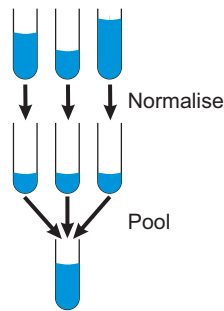


## Normalisation and Pooling

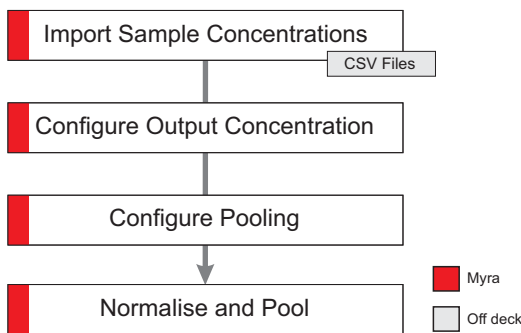
### Myra for Normalisation and Pooling

Normalisation and pooling are critical steps in various laboratory workflows in applications such as Next Generation Sequencing (NGS) library preparation and quantitative PCR (qPCR). Multiplexing of libraries for NGS improves the use of the ever-increasing capacity of sequencing instruments allowing multiple libraries to be run on a single flow cell, which reduces run times and drives down costs. As libraries are multiplexed onto a single flow cell, it is important that each library is represented equally and sequenced to sufficient depth. Normalisation prevents under- or over-representation of individual libraries after pooling.



### Setup Workflow

No calculations required!



### Key Features

- **Flexible Workflow:** Users can upload sample information, including sample name, concentration, starting volume, and position, minimising manual data input. The software automatically calculates the required volumes of sample and diluent based on original and final concentrations, making normalisation calculation seamless.
- **Time and Labor Efficiency:** Myra reduces user errors and repetitive tasks, freeing up researchers' time. Further, Myra can pool a variable volume of each sample into a single well allowing for normalisation and pooling to occur in a single step, further streamlining your workflow.

### Overcome the Challenges of Normalisation with Myra Liquid Handling System

Myra's advanced pipette and intelligent software provide accurate and consistent volume dilutions and transfers during normalisation and pooling. This reduces pipetting inconsistencies and improves sequencing data quality. Our results demonstrate that the Myra liquid handling system effectively normalised samples with high precision and accuracy, even with large sample numbers and DNA concentrations (Figure 1). The automated process significantly reduced hands-on time and minimized variability across the 96-well plate.

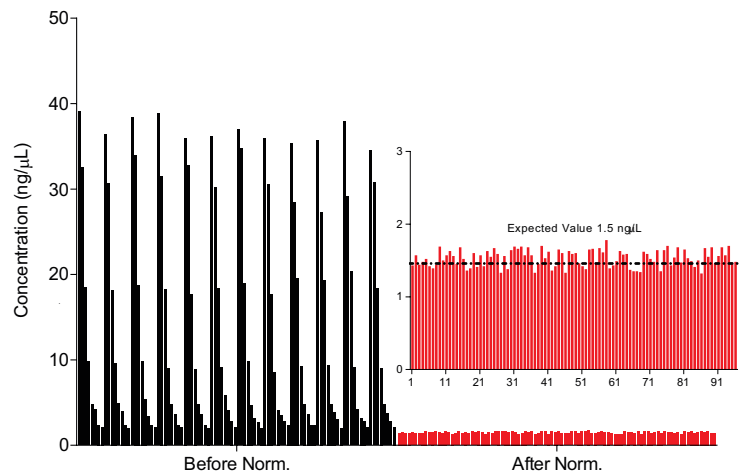


Figure 1. Concentrations of samples before and after normalisation with Myra. A set of 96 samples, with initial concentrations ranging from 2 to 40 ng/μL were normalised to a target concentration of 1.5 ng/μL, with a total volume of 25 μL. The coefficient of variance (CV) post normalisation was 7.5%.

### Summary

The Myra Liquid Handling System provides a reliable and efficient solution for automating normalisation and pooling workflows in the laboratory. By leveraging robotics and intuitive software, Myra eliminates manual pipetting errors, reduces variability, and streamlines workflows, significantly enhancing overall data reliability. Its precise and accurate pipetting, combined with easy importation of sample data, ensures high accuracy and increased reproducibility in library normalisation and pooling. This not only accelerates research and discovery across various fields but also frees up substantial time and energy for laboratory personnel.

# Case Study: Normalisation and Pooling by Ramaciotti Centre for Genomics

Accurate normalisation and pooling of whole genome libraries prior to used on the Illumina NovaSeq X.

## A Strong Preference for Myra

The Ramaciotti Centre for Genomics is a leading Australian research facility that provides advanced genomics services including whole-genome sequencing, transcriptome analysis, and single-cell sequencing, utilizing state-of-the-art technologies to support diverse research needs in medicine, agriculture, and environmental science. Being one of the country's leading sequencing service providers means the Ramaciotti Centre are tasked with providing high quality sequencing reads for all their clients. Failure to achieve sufficient sequencing data can result in lost time, loss of precious sample and additional costs to re-run the sequencing.

Here we provide an example of how the Ramaciotti Centre utilise Myra for all their normalisation and pooling of whole genome library samples prior to running them on highly multiplexed sequencers such as the Illumina NovaSeq X. We show why Myra earns their trust and has become their go to instrument.

## Experiment

Human genomic DNA samples were processed using the Illumina DNA library prep kit for use in whole genome sequencing. For each run 24 samples were normalised and pooled using Myra to a starting concentration of 2 nM. The pooled samples were run on the Illumina NovaSeq X Plus platform. The read depth for each sample was analysed post sequencing as part of the quality control for each run. A total of 4 runs were conducted for this study.

The screenshot shows the Myra software interface. The 'Normalisation / Dilution' section has 'Specific concentration' set to 2 nM (circled in red) and 'Total volume' set to 120 µL. The 'Destination Wells' table is as follows:

Wells	Sources	Concentration (nM)	Total Volume (µL)
1	Sample 1; Sample 2; Sample 3	2	120
	Source		Source Volume (µL)
	Diluent		66.27
	Sample 1	2	2.53
	Sample 2	2	5.11

Figure 2. The concentration of each sample is imported into the Workbench software. Using the Simple Transfer feature, the desired concentration, number of pooled samples and volumes for pooling are entered (red circles). The software will automatically calculate the amount of sample required in the pool and devise dilution factors and volumes for samples that are too high for direct volume transfer. The process is simple and intuitive.

## Results

Statistically consistent read depths were demonstrated in each of four separate sequencing runs following normalisation and pooling using Myra (Figure 3). This indicates the reliable and reproducible performance of Myra in normalising and pooling samples for multiplexed NGS.

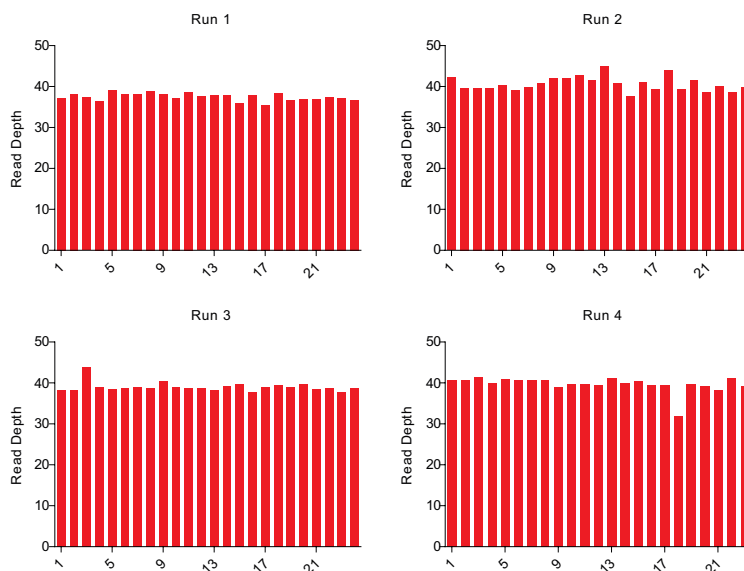


Figure 3. The read depth for each sample across 4 runs performed on the NovaSeq X Plus, where the Myra Liquid Handling System was used to simultaneously normalise and pool 24 samples to a concentration of 2 nM in a single tube. CV% was < 5% across samples and runs. Data was provided by Ramaciotti Centre for Genomics at UNSW.

The Ramaciotti Centre trusts Myra over other liquid handlers for consistently reliable results. Unlike other liquid handlers that require constant monitoring to ensure correct volume transfers, Myra uses a pressure-based liquid level sensing system to effectively normalise samples and trigger warnings if issues arise. This added assurance ensures accuracy and consistency in sample handling, maintaining high standards of data integrity.

Taking into consideration that each highly multiplexed NovaSeq X run can cost well above \$10,000 USD; any lost data can quickly become a financial burden for a sequencing service provider. Not to mention the lost time each client would experience along with their important samples. Myra's pipetting accuracy and ease of use gives the Ramaciotti group the peace of mind they need to continue their valuable work today and into the future.