1 OVERVIEW

The Rapid Barcoding Kit 24 V14 by Oxford Nanopore Technologies is a NGS Library Prep kit designed to swiftly attach unique barcode sequences to DNA samples for NGS sequencing. Unlike the Native Barcoding Kit, which directly links barcodes to DNA without amplification, the Rapid Barcoding Kit uses a PCR-based approach after DNA amplification. This method enables the simultaneous barcoding of multiple samples in a single sequencing run, making it perfect for high-throughput experiments where efficiency and speed are crucial. By streamlining the barcoding process, this kit accelerates experimental workflows, saving researchers time and resources while ensuring accurate sample identification.

The Rapid Barcoding Kit 24 V14 generates sequencing libraries from extracted gDNA using a transposase which simultaneously cleaves template molecules and attaches barcoded tags to the cleaved ends. The barcoded samples are then SPRI cleaned and pooled before adding the Rapid Sequencing Adapters to the tagged ends.

The Rapid Barcoding Kit workflow is demonstrated below:

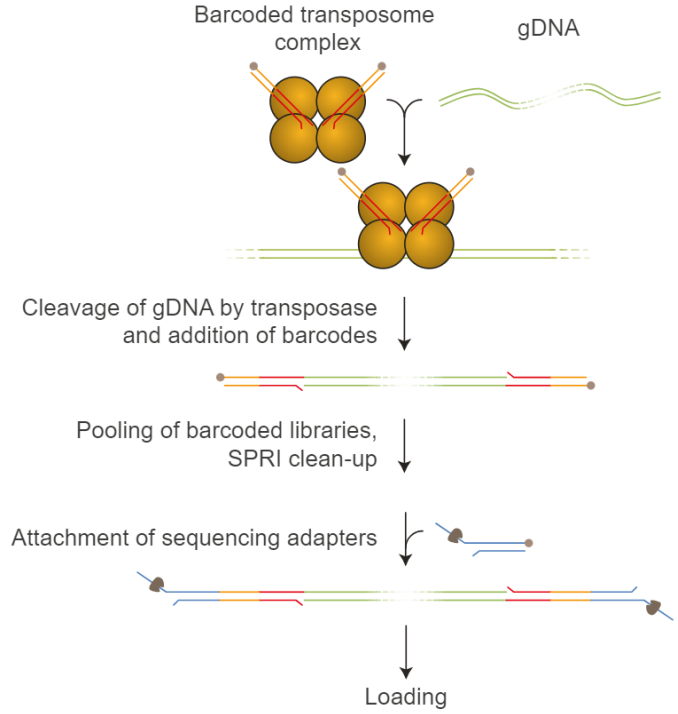


Figure 1. Image extracted from Oxford Nanopore Technology website (https://store.nanoporetech.com/rapid-barcoding-sequencing-kit-24-v14.html).

Preparing a high-quality Next Generation Sequencing (NGS) library is a complex process that requires a significant investment of time, money, and expertise. The whole workflow typically consists of multiple stages, each with hundreds of pipetting steps and require precision. As pipetting precision depends on the user, variability is introduced, which negatively affects the reproducibility. The Myra can streamline this labour-intensive process with minimal user intervention and setup time, in which we have provided a protocol to minimize programming and get you up and running quickly.

This protocol semi-automates Rapid Barcoding Kit 24 V14 for up to 24 libraries on the Myra where the 96 Well Magnetic Station is used for the clean-up steps and the heating steps are performed on an external thermal cycler. By automating the pipetting-intensive steps, human error can be eliminated, thereby providing high-quality reproducible results, and increasing overall productivity.

2 NOTES

Rapid Barcoding Kit 24 V14 v2.0 scripts can only be used with BMS Workbench v1.4.8 and later.

The following components need to be aliquoted or prepared into tubes that can be appropriately positioned on deck:

- 80% Ethanol (EtOH) in Generic 2 mL Screw Cap Tube
- Nuclease-free Water (H_2O) in Generic 2 mL Screw Cap Tube
- AMPure XP Beads (AXP) in Generic 2 mL Screw Cap Tube

It is important to prepare ethanol tubes with **AT LEAST 10% OVERAGE** as ethanol is hygroscopic where prolonged exposure can lead to volume loss due to evaporation as well as a more diluted concentration than initially prepared.

If reagents are required to be kept cold during the run, the *Myra Multipurpose Loading Block* can be placed in the freezer prior to the run to provide passive cooling for reagents.

2.1 SAMPLE EDITOR

If samples are required to be transferred to the Magnet Station, the location of samples in the 96 Well Plate will need to be defined in the *Sample Editor*. If samples are preloaded into the Magnet Station, ensure the correct starting volume is present in these tubes.

A Rapid Barcode also needs to be allocated to each sample. If barcodes are found in a plate format, the script will automatically determine the location of each Rapid Barcode on the plate.

Samples (24)

Search

	Name	Source Well ID - Source Plate Only	Rapid Barcode
1	Sample 1	A1	RB01
2	Sample 2	B1	RB02
3	Sample 3	C1	RB03
4	Sample 4	D1	RB04
5	Sample 5	E1	RB05
6	Sample 6	F1	RB06
7	Sample 7	G1	RB07
8	Sample 8	H1	RB08
9	Sample 9	A2	RB09
10	Sample 10	B2	RB10
11	Sample 11	C2	RB11
12	Sample 12	D2	RB12
13	Sample 13	E2	RB13
14	Sample 14	F2	RB14
15	Sample 15	G2	RB15
16	Sample 16	H2	RB16
17	Sample 17	A3	RB17
18	Sample 18	B3	RB18
19	Sample 19	C3	RB19
20	Sample 20	D3	RB20
21	Sample 21	E3	RB21
22	Sample 22	F3	RB22
23	Sample 23	G3	RB23
24	Sample 24	H3	RB24

Spin down the Index Adapter Plate before placing on deck.

Myra will pierce the foil before pipetting the Rapid Barcode (if specified to do so in *User Configurable Settings*).

2.2 USER CONFIGURABLE SETTINGS

At the beginning of the script, there is a *User Configurable Settings* section that allows you to customise the run:

- **sourcePlateType**: Input the source plate type or use *None* for samples placed on the Magnet Station with the correct starting volume.
- **amplicon**: Set to *True* to follow workflow for amplicons (i.e. no DNA-repair); *False* to follow workflow for gDNA.
- **barcodePlate**: Set to *True* for Native Barcodes in a plate format; *False* for tube format (tubes will need put into the *36 Well Microcentrifuge Tube Loading Block*).
- **pierceFoil**: Set to *True* if the index adapter plate has a foil covering. In this case, the Myra will pierce the foil prior to taking an aliquot.
- **hulaShaker**: Set to *True* to place reactions on the Hula Shaker after bead addition without mixing. Set to *False* for reactions to be mixed after bead addition and remain on deck for the incubation step.

```
sourcePlateType = "Generic 96 Well 0.2 mL Skirted Plate"    # Input Source Template plate type. For samples preloaded onto magnet station use: sourcePlateType = None
amplicon = False                                            # Set to True if Source Templates are amplicon; False if source templates are gDNA
barcodePlate = True                                         # Set to True if Barcodes come in plate; False if Barcodes come in 0.5 mL Screw Cap Tubes
pierceFoil = False                                          # Set to True if the Barcode plate has a foil covering that needs to be pierced on-deck
hulaShaker = True                                           # Set to True if tubes need to be put on Hula Shaker during bead incubation
```

3 DECK LAYOUT

3.1 REQUIRED BLOCKS AND INSERTS

Item	Image
Myra Multipurpose Loading Block	
Myra 96 Well Magnetic Station	
Myra 36X Microcentrifuge Tube Loading Block (if required)	

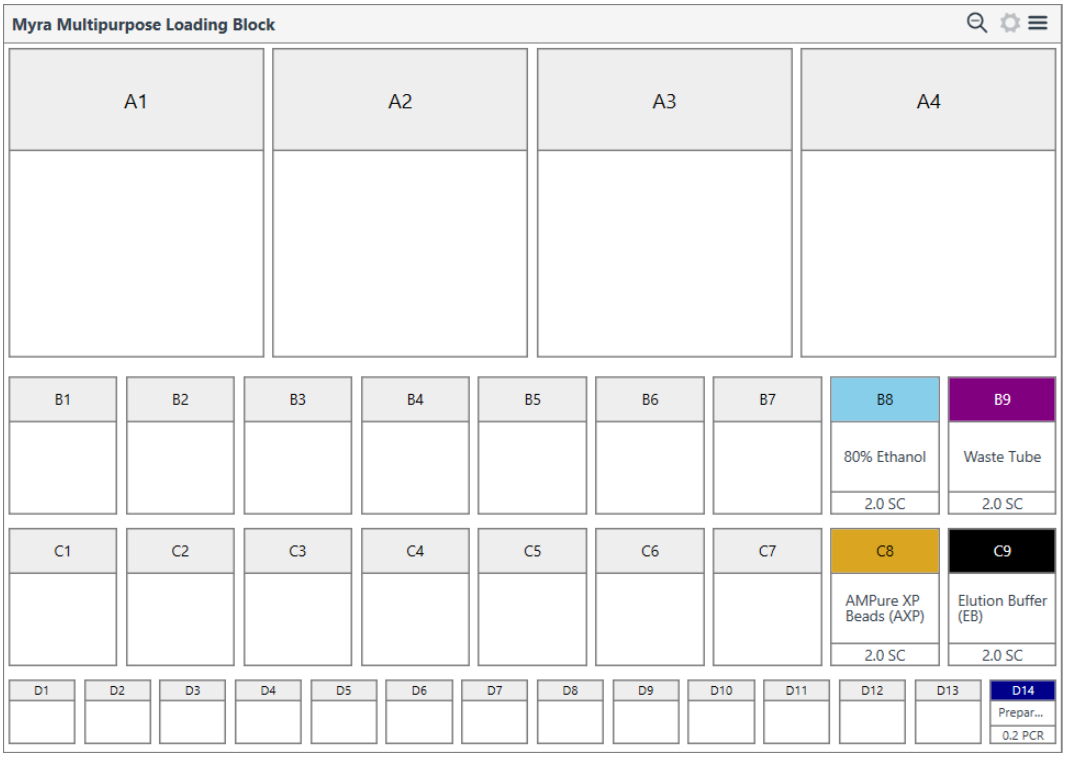
3.2 COMPONENTS AND CONSUMABLES REQUIRED

Components Required
Rapid Barcoding Kit 24 V14 (SQK-RBK114.24) OR Rapid Barcoding Kit 96 V14 (SQK-RBK114.96)
80% Ethanol

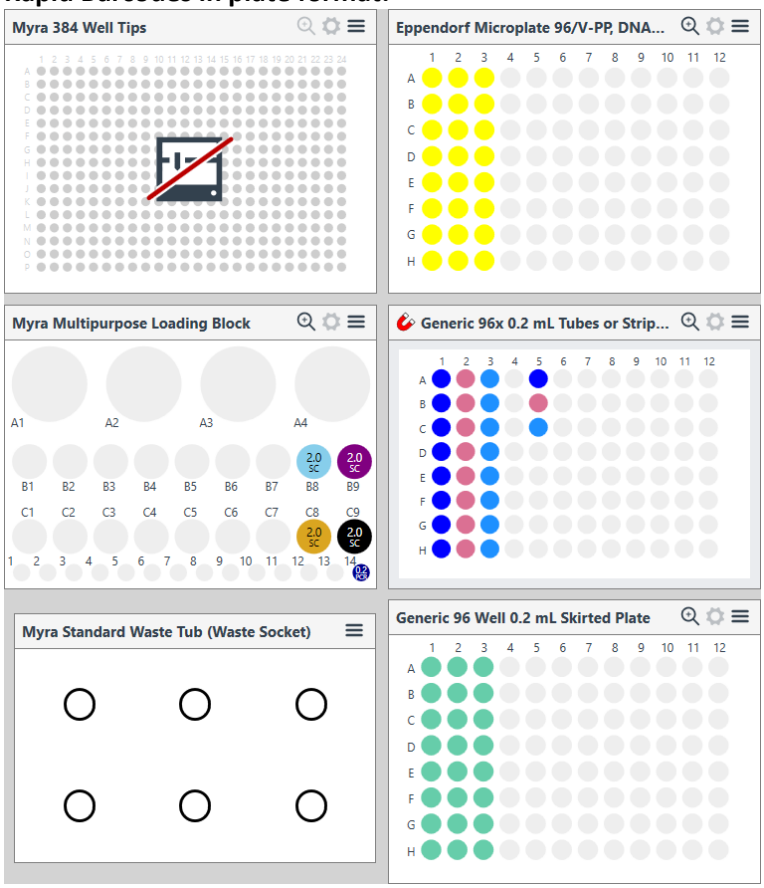
Consumables Required	Qty
Myra 384 Well Tips	1 rack
Generic 2 mL Screw Cap Tube as Waste Tube	1
0.2 mL PCR Tubes	28

3.3 DECK PLACEMENT

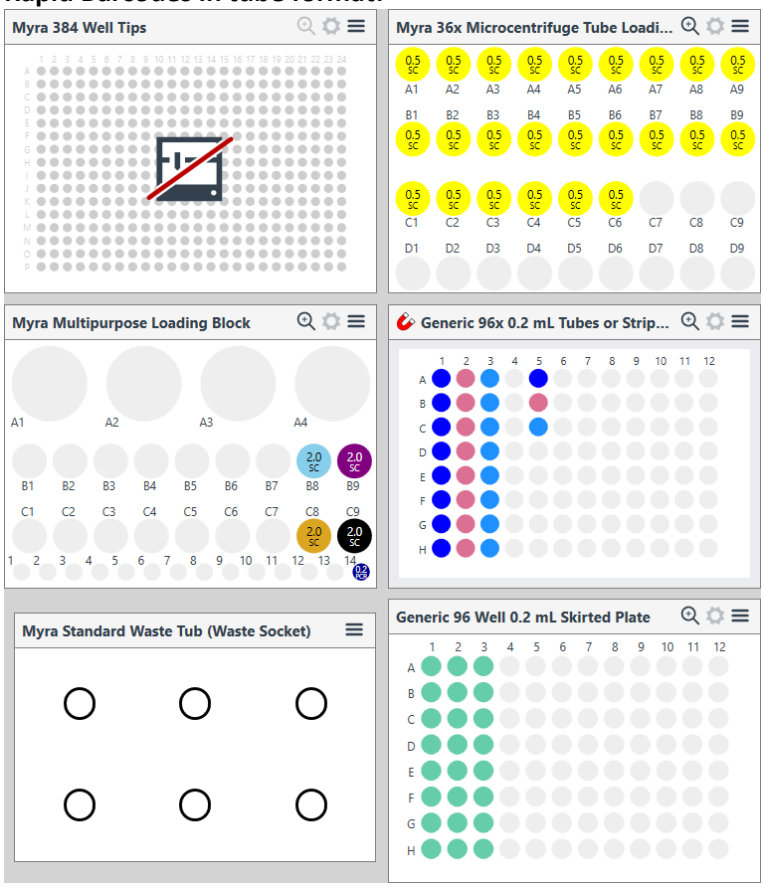
Socket A	Socket C
Myra 384 Well Tips	Rapid Barcodes in either 0.5 mL Screw Cap Tube in a 36X Microcentrifuge Tube Loading Block or in 96 Well Plate Format
Socket B	**Socket D**
Myra Multipurpose Loading Block with B8: 80% Ethanol in Generic 2 mL Self-Standing Screw Cap Tube B9: Empty Generic 2 mL Self-Standing Screw Cap Tube C8: AMPure XP Beads in Generic 2 mL Self-Standing Screw Cap Tube C9: Elution Buffer (EB) in Generic 2 mL Self-Standing Screw Cap Tube D14: Empty 0.2 mL PCR Tube for final Pooled Library	Generic 96x0.2 mL Tubes or Strips without Attached Caps sitting on top of Myra 96 Well Magnet Station
Socket Waste	**Socket E**
Standard Waste Tub	Generic 96 Well 0.2 mL Skirted Plate containing samples (if required)



Rapid Barcodes in plate format:



Rapid Barcodes in tube format:



4 REVISION HISTORY

Date	Revision	Changes
3rd June 2024	v1.0	Initial version
23rd August 2024	v2.0	Improved mixing and pipetting parameters to increase final yield produced