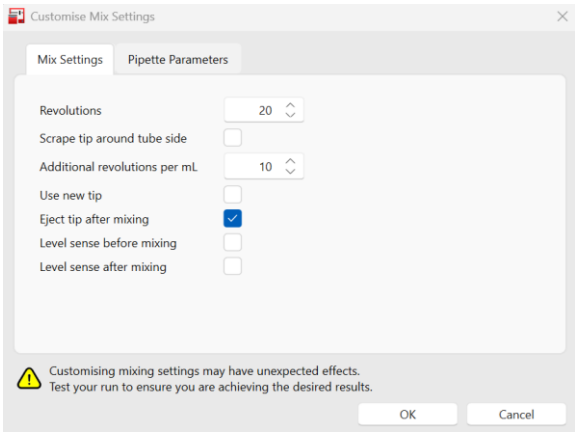
- **Preset:** Default configurations such as Normal to restore standard pipetting behaviour.
- **Aspirate Speed:** Speed (in µL/s) at which the pipette draws up liquid. Lower speeds improve accuracy for viscous or volatile liquids.
- **Aspirate Delay:** Pause (in ms) after aspiration before the tip is moved. Useful for allowing liquid to fully enter the tip.
- **Dispense Speed:** Speed (in µL/s) at which liquid is dispensed.
- **Dispense Delay:** Delay before tip withdrawal post-dispense. Auto uses intelligent settings based on volume and viscosity.
- **Air Volume:** Volume of air gap (in µL) aspirated above the liquid to prevent dripping or splashing.
- **Safety Margin Volume:** Additional buffer volume aspirated to ensure sufficient liquid is available, especially useful in back-to-back dispenses.
- **Maximum Tip Volume:** Defines the maximum volume allowed per tip. Auto selects the value based on the pipette type in use.
- **Reverse Pipette Volume:** Volume re-aspirated after dispensing to clear the tip of residual liquid, typically used for viscous reagents.

ATTENTION

Customising transfer settings may have unexpected effects. It is strongly recommended to test your run to ensure the desired results are achieved.

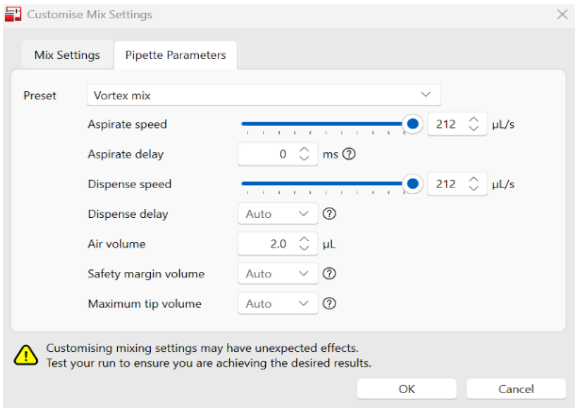
Mix Before Use provide full flexibility to optimise mixing and pipetting behaviour for different reagents and labware. Parameters are grouped into two categories: **Mix Settings** and **Pipette Parameters**. These should be configured carefully, as they directly influence tip positioning, accuracy, and liquid handling performance.

Mix Settings defines how mixing is executed, including the number of mixing cycles, tip handling, and level sensing behaviour. It has the following parameters available:



- **Revolutions or Mix Cycles**: Sets the base number of aspirate-dispense cycles (mixing strokes) performed during mixing. Higher numbers result in more thorough mixing but increase run time.
- **Scrape Tip Around Tube Side (Vortex only)**: When enabled, causes the tip to move in contact with the side wall of the tube or well during mixing. Useful for sticky or high-viscosity reagents to dislodge material from the walls.
- **Additional Revolutions per mL (Vortex only)**: Adds extra mix cycles for each millilitre of liquid being mixed. Helps scale mixing effort with volume, ensuring larger volumes are mixed adequately.
- **Use New Tip**: When selected, forces a fresh tip to be used specifically for the mixing step, even if the same tip was used for aspiration or dispensing. Minimises cross-contamination risk.
- **Eject Tip After Mixing**: When enabled, automatically discards the tip following the mix operation. Recommended when mixing sensitive reagents or when contamination control is critical.
- **Level Sense Before Mixing**: Performs a liquid level sense prior to mixing. Useful when variable volumes may be present and tip position must be accurate before starting the mix.
- **Level Sense After Mixing**: Performs a level sense following mixing. Can be used for downstream volume verification or to prepare for subsequent pipetting operations requiring precise level information.

Pipette Parameters controls flow rate, timing, and volumes. It has the following parameters available:



- **Preset:** Default configurations for mix type selected.
- **Aspirate Speed:** Speed (in µL/s) at which the pipette draws up liquid. Lower speeds improve accuracy for viscous or volatile liquids.
- **Aspirate Delay:** Pause (in ms) after aspiration before the tip is moved. Useful for allowing liquid to fully enter the tip.
- **Dispense Speed:** Speed (in µL/s) at which liquid is dispensed.
- **Dispense Delay:** Delay before tip withdrawal post-dispense. Auto uses intelligent settings based on volume and viscosity.
- **Air Volume:** Volume of air gap (in µL) aspirated above the liquid to prevent dripping or splashing.

- **Safety Margin Volume:** Additional buffer volume aspirated to ensure sufficient liquid is available, especially useful in back-to-back dispenses.
- **Maximum Tip Volume:** Defines the maximum volume allowed per tip. Auto selects the value based on the pipette type in use.

ATTENTION

Customising mixing settings may have unexpected effects. It is strongly recommended to test your run to ensure the desired results are achieved.

Enter the source names.
Use the Fill down or Auto fill options to simplify annotation, available on the top right of the toolbar.

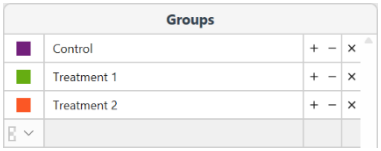
The same toolbar section also offers options to import samples. Refer to Importing Data for more information on how to import samples.

For plates you can use the Well Filter to highlight specific wells only.

For tube sources, add additional tubes by typing in new rows of the source wells.

Provide a concentration for normalisation/dilution. (optional)

Allocate one or more groups to your samples, begin by entering the required group names in the Groups table, then assign groups to your samples. (optional)
Use sample groups to allow you to calculate statistics for a collection of samples that are not replicates (e.g., Treatment or Control). You will be required to create and allocate groups when using Relative Quantification analysis.



To allocate a group, click on the Groups column cell of the relevant sample and select the desired group from the drop-down list. If you wish to assign a group to multiple samples, you can highlight multiple cells in either the Groups or Name column. To select non-adjacent samples, hold Ctrl while clicking. Select the + button next to the group name under the Groups table on the bottom left corner to allocate the desired group/s.

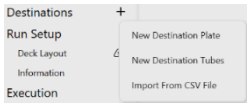
Alternatively, the name of the group can be typed directly into the Groups column. If the group exists, a list of options beginning with the first set of characters will appear. If the group does not exist, it will be captured in the group list after you have completed entering the name and exited out of the cell.

You can remove groups assigned to Samples by using the Delete button on your keyboard or the × button next to the group name in the Groups column of the Samples Editor. Alternatively, you can use the – button next to the group name in the Groups table.

The colour for each group can be edited in the Groups table and can be used to display on the reaction plate on the Deck Layout.

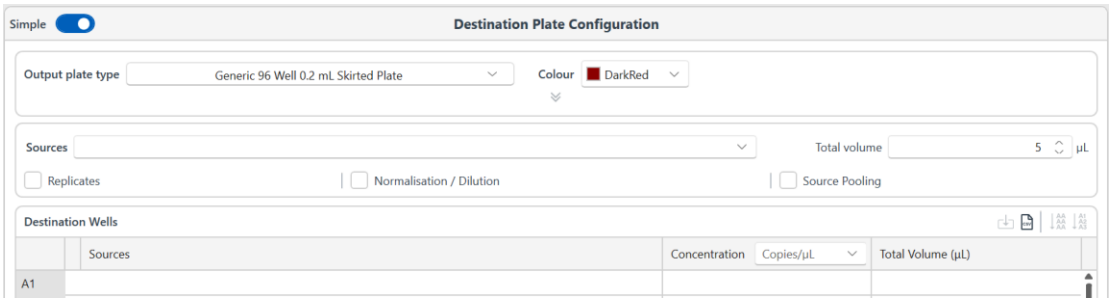
Destinations

Add the destination to transfer to by clicking on + button to the right of Destinations.

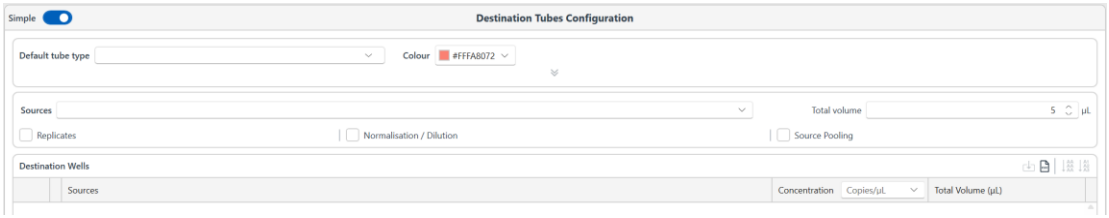


There are three options available outputs:

- **New Destination Plate**: Choose the Output plate type from the Plate Library in the drop-down menu.

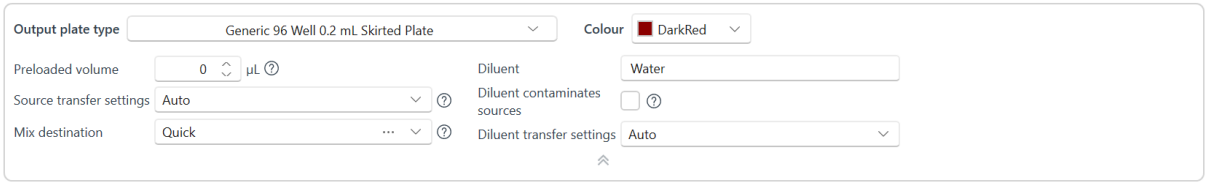


- **New Destination Tubes**: Choose the Default tube type from the Select Tube window.



- **Import from CSV File**: refer to Importing Data.

The input section can be expanded to display source configuration options:



- **Colour:** Used to display destination wells in the Deck Layout for improved visual tracking.
- **Preloaded Volume:** Indicates the volume already present in the output wells. This is used to optimise tip usage and mixing logic. If volumes vary across wells, input the *minimum* volume.
- **Source Transfer Settings:** Transfer settings to apply for transfer of sources to their destinations. Options include:
    - **Auto**: Uses MyraSense to optimise pipetting based on liquid type and volume.
    - **Default**: Applies standard pipetting and transfer parameters.
    - **Viscous**: Slows pipetting to improve accuracy with viscous liquids.
    - **Bubbly**: Reduces level sensing frequency to prevent foam and bubbles and increases immersion depth to avoid bubbles that may be present in bubbly sources.
    - **Non-contact**: Uses non-contact dispensing to minimise contamination and avoid tip contact.
    - **Ethanol**: Reduces the maximum volume to prevent ethanol wicking up into the filter.

  Aspirate level, dispense level and pipette parameters are fully customisable. More information on the available configurations can be found in **Sources**.
- **Mix Destination:** Defines how the destination well is mixed after transfers. Options include:
    - **None**: No mixing performed.
    - **Auto**: Uses optimum mixing type for the volume and tube type to be mixed.
    - **Auto without vortexing**: Same as Auto but excludes vortex mixing.

- **Vortex**: High-speed circular pipette movements with synchronised Z-axis motion; ideal for rapid and consistent mixing.
- **Advanced**: Thorough mixing using synchronised pipette and Z-axis motion at multiple heights.
- **Simple**: Basic aspirate/dispense cycles at top and bottom of well; suitable for small volumes.
- **Quick**: A shortened version of Simple; used for light mixing of small volumes.
- **Custom**: User-defined parameters for fully tailored mixing control.

Mix settings and pipette parameters are fully customisable. More information on the available configurations can be found in **Sources**.

- **Diluent**: Specifies the name of the diluent to be added to the destination well.
- **Diluent Contaminates Sources:** When enabled, this ensures that any source transferred into a well containing diluent will *not* reuse tips, preventing potential cross-contamination.
- **Diluent Transfer Settings:** Specifies transfer parameters for diluent addition. All options available under Source Transfer Settings are also applicable here.

Select the Sources being used from the drop-down menu.
You can choose any combination of Sources, including items and groups, or use the Select: All option to automatically choose all Source items.

Destination Wells will auto-populate based on information input.

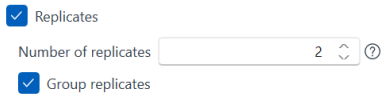
Enter the total volume required for the Destination Wells.
Workbench will calculate the source volume requirements.

For all the above modes, you can toggle between Simple and Custom by using the switch on the top left.
Simple mode offers Workbench complete control over setting up Replicates, Normalisation / Dilution and Source Pooling, thereby simplifying these processes and minimising error rates in the process.

Refer to the sections below for an understanding on how to use these functions. Alternatively, to learn more about Custom Mode, refer to Custom Mode Setup.

Replicates

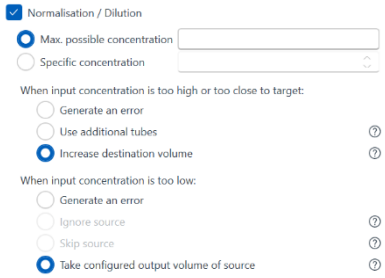
You can create replicates by ticking the Replicates checkbox.
Define the number of replicates to be made for each sample.



You can also choose to Group replicates to create replicates side-by-side in a destination plate. If left un-checked, replicates will only be created once the first batch of sources have been added.

Normalisation / Dilution

You can normalise samples to a set concentration by ticking the Normalisation/Dilution checkbox.
A normalisation step may be used to adjust source sample concentrations to a single (normalised) concentration.



You may choose to transfer either the Max. possible concentration, or you may enter a Specific concentration as the normalisation value.

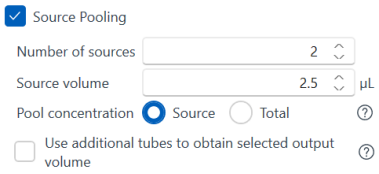
The maximum possible concentration to normalise samples to will be the lowest concentration of source that will be used (as sources may be diluted to reduce their concentration, but may not be concentrated).

Similarly, any specified concentration must be lower than the lowest concentration of source being used to construct that concentration in the destination.

You can also now specify how samples should be handled when an input concentration is too high or too close to the target, opting to Generate an error, Use additional tubes or Increase destination volume. When an input concentration is too low, you can opt to Generate an error, Ignore source, Skip source or Take configured output volume of source.

Source Pooling

You can pool multiple sources into a single destination by ticking the Source Pooling checkbox.



Choose the Number of sources to be pooled for each destination.
A single destination will be created if the number of sources to be pooled is the total number of sources that have been selected, otherwise multiple destinations will be used to complete the pools across all selected sources.

You may also specify Source volume requirements. Doing so will update the Total volume for the destination wells.

Select whether the Pool concentration should be using Source concentration or Total concentration.
This selection will also be reflected in the concentration of each output pool.

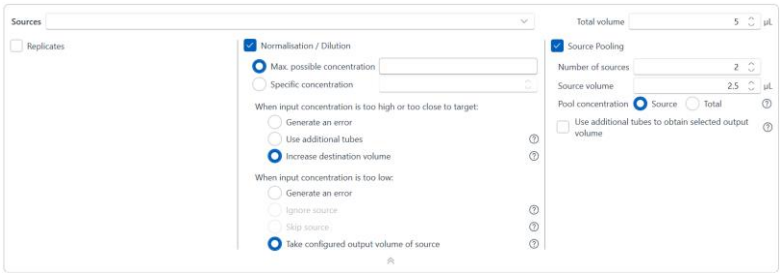
Tick if you would like to Use additional tubes to obtain selected output volume.
If the requested volume of the destination well is less than the minimum volume required to construct the pool successfully, this option will construct the pool in an additional tube. An aliquot will be taken from this tube to construct the destination with the requested volume.

Normalisation / Dilution and Source Pooling Simultaneously

There is an option for Normalisation / Dilution to be performed at the same time as Source Pooling.

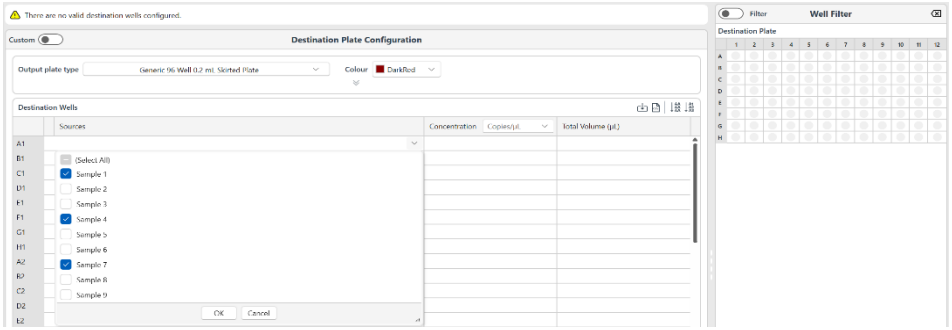
This option can be useful for applications such as next generation sequencing, where prepared libraries can have different concentrations that need to be normalised to the required starting concentration of the sequencing system, followed by pooling of normalised libraries ready to be denatured and diluted to the final loading concentration of the sequencing system. For more information on how to set this up, refer to **Workbench Application Note 4 – NGS Library Preparation: Simultaneous Normalisation and Pooling** found under the Quick Links section on the Start Page.

You can normalise and pool multiple sources simultaneous by ticking both Normalisation / Dilution and Source Pooling checkboxes and follow the prompts as above.



Custom Mode Setup

Toggling Custom Setup mode will allow you to define what sources are pooled together, along with their custom defined output concentration and volume.



Use the drop-down menu in each of the Sources to select the number of samples to pool.
Unlike Simple mode, in which the software configures which samples to pool numerically, you can choose any order you wish.

Define the final Concentration in each Destination Well. (optional)
The displayed pool concentration will always be the concentration of each source.

Define the Total Volume (µL) per Destination Well.
Equal volumes will automatically be taken for each source defined if the final concentration has been left blank.

Run Setup

Deck Layout

Refer to the **Deck Layout** section above for more information on how to setup your deck.

Information (Additional Settings)

Refer to the **Information** section above for more information on how to access **Advanced Settings** and **Checklist Settings**.

The run can now be commenced.

MyraScript Run

MyraScript Run is a Python-based run that can be used to develop fully customisable protocols. Full control of liquid transfer parameters, including pipetting speeds, delays, level sensing, tracking, and mixing can be programmed by the user. Python scripting is used in combination with a MyraScript API to configure plasticware and reagents on Deck Layout, and compile a list of operations for execution.

For more details, including examples, please refer to the MyraScript API documentation found under the **Help Icon** in the **Start Page** toolbar. Visit https://biomolecularsystems.com/myra-script-library for access to an extensive collection of completed script runs for various protocols including serial dilutions, normalisation, cell cultures, next-generation sequencing (NGS) applications and magnetic bead clean-up.

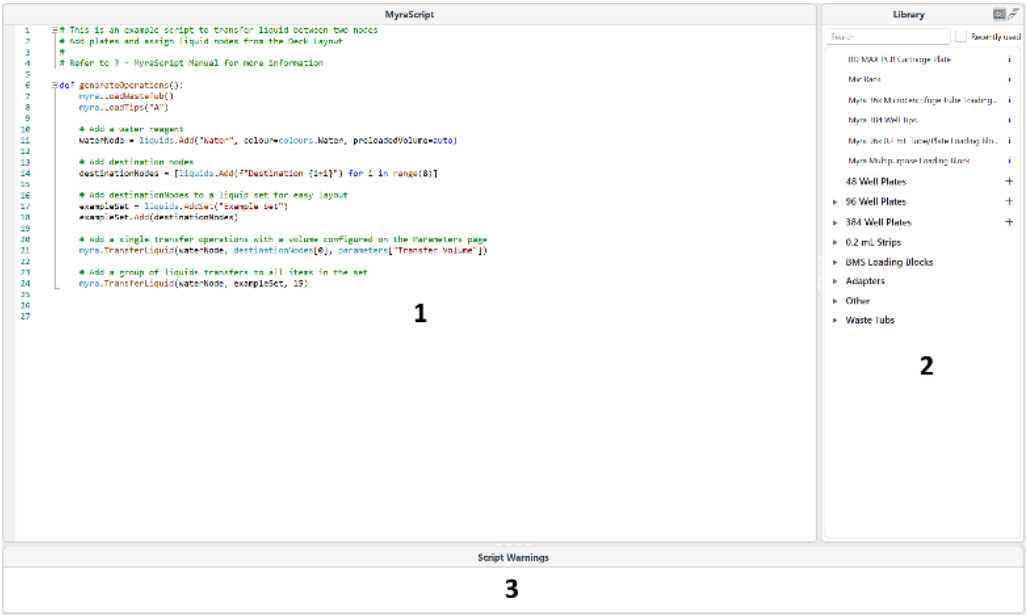
For a guide on a sample MyraScript Run, including an example of an NGS library prep, refer to **Workbench Application Note 4: NGS Library Preparation: Simultaneous**, found under the Quick Links section on the Start Page.

MyraScript

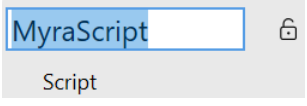
Script Editor

The default screen contains three windows:

1. **MyraScript**: This panel displays the script, written in Python 3, which defines the deck layout and run operations. A sample MyraScript is provided by default to illustrate the script structure and logic.
2. **Library**: Contains a list of all available deck ware, including plates and tubes. Items can be dragged and dropped into position during script creation.
3. **Script Warnings**: Displays any syntax errors or other issues identified in the script. Each error includes a line number for reference. Corresponding warning icons also appear in the navigator panel.



The **MyraScript** heading in the Navigator Bar can be renamed by double clicking on the title. Once the script has been fully developed, it can be locked to prevent any further modifications using the lock icon.



For more information on how to configure and write a MyraScript Run, please refer to the **MyraScript Manual**, found under the **Help Icon** on the tool bar or under Quick Link on the Start Page.

Configuration

The **Configuration** section provides a user-friendly interface for defining parameters used within the **MyraScript**. It is designed to make scripting more accessible by allowing commonly adjusted settings, such as pipetting volumes, transfer types, mixing options, incubation settings and source configurations, to be modified without editing the script directly. This eliminates the need to navigate the Script window for routine adjustments and therefore minimising the risk of altering script logic.

Multiple parameter pages can be created using the + button to logically group related settings (e.g., sample editor, pipette settings, reagent configuration etc), further streamlining the scripting experience.

Import from CSV is also supported to provide integration with external systems; refer to Importing Data on how to import a CSV file.

Run Setup

Deck Layout

Refer to the Deck Layout section above for more information on how to setup your deck.

The run can now be commenced. If you wish to define additional settings, you can do so in Information.

Information (Checklist Settings)

Refer to the Information section above for more information on how to access Checklist Settings.

Appendix A – Running BMS Workbench on MacIntosh Operating Systems

Workbench is currently not compatible with Macintosh systems. We recommend using a virtual machine program, ideally Parallels®, VMware® Fusion or Oracle® VM VirtualBox to run Workbench on a Mac without rebooting. This method will allow you to run macOS and Windows applications concurrently.

To dual-boot between macOS and Windows, use Apple's boot camp (https://support.apple.com/en-au/HT201468). This method will only allow your computer to switch between booting up macOS or Windows partitions.

Appendix B – User Permissions

The Workbench system offers three levels of user permissions to allow different users varied levels of access when it comes to the control of the instrument and software features. The three Windows User Group permission levels are:

- BMS Administrator
- BMS Analyst
- BMS Technician

When the Workbench software starts, it will discover the Windows User Groups of the logged in user and attempt to match the user's group level to the permission levels built into the software. It will allocate the highest priority user permission level with a name that matches one of the user's Windows groups. The software will by-pass the permission system if none of the user groups are found on the computer.

User Group Configuration

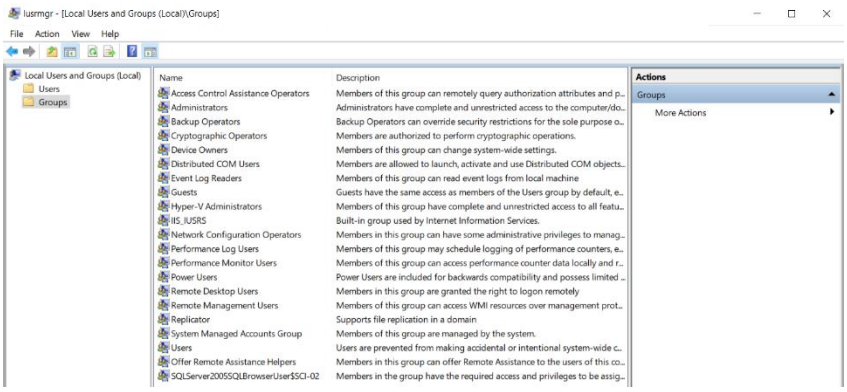
Configuring the permissions for a user is simply a matter of using the built-in Windows tools for creating groups and allocating a user to groups. Generally, there are two types of Windows groups.

- Local Windows groups are configured on a single computer. A user with local administrator privileges can add, remove, and/or manipulate groups of this type. The instructions below detail how to configure local groups.
- Non-local Windows groups are generally shared across multiple computers in an organisation, such as Windows Domain groups. If you wish to use non-local groups, you will need to contact your local IT support for assistance.

Both local and non-local Windows groups can be used for allocating user permissions.

Configuring local Windows User Groups

The following steps will need to be performed as an administrator on the local machine. The configuration described below will affect only the local computer. This procedure will need to be repeated on any other computer the user will need to access. If the non-local user is being added or removed from a local group, ensure that the computer has network access to the domain controller.

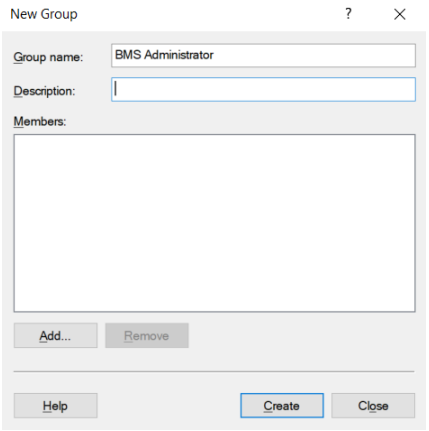


If not already open, open the 'Local Users and Groups' tool
Press Windows+R to open Run, enter lusrmgr.msc in the blank box and click on OK.

Click on the Groups folder on the left-hand pane.

Click on the More Actions menu item at the top of the tool then select New Group from the drop-down menu.
A New Group dialog will appear in the Group Name field.

Enter the Group name of the permission level.
The name should be one of BMS Analyst, BMS Technician or BMS Administrator.

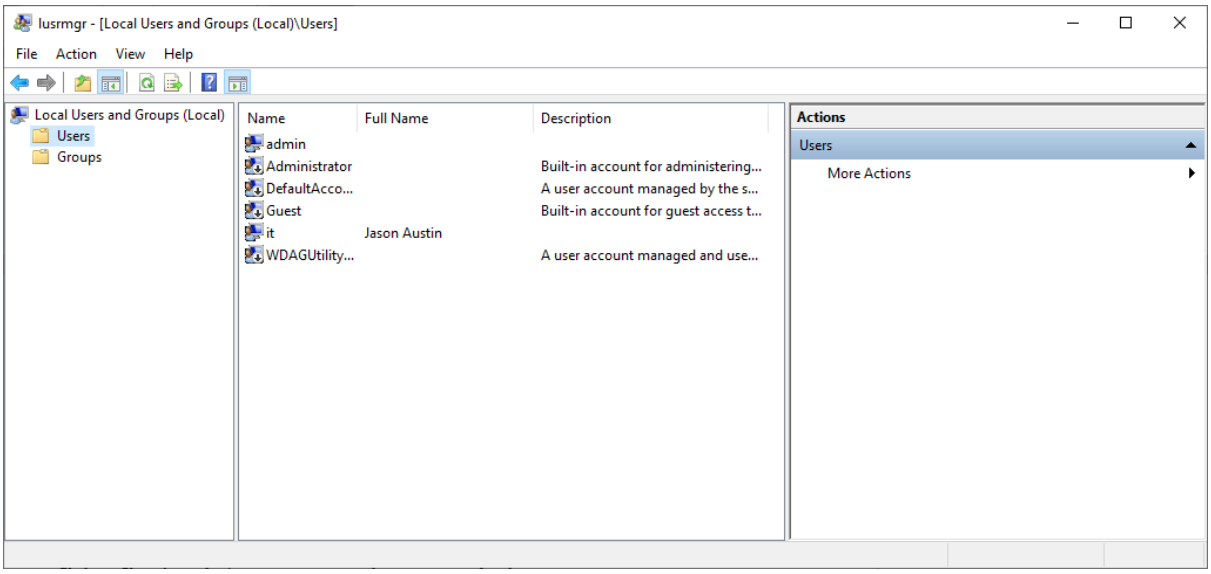


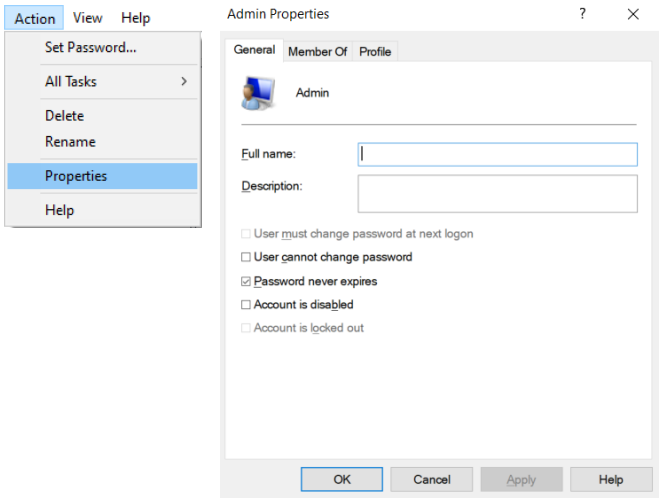
Once you have entered the group name, click *Create*.
Click on *Close* if you do not want to create another permission level group. The user may need to logout for the group change to take effect.

To add a user to a permission level group, open the 'Local Users and Groups' tool, click on the *Users* folder on the left-hand pane.

Click on the user of interest in the middle pane

Click on the Action menu item at the top of the tool then select Properties from the drop-down menu.
A <user> Properties dialog will appear.





Click on the Member Of tab.

The list of the groups the user is a member of will appear in the Member Of: panel.

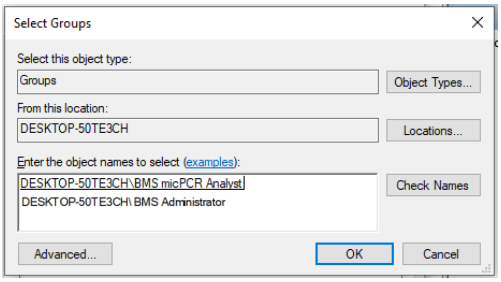
Click on the Add button.

Select Groups dialog will appear.

Type the name of the permission level group into the Enter the object name to select panel.

The name should be one of BMS Analyst, BMS Technician or BMS Administrator.

Click on the Check Names button.



The username should then re-appear with the computer or domain name prepended, **click on OK**. The Select Groups dialog will disappear.

The name of the group will now appear in the Member Of: panel, click on OK. The <user> Properties dialog will disappear.

Select Apply and then OK to exit.

The user may need to logout for the group change to take effect.

To remove a user from a permission level group, in the Member of: panel select the group to remove.

Click on the Remove button and click on OK.

The <user> Properties dialog will disappear. The user may need to logout for the group change to take effect.

To add a domain user to a permission level group, ensure the computer is connected to the domain control. Check with your IT department how this is best done.

Click on the Groups folder on the left-hand pane and select the group of interest in the middle pane.

Click on the Action menu item at the top of the tool then select Add to Group from the drop-down menu. A <group> Properties dialog will appear; click on the Add... button.

A Select Groups, Computers, Service Accounts or Groups dialog box will appear. Ensure the domain name appears in the From this location field. If the domain name is not correct click on the Locations... button to correct it.

Type the username into the Enter the object name to select panel.

Click on the Check Names button.

The username should then re-appear with the computer or domain name added. Click on OK. The Select Groups, Computers, Service Accounts or Groups dialog box will appear. The username will now appear in the Members: panel, click on **OK**. The <group> Properties dialog will disappear. The user may need to logout for the group change to take effect.

To remove a domain user from a permission level group, perform steps 1 to 5 in Add a domain user to a permission level group.

In the Members: panel select the group to remove.

Click on the Remove button then click on OK. The <group> Properties dialog will disappear. The user may need to logout for the group change to take effect.

User Levels and Permissions

The following are the BMS Technician permissions.

Creating files	Can create new runs from a template. Cannot create assays or run templates.
Saving files	Can save run files. Can excel export run files. Cannot save assays or run templates.
Opening files	Can open runs. Can add assays to runs before runs start.
Assays	Can add and remove assays to run before the run starts. Can only view the assay information page. Cannot edit. Cannot see the assay profile. Cannot see the assay analysis settings. Cannot save an assay. Cannot change the assay name in navigator.
Samples	Can view and edit the sample information before the run starts. Can edit the sample information at any time. Can import sample values from a text file before the run starts. Can export sample information to CSV file and copy to clipboard.
Run Control	Can start runs will no sample editor warnings. Can abort runs. Can recover runs.
Run Profile	Cannot view or edit the run profile.
Information	Can view and edit the information page.
Run data	Can view the raw run data page to see the raw fluorescence traces.
Analyses	Cannot create analyses. Cannot delete analyses. Can view existing analyses in files created/edited by analyst or administrator. Can view child analyses. Can view analysis settings. Cannot edit analysis settings. Cannot enable or disable samples.
Reports	Cannot create reports. Cannot delete reports.

	Can view existing reports in files created/edited by analyst or administrator. Cannot enable or disable visibility of report sections. Can export and print reports.
Instrument Properties	Can view instrument properties. Cannot edit instrument properties. Cannot upgrade firmware. Cannot run TVS.
Miscellaneous	Can create support package. Can view manual. Can discover instruments.

The following are the BMS Analyst permissions.

Creating files	Can create new runs from a template. Cannot create assays or run templates.
Saving files	Can save run files. Can excel export run files. Cannot save assays or run templates.
Opening files	Can add assays to runs before runs start.
Assays	Can add and remove assays before the run starts. Can only view the assay information page. Cannot edit. Can see the assay profile. Cannot edit the assay profile Can see the assay analysis settings. Cannot edit the assay analysis settings. Cannot save an assay. Cannot change the assay name in navigator.
Samples	Can view and edit the sample information before the run starts. Can edit the sample information at any time. Can import sample values from a text file before the run starts. Can export to CSV file and copy to clipboard.
Run Control	Can start runs will no sample editor warnings. Can abort runs. Can recover runs.
Run Profile	Can view the run profile. Cannot edit the run profile
Information	Can view and edit the information page.
Run data	Can view the raw run data page to see the raw fluorescence traces.
Analyses	Can create, view, and delete analyses. Can view child analyses. Can view and edit analysis settings. Can enable and disable samples.
Reports	Can create, view, and delete reports. Can enable and disable visibility of report sections. Can export and print reports.
Instrument Properties	Can view instrument properties. Cannot edit instrument properties. Cannot upgrade firmware. Cannot run TVS.
Miscellaneous	Can create support package. Can view manual. Can discover instruments.

The BMS Administrator will have access to all levels of control in the application software.

Appendix C – Laboratory Information Management System (LIMS) Settings

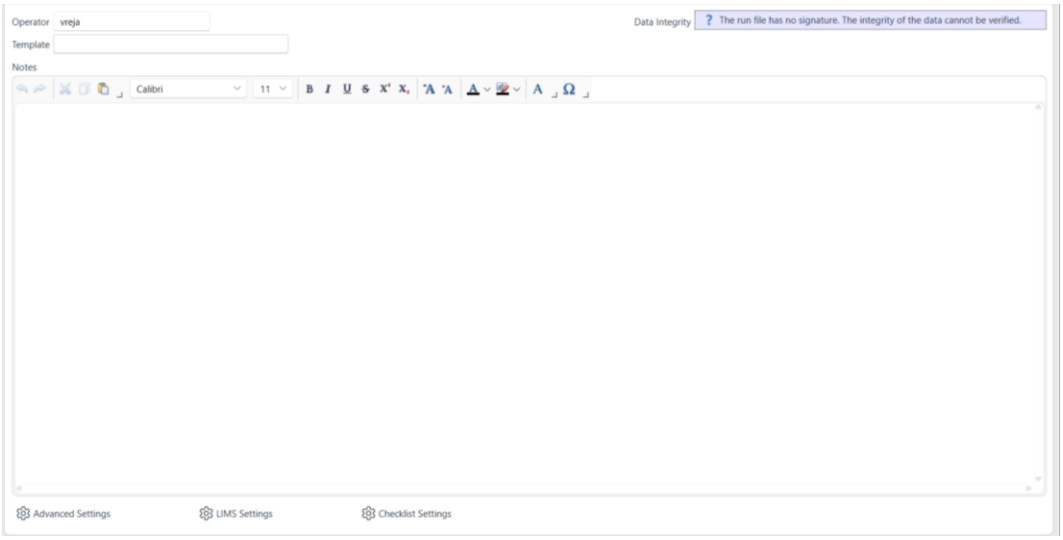
LIMS Import

It is possible to import sample and assay information from a file generated from a LIMS to Workbench for setting up a qPCR run. Workbench then controls the instrument to perform the experiment and required analysis. Run information can then be exported from Workbench and fed back to the LIMS, to archive completed run information.

Files imported into Workbench are required to be in either .csv,. tsv or .txt format. Once the file is imported into Workbench, a table will display all the fields in the title, where certain fields can be imported by linking the column from the file to a column in the Sample Editor (e.g., Well, Name, Type, Groups, Assay, Standards Concentration, Input DNA Concentration, Input RNA Concentration, RIN, and Lot Number).

LIMS Export

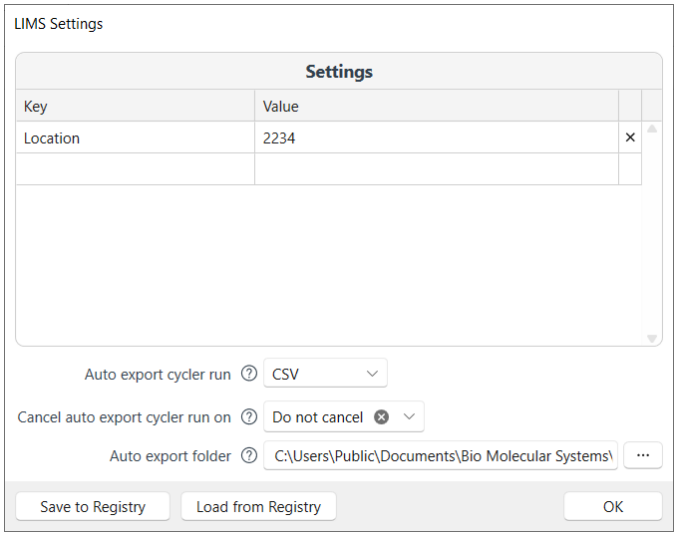
Results generated from a Mic can be automatically exported to a LIMS. When a Myra is utilised for reaction setup for a **qPCR Run** on a Mic, LIMS can be setup to export information on the Myra instrument. The Mic **qPCR Run** file will be exported in either .xlsx or .csv formats. The LIMS Settings option is found in the **Information** heading.



Each run file contains its own set of keys and value meta data that is editable.

Click on the LIMS Settings option under the Information heading. Enter the required Key labels and associated Value(s) in the table.

Any user defined key and value combinations will be stored in the run file and exported.



It is possible to automatically save the data from the qPCR run to a folder that is linked to a LIMS by clicking on the icon next to Auto save mi run folder and selecting the desired folder. The run data can be auto exported in either .csv or .xlsx format by selecting it in the drop-down menu for Auto save mic run.

There is also the option to have the auto export be cancelled if an error is detected in any auto generated analysis. If an error is detected a warning should be displayed allowing you to possible rectify or otherwise process the run file. This can be done by selecting one of the following options in the Cancel auto save mic run on menu: Analysis error, Optical Failure, Reportable Warning, and/or Run Failure.

There is also a common LIMS metadata registry which is stored on the computer.

- **Save to Registry** will overwrite the values in the CommonMetadata.xml file from the run.
- **Load from Registry** will overwrite the values in the run from the CommonMetadata.xml file.

When a new Workbench file is created, it will load the values from the common CommonMetadata.xml.

When a new Workbench file is created from a Template it will use the meta data values stored in the template. It will not load the values from the common CommonMetadata.xml.

Each document contains the following fixed worksheets:

- **General Information**: Information about the run, such as date and time, file storage, file status, operator, instrument name and serial number, firmware version and user defined values, run start and end times.
- **Run Profile**: Information on temperature control, cycling conditions, reaction volume and logical file)
- **Sample**: Information on the well, name, type, groups, assay, standards concentration, input DNA concentration, input RNA concentration, RIN, and Lot number.
- Assay: Information on the Assay name and type, target name, probe name, report type and channel(s) required for acquisition)

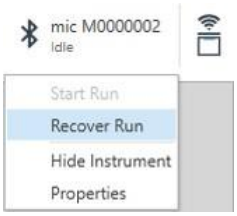
Each document will also produce the following non-fixed worksheets if data is present:

- **Raw data**: A separate worksheet for each channel on which cycling and melt data was acquired.
- **Analysis**: A separate worksheet for each analysis created.

Appendix D – Retrieving Data from a Disconnected Mic Device

The Mic will collect data during operation and transmit it to Workbench. To provide a failsafe against the loss of data, if the connection between a Mic and the Workbench software is disrupted, there are 3 possible ways to retrieve data:

1. **Resume connection before the run has finished**. Workbench will automatically update data to the current run status. When a Bluetooth® connection is in range, this will occur automatically.
2. **Resume the connection after the run has finished using the same computer that started the run**. Once the connection is re-established, click on the instrument icon in the top right-hand corner of the software and select the **Recover Run option** from the drop-down menu. This will retrieve data from the previous run.



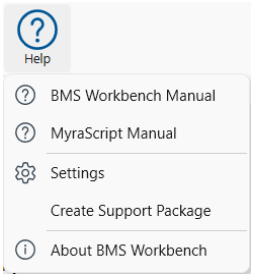
3. **Resume the connection after the run has finished using a different computer to the one that started the run**. Once connected, click on the instrument icon in the top right-hand corner of the software and select the **Reconstruct Run** option from the drop-down menu. This will retrieve data from the previous run.

Note that if there is data stored on a device there will be a warning message displayed to avoid the loss of data. Starting a new run will erase any stored data.

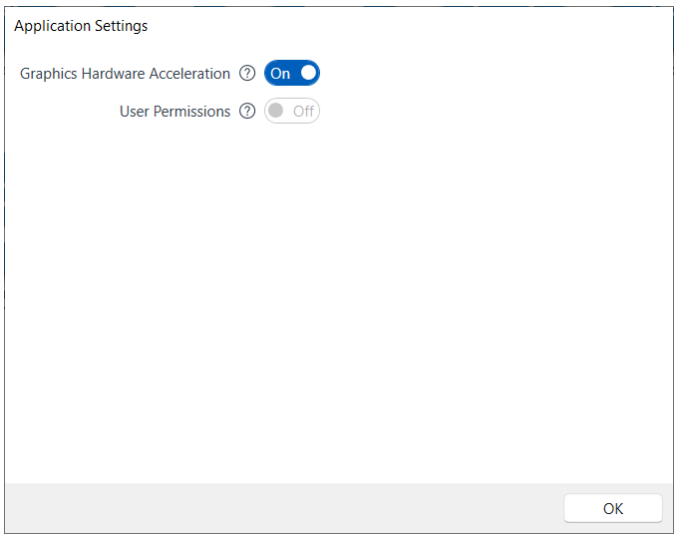
Note also that options to **Recover** or **Reconstruct Run** are only available when the previous run has not been transmitted successfully to Workbench. Data that has been moved to Workbench will not be stored on the machine.

Appendix E – Disabling Hardware Acceleration Graphics for the Mic

Occasionally, users may experience an issue where traces on run data and analysis charts from a Mic qPCR device do not update automatically when sample selection is changed. The root cause of this problem is a display driver for the Intel Iris Xe graphics card, with other similar graphics drivers also known to cause this issue. Workbench offers the option to disable hardware acceleration graphics, allowing for compatibility with these graphics' drivers. To use this feature, click on the Help icon and then Settings.



This will open the Workbench **Application Settings** dialogue box. Simply toggle the Graphics Hardware Acceleration option to Off.



The software may need to be restarted for these changes to take effect. If the issue persists, please contact BMS Support.

Appendix F – Post-PCR Sample Recovery from Mic Tubes

The Mic qPCR cycler incorporates an oil overlay in the tubes for superior results and eliminates the need for a heated lid. The oil overlay maintains temperature stability of the samples and prevents sample evaporation and condensation during a qPCR run. Oil present in PCR products can be unfavourable for some downstream applications, such as DNA sequencing. This document discusses four methods for sample recovery and their level of appropriateness with Mic tubes.

Method	Description	Advantages (+) or Disadvantages (-)	Applications
Direct pipetting	Pushing a narrow bore pipette tip against the wall of the tube and inserting just below the oil surface enables large volumes of sample recovery with minor oil contamination.	+ Volumes up to 3 µL less than the original reaction volume (excluding oil) can be pipetted. + Least number of materials required. - There may be minor residue leftover on the tip.	Sequencing Gel electrophoresis
Using parafilm	When the mixture (sample and oil) is first pipetted onto parafilm, the oil sits below the PCR product due to the parafilm's hydrophobic nature. Pipetting up to 5 µL less than the original reaction volume ensures that the pipette tip is not touching the oil.	+ Volumes up to 5 µL less than the original reaction volume can be pipetted. + Reduces the likelihood of oil contamination compared to pipetting under the oil. - Reduced sample recovery compared to pipetting under the oil and purification kits.	Sequencing Gel electrophoresis
Freezing sample	It is advised not to use this method. Unpredictable freezing and quick melting of samples leads to difficult extraction of pure oil.	- The small sample volumes result in instant melting upon touching the tube. - Difficult to pipette pure oil, leading to large sample loss. - Samples freeze unevenly. - Requires a long wait time.	Not Advisable.
Purification kits	PCR product isolation using a silica spin column. The spin column method typically provides more rapid and high-yielding purification of PCR products. It is important that ethanol is completely removed prior to DNA elution as it can inhibit downstream reactions.	+ Produces a highly pure PCR product (also remove enzymes). + Up to 95% sample recovery. + Kits can be fully automated. - Costly compared to other methods. - Timelier as there are multiple steps to the process.	Cloning Sequencing Microarray Ligation Labelling RE digestion

There are three recommended methods for sample recovery and the decision made may depend on the final volume and purity required. Pipetting under the oil requires the least number of materials and will allow the user to pipette a larger volume than using the parafilm method. Purification kits should be used if a completely pure PCR product or enzyme removal is required. Freezing samples is not recommended as extraction of pure oil is difficult due to unpredictable freezing and quick melting of samples.

Detailed Methods

Batches of 10, 15, 20, and 25 µL reactions (solution of water, gBlocks, 1x PCR buffer and blue food dye) were pipetted into Mic tubes. The tubes were run in the Mic qPCR Cycler to mimic the result of a real-life run (Table 1).

Table 1: PCR Conditions on the Mic.

Step	Temperature and Time	Cycles
Initial denature	95°C for 2 min	1
Denature	95°C for 5 sec	40
Anneal/Extension	65°C for 10 sec	
Melt	72°C - 95°C in 1°C increments	

Method 1: Direct Pipetting

A 20 µL narrow-bore pipette tip (Eppendorf Research Plus - Research 20 G21370D) was pushed against the wall of the tube and inserted just below the surface of the oil (Figure 1). There is a visible distinction between the oil overlay and the aqueous solution due to the higher viscosity of the oil. The sample was aspirated and placed in a new empty Mic tube without oil.

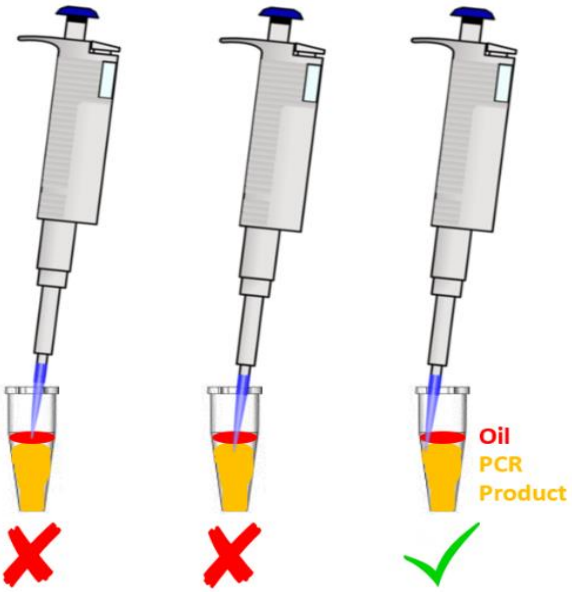


Figure 1. It is recommended to use a narrow-bore pipette tip for extracting PCR product from Mic tubes. Pushing the pipette tip against the wall of the tube and pipetting below the oil surface will allow the most efficient extraction of pure PCR product for downstream applications. It is recommended to pipette no more than 3 µL less than the original reaction volume.

Method 2: Using Parafilm

The total tube volume was aspirated and pipetted onto parafilm (PM996). As parafilm is hydrophobic in nature, a droplet is formed with the aqueous solution sitting above the oil. The tip of a 20 µL pipette was positioned on top of the droplet to avoid contact with the oil layer. The aqueous solution was aspirated and pipetted in a new empty Mic tube without oil (Figure 2).

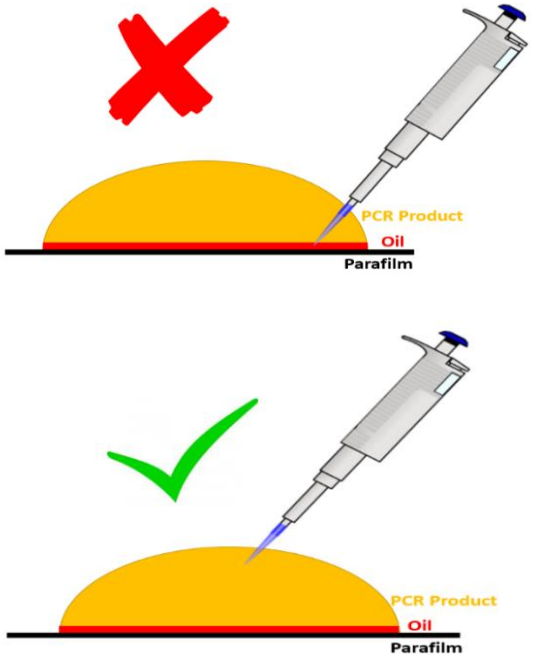


Figure 2. Due to the hydrophobic nature of parafilm, the oil sits on the bottom of the droplet whilst the aqueous solution sits on top. It is important to avoid touching the parafilm with the pipette tip due to the oil sitting on top of the parafilm. For the most efficient sample recovery, it is recommended to draw the sample from the top centre of the droplet to avoid extracting the oil that sits on the bottom. It is recommended to pipette no more than 5 µL less than the original reaction volume.

Method 3: Freezing Sample

The Mic tubes were placed in an aluminium loading block and put in the freezer at -20°C for various amounts of time (20, 40, 60 minutes). After the indicated time, the non-frozen surface liquid (oil) was drawn using a 20 µL pipette tip. Remaining aqueous solution was then transferred to a new Mic tube.

Method 4: Purification Kits

The manufacturers protocol for the QIAquick PCR Purification kit was followed. The elution step was transferred into new empty Mic tubes without oil.

Evaluating Recovery

To determine the maximum volume for sample recovery, various volumes (Table 2) of aqueous solution were aspirated from the reaction tubes and dispensed into empty Mic tubes with no oil. The new Mic tubes were then placed in a Mic qPCR Cycler and 10 cycles were run using the above cycling conditions. These tubes were inspected for effective sample recovery.
The criteria for effective sample recovery after the new Mic tubes were run in the Mic qPCR Cycler was for evaporation and condensation to occur within the Mic tubes (Figure 3). This indicates that there is no oil overlay present to prevent sample evaporation and effective sample recovery has been successful. Experimental tubes were compared to a control set of tubes containing the reaction solution (never had oil). The largest volume that passes the criteria is considered the largest volume that can be used for effective sample recovery for that method.

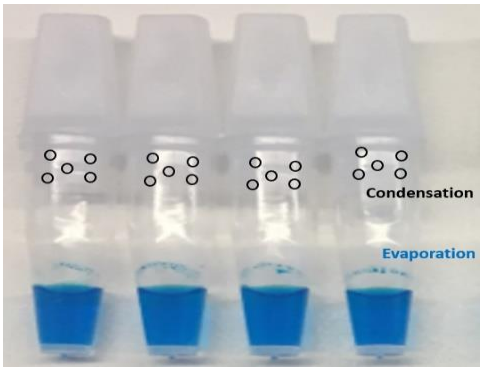


Figure 3. When there is no oil overlay present in the *Mic* tubes, evaporation (indicated by the blue residue line) and condensation (liquid bubbles present in the lid) occur.

Table 2: The volumes pipetted in methods 1 and 2.

Initial Reaction Volume	Volumes Attempted to be Aspirated	Max Successful Volume
25 µL	20, 22, 23, 24 µL	Method 1 = 22 µL Method 2 = 20 µL
20 µL	15, 17, 18 µL	Method 1 = 17 µL Method 2 = 15 µL
15 µL	10, 12, 13 µL	Method 1 = 12 µL Method 2 = 10 µL
10 µL	5, 7, 8 µL	Method 1 = 7 µL Method 2 = 5 µL

Method 4 resulted in complete removal of reaction for all volumes tested. For method 3, none of the volumes tested passed our criteria.

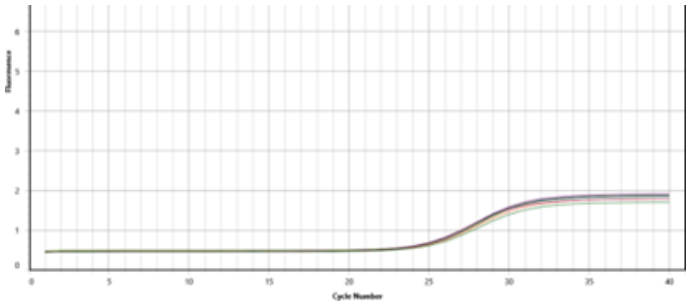
Appendix G – Removing old reaction tubes from Mic rotor

The Problem

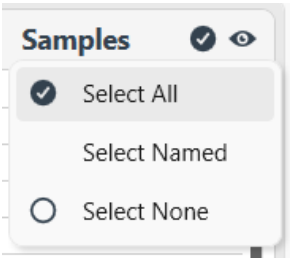
Let us assume you did a full rotor run and then in the next run you used just 8 samples. You then observe that the signals are low, as shown below. You might wonder if this is due to bad probe design, low concentration of probes in your reaction, or just poor set up. But in this case the issue is quite simple.

The Issue

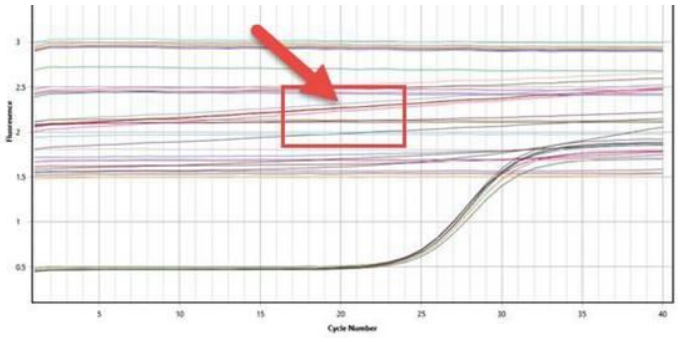
Low signals are all too often caused by old reactions left in the rotor from the previous run.



To find out, click on **Select All** in the **Samples** selector.



Now you can see that most of the unused reactions have a higher baseline signal. The highest signal starts at a value set for the gain adjustment (in this example it is 3 units, which is the default for intercalating dyes).



Why Does This Happen?

When the instrument starts, it will adjust the gain to ensure the data is within an appropriate scale for the chemistry used. By default, the instrument will adjust the gain using the tube with the highest fluorescence reading. Therefore, tubes that are left inside from a previous run, will have a higher fluorescence signal than the new reactions. So, the gain for the new reactions will be set too low.

How to Avoid This

Always take out the reactions from the previous run. It is also good laboratory practice as it reduces the chance of accidental contamination.

Ask yourself, what are the reaction volumes I normally use? Then prepare a set of water load tubes, at that volume, to use in subsequent reactions. As the tubes contain an oil overlay, they can be used for over a month without any loss of volume. Store them near the instrument in the provided plastic racks. You can also prepare different volumes if required.

Alternatively, select a specific tube to run the gain adjustment on. This is done through the Profile Editor for the specific run (independent of the assay profile).

Appendix H – Compatible Dyes for Mic and Myra+ Cyclers

The following is a list of commonly used dyes for qPCR and the channels Mic can read them on. Most of the dyes will be optimal for detection on a Mic 4-Channel, including a Myra+ Cycler.

Each channel will have its own excitation and emission (LED and photodiode). These sit side by side in a fixed optical path (see Figure 1) and work together to provide fast acquisition times (1 second). They are also very robust and do not require calibration or any form of colour compensation or reference dyes. The dyes have a very broad range of spectra. For the optical channels, they have a band width that can be up to 20 nm wide. This ensures each channel can pick up many different dyes while avoiding cross talk.

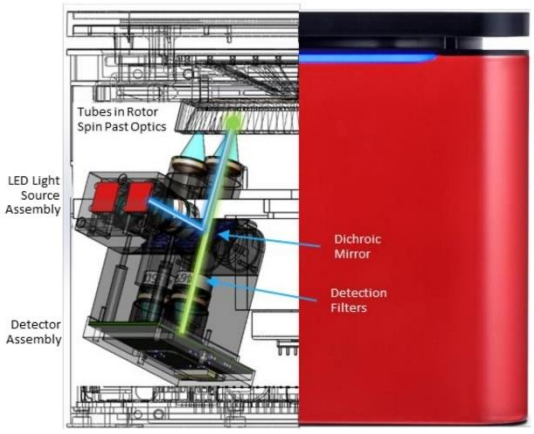


Figure 1. LED light passes through an excitation filter. Dichroic mirror reflects the excitation light onto the tubes and then only allows the emission light to pass through to the detector.

Dye	Ex.	Em.	Channel	Application
BEBO	468	492		Intercalating
LC Green®	455	495		HRM dye
SYTO® 9	483	503		HRM dye
FAM™ (best)	494	515		Conjugated label
SYBR® Green I	494	521		Intercalating
RiboGreen®	500	520		RNA label
PicoGreen®	502	523		dsDNA label
EvaGreen®	503	527		HRM dye
TET™	521	536	suboptimal	Conjugated label
CAL Fluor® Gold 540	522	541	suboptimal	Conjugated label
JOE™	520	548	suboptimal	Conjugated label
VIC®	538	554		Conjugated label
HEX™	535	555		Conjugated label
CAL Fluor Orange 560 (best)	540	561		Conjugated label
Quasar® 570	548	566		Conjugated label
Cy™3	550	570		Conjugated label
NED™	546	575		Conjugated label
TAMRA™	555	576		Conjugated label
CAL Fluor® Red 590	565	588	X	Conjugated label
ROX™	573	602		Conjugated label
Texas Red®	583	603		Conjugated label
CAL Fluor® Red 610 (best)	590	610		Conjugated label
LC® Red 640	620	635	suboptimal	Conjugated label
Quasar® 670 (best)	647	667		Conjugated label
Cy™5	651	674		Conjugated label
Cy™5.5	675	694		Conjugated label
Quasar® 705	690	705	X	Conjugated label

Appendix I – Temperature Verification System (TVS) for Mic qPCR Cycler and Myra+ Cycler

The **Mic TVS** can be used to ensure that the Mic or Myra+ Cycler is operating to specification regarding temperature control.

CAUTION	
⚠	**TVS sensor is fragile and connected via a very thin cable.** Please handle with care to avoid breakage. Hold the device only by its sides.

Verification Environment

For optimal results, it is best to perform the tests away from sources of airflow; for example, fans and air vents. The ambient temperature may also influence the measurement. We recommend testing under standard lab conditions (22 – 28°C).

Hardware and Software Installation

Connect the Mic or Myra+ Cycler to be verified to your computer and switch it on.

These can be connected via USB cable. For the Myra+ Cycler, connect using the Myra+ instrument icon.

Take the TVS out of the protective case and connect it to your computer through USB.

Open the BMS Workbench software.

Version must be at least 1.0.0.

Once the Mic or Myra+ Cycler and TVS are connected to your computer, scan for devices by clicking on the Communication icon.

Select the instrument to test, and select Temperature Verification from the drop-down menu.

Follow the prompts on the TVS Wizard.

During these steps, keep the TVS placed beside your Mic instrument.

When prompted by the software, remove the sensor from the foam slot and place it in wells 1 – 4 with the tab facing inwards.

Keep all other wells empty. Once the sensor is secured in with the tube clamp, close the lid and continue following the prompts on the screen. Verification may take up to 25 minutes.

At completion, you will be given the option to save or print your verification report.

bio molecular systems

MIC Temperature Verification Report

Date Completed: 23/05/2017

Instrument: M0000002
Verified By: TVS00006

Verification Check			
	Ring Temp. (°C)	**Actual Temp. (°C)**	**Measurement Error (Max±0.25°C)**
Pt 60°C	60.01	60	0.01
Pt 95°C	95.03	94.97	0.06

This machine has been verified to be working according to specification

Name

Signature

Carefully remove the sensor from the instrument and place it back into the foam slot.

Unplug the TVS from the computer and place it back into the protective case.

Appendix J – Mic in the Field: Alternative Power Sources

The portability and robustness of Mic is a huge advantage for anyone needing to do qPCR in the field. We have compiled some information on alternative power sources for getting the best performance from your instrument.

Running from batteries or solar power

Mic has proven to run reliably off batteries (car, truck or solar powered) using an inverter to supply between 100V and 240V AC.

When purchasing an inverter look for the following:

1. The output must be a Pure Sinewave. Square wave inverters **DO NOT** work.
2. Minimum continuous output power of 360W.
3. You may need to turn off the slow start up function. There is often a switch on the inverter.

Figure. Example Pure Sinewave Inverter

Running from an unreliable power source

If you have a power source that intermittently stops or changes such as a generator, you can run Mic from an uninterruptible power supply (UPS) to provide clean and stable power.

When purchasing a UPS look for the following:

1. Minimum 360W output power
2. 'Pure Sinewave Online' is the preferred type.
3. Consider the battery capacity to give the run time you require.

As an example, the following specifications allow for one Mic to be run for up to 82 minutes or two Mics to be run for up to 38 minutes.

Rating	1000VA/700W
Battery Capacity	3x 12V 7Ah SLA

Appendix K – 21CFR11

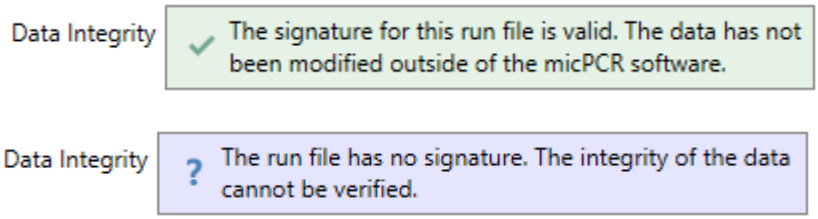
The Code of Federal Regulations Tile 21 part 11 (21CFR11) of the U.S Food and Drug Administration, covers the regulations on electronic records and electronic signatures to ensure trustworthy, reliable, and equivalent to paper records. We have implemented several features into our software that cover some of the requirements of **21CFR11 section 11.10** for closed systems. The procedures covered are listed and described below. The remaining procedures not covered by us, have a valid reason for each described below.

21CFR11 (section 11.10) states:

"Persons who use closed systems to create, modify, maintain, or transmit electronic records shall employ procedures and controls designed to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine. Such procedures and controls shall include the following":

"(a) Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records."

The software contains run file signatures that ensure validity of the records. Any tampering of the run file through external software will break the signature, resulting in a notification by the software. The Data Integrity message can be found in the **Information page** of the run file.



"(b) The ability to generate accurate and complete copies of records in both human readable and electronic form suitable for inspection, review, and copying by the agency."

This control is met through the generation of pdf reports.

"(e) Use of secure, computer-generated, time-stamped audit trails to independently record the date and time of operator entries and actions that create, modify, or delete electronic records. Record changes shall not obscure previously recorded information. Such audit trail documentation shall be retained for a period at least as long as that required for the subject electronic records and shall be available for agency review and copying."

The following will be logged and will appear in the Messages page of the application and the **Event log** section of the report or Excel report when the run is saved or when a report is switched to:

- Changes to all parameters of analyses since the log update for analyses created before the last log update
- Changes from default parameter values for analyses created since the last log update
- Removal of analyses
- Changes in Analysis sample selection

There will be a single entry per analysis in the Message panel summarising changes. The time stamp of the entry is the time of the Run Save not of the analysis parameter change.

The remaining procedures we have no control over, especially as some of them relate to the whole standard operating procedure for a test and/or quality management process, not just to the software. These include:

"(c) Protection of records to enable their accurate and ready retrieval throughout the records retention period."

Provided by organisation document management system (see note below).

"(d) Limiting system access to authorized individuals."

Provided by organisation document management system (see note below).

"(f) Use of operational system checks to enforce permitted sequencing of steps and events, as appropriate."

This is an organisation operational requirement.

"(g) Use of authority checks to ensure that only authorized individuals can use the system, electronically sign a record, access the operation or computer system input or output device, alter a record, or perform the operation at hand."

Provided by organisation document management system (see note below).

"(h) Use of device (e.g., terminal) checks to determine, as appropriate, the validity of the source of data input or operational instruction."

This is an organisation operational requirement.

"(i) Determination that persons who develop, maintain, or use electronic record/electronic signature systems have the education, training, and experience to perform their assigned tasks."

This is an organisation operational requirement.

"(j) The establishment of, and adherence to, written policies that hold individuals accountable and responsible for actions initiated under their electronic signatures, in order to deter record and signature falsification."

This is an organisation operational requirement.

"(k) Use of appropriate controls over systems documentation including:

(1) Adequate controls over the distribution of, access to, and use of documentation for system operation and maintenance.

(2) Revision and change control procedures to maintain an audit trail that documents time-sequenced development and modification of systems documentation."

This is an organisation operational requirement.

File management is up to the end user. They must configure appropriate permissions and file storage such that it meets these requirements. Windows allows you to have separate permissions to create files or write data and delete. However, it does not distinguish between writing a file and overwriting a file so there is nothing to prevent you from saving one file over the top of another.

It would be our recommendation to use a proper enterprise quality management software system like MasterControl™ (MasterControl Inc., Utah, USA) to meet all the 21CFR11 requirements.

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Abbreviations

CI:	Confidence interval
C*q*:	Quantification cycle
CV:	Coefficient of variation
FRET:	Forster Resonance Energy Transfer
HRM:	High resolution melting
IAC:	Internal amplification control
IRC:	Internal run control
LoD:	Limit of detection
NAC:	No amplification control
NRT:	No reverse transcriptase
NTC:	No template control
PCR:	Polymerase chain reaction
qPCR:	Real time PCR
RIN:	RNA integrity number
RT-qPCR:	Reverse transcription real time PCR
SD:	Standard deviation
T_m:	Melting temperature
UDG:	Uracil DNA glycosylase
W-o-L:	Window of linearity

Glossary

Analytical Accuracy: the difference between the experimentally measured and actual concentrations.

Analytical Repeatability: precision of the assay within the same samples repeatedly measured in the same assay. Also, referred to intra-assay variance it is expressed as the SD for the C*q* variance or CV of the copy number/ concentration variance.

Analytical Reproducibility: the variation in results between runs or different laboratories. It is also referred to as inter-assay variance and is expressed as the SD or CV of copy number or concentration. As C*q*'s typically vary between runs the reporting of inter run variation is not appropriate.

Analytical sensitivity: the minimum number of copies in a sample that can be measured accurately with an assay.

Analytical specificity: assay detecting the specific target sequence rather than another, nonspecific, target. Use of NTC's helps determine analytical specificity.

Dual hybridisation probes: rely on FRET of a reporter dye, attached to the 3' end of one probe (donor), to a quencher molecule, attached to the 5' end of a second probe (acceptor). When the two probes are hybridised adjacently to each other, energy transfer results in a reduction in the reporter dye fluorescence due to the proximity of the quencher molecule. Another iteration of dual hybridisation probe uses a dye as the acceptor, resulting in an increase in the emission signal of the acceptor dye due to the FRET.

Hydrolysis probes: are short oligonucleotides with a fluorescence reporter dye at the 5' end and a quencher molecule at the 3' end. When the probe is intact, the proximity of the reporter dye to the quencher results in little fluorescence being detected. During extension, the polymerase will cleave the probe through exonuclease activity separating the reporter dye from the quencher, resulting in increased fluorescence.

Intercalating dye: intercalating dyes, such as SYBR® Green I, bind to double stranded DNA. The unbound dye exhibits little fluorescence in solution, but upon binding to double stranded DNA, the fluorescence is enhanced.

Limit of Detection (LoD): the LoD is the minimum concentration that can be detected with reasonable certainty (typically 95% probability).

Linear dynamic range: is the highest to the lowest quantifiable copy number determined by means of a standard curve.

Magnetic induction: when a conductor such as a metal is exposed to a magnetic field it produces a circular electric current, also known as an eddy current, in the conductor. Due to the resistance of the conductor, it becomes hot. In the MIC instrument the rotor is the conductor that becomes hot under as it is exposed to a magnetic field.

Quenched FRET: occurs when an excited dye, near a quencher molecule, transfers its emission energy to the quencher molecule, resulting in a reduction in signal from the reporter dye.

Reference gene: are genes that are stably expressed within the experimental samples and are used to normalise for variations in extraction yield and reverse-transcription yield.

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