



Rapid PCR barcoding DNA V14 – automated ElysION MinION (SQK-RPB114.24)

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This is an automated rapid PCR barcoding method using the ElysION™ device with a MinION™ setup, outlining library preparation and sequencing.

This protocol:

- Is an automated method using the ElysION device with a MinION sequencing setup
- Uses genomic DNA
- Has a low input requirement
- Involves tagmentation, barcoding, and PCR amplification
- Allows multiplexing of 1–24 samples
- Is compatible with R10.4.1 flow cells

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1. Overview of protocol

Introduction to the automated rapid PCR barcoding DNA protocol using ElysION with a MinION sequencing setup

The ElysION automated Rapid PCR Barcoding Kit 24 V14 (SQK-RPB114.24) workflow is optimised for simplicity and speed to automatically prepare and sequence between 1 to 24 purified DNA samples. This method is PCR-based, enabling low DNA input and contains 24 unique barcodes which allows you to pool up to 24 different samples into one sequencing experiment.

After sequencing, the ElysION can perform downstream analysis using the EPI2ME metagenomic workflow ([wf-metagenomics](#)) for taxonomic classification of your metagenomic samples. If you wish for the analysis to be automatically performed after sequencing, use the **Metagenomics** assay.

Steps in the sequencing workflow:

Prepare for your experiment

You will need to:

- Ensure your ElysION device is installed with the required hardware and software package for this workflow.
- Ensure you have your sequencing kit, the correct equipment and third-party reagents.
- Check your flow cell during the ElysION run setup to ensure it has sufficient pores for a good sequencing run.

Automated library preparation, sequencing, and analysis

The Table below is an overview of the steps automated by the ElysION device:

Library preparation step	Process
DNA tagmentation	Tagmentation of the DNA using the Fragmentation Mix provided in the kit.
PCR amplification and barcoding	PCR amplification and barcoding of the DNA using barcoded primers supplied in the kit.
Sample pooling and clean-up	Pooling of barcoded libraries and AMPure XP bead clean-up.
Adapter ligation	Attachment of sequencing adapters to the DNA ends.
Priming and loading the flow cell	Prime the flow cell and load the prepared library for sequencing.
Sequencing	The sequencing run uses the MinKNOW software which will collect raw data to basecall and demultiplex the barcoded reads.
Data analysis	Once sequencing is complete, there are a number of downstream analysis options available for your data through EPI2ME.
Flow cell wash or flush	Wash the flow cell using the Flow Cell Wash Kit (EXP-WSH004) for reuse or flush the flow cell for return to Oxford Nanopore for recycling.



Compatibility of this protocol

This protocol should only be used in combination with:

- Rapid PCR Barcoding Kit 24 V14 (SQK-RPB114.24)
- R10.4.1 MinION/GridION Flow Cells (FLO-MIN114)
- Flow Cell Wash Kit (EXP-WSH004)
- Flow Cell Priming Kit V14 (EXP-FLP004)
- Sequencing Auxiliary Vials V14 (EXP-AUX003)
- Rapid Adapter Auxiliary V14 (EXP-RAA114)
- ElysION device with MinION integration

2. Equipment and consumables

Materials	25 ng gDNA in 15 µl per sample (concentration: ~1.66 ng/µl) in a Hard-Shell® 96-Well PCR Plate, low profile, thin wall, skirted, red/clear (Bio-Rad™, HSP9611) Rapid PCR Barcoding Kit 24 V14 (SQK-RPB114.24) Flow Cell Wash Kit (EXP-WSH004)
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Consumables	MinION/GridION Flow Cell (FLO-MIN114) Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, red/clear (Bio-Rad™, HSP9611) Nunc™ 96 Well Polypropylene 2 ml DeepWell™ Plate (ThermoFisher, 95040452) Reservoir Plate (Porvair, 390015) Azenta PCR Plate Lid (Azenta, 4ti-0291) Screw cap micro tube 2 ml, sterile (Sarstedt™, 72.694.006) Screw cap micro tube 0.5 ml, sterile (Sarstedt™, 72.785.005) LongAmp Hot Start Taq 2X Master Mix (NEB, M0533) Bovine Serum Albumin (BSA) (50 mg/ml) (e.g. Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616) Freshly prepared 80% ethanol in nuclease-free water Nuclease-free water (e.g. ThermoFisher, AM9937) 1.5 ml Eppendorf DNA LoBind tubes 1000 µL Disposable Tips - Filtered, Pure, Single Stack (Tecan, 30057817) 200 µL Disposable Tips - Filtered, Pure, Single Stack (Tecan, 30057815) 50 µL Disposable Tips - Filtered, Pure, Single Stack (Tecan, 30057813) 10 µL Disposable Tips - Filtered, Pure, Single Stack (Tecan, 30104974) LiHa empty Disposable Tip Box Large (Tecan, 30058507) LiHa empty Disposable Tip Box Small (Tecan, 30058506) Waste bin (Tecan, 30185747) Qubit™ dsDNA HS Assay Kit (Invitrogen, Q23851) - optional Qubit™ Assay Tubes (Invitrogen, Q32856) - optional
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Equipment	ElysION device with MinION integration Microfuge Vortex mixer Microplate centrifuge Multichannel pipette and tips P1000 pipette and tips P200 pipette and tips P100 pipette and tips P20 pipette and tips P2 pipette and tips Ice bucket with ice
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Optional equipment

Qubit™ fluorometer (or equivalent for QC check)



For this protocol, 25 ng high molecular weight gDNA in 15 µl is required per sample as input.

The starting concentration of your sample(s) should be ~1.66 ng/µl in 15 µl of volume.

Note: Your input DNA must be at least 4 kb in length to ensure correct tagmentation and PCR amplification.

Third-party reagents

We have validated and recommend the use of all third-party reagents used in this protocol. Alternatives have not been tested by Oxford Nanopore.

For all third-party reagents, we recommend following the manufacturer's instructions to prepare the reagents for use.

Input DNA

How to QC your input DNA

It is important that the input DNA meets the quantity and quality requirements. Using too little or too much DNA, or DNA of poor quality (e.g. highly fragmented DNA or DNA containing RNA or chemical contaminants) can affect your library preparation.

For instructions detailing how to perform quality control of your DNA sample, read the [Input DNA/RNA QC](#) protocol.

Chemical contaminants

Depending on the DNA extraction method used, certain chemical contaminants may remain in the purified DNA, which can affect library preparation efficiency and sequencing quality. Read more about contaminants on the [Contaminants](#) page on the Community.

Check your flow cell

The number of pores in your flow cell will be checked by the ElysION device at the start of your assay. MinION/GridION Flow Cells should be checked within 12 weeks of purchase.

Oxford Nanopore will replace any unused flow cell with fewer than the number of pores listed in the Table below, when the result is reported within two days of performing the flow cell check, and when the storage recommendations have been followed. To do the flow cell check, follow the instructions on the ElysION on-screen display.

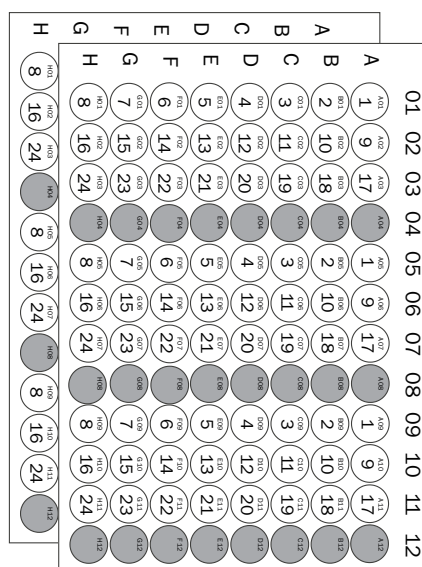
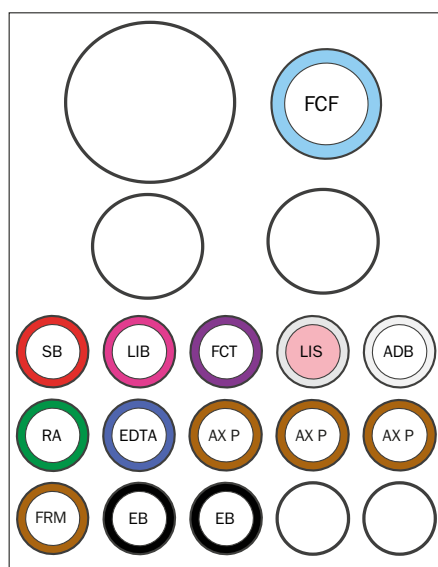


The Rapid Adapter (RA) used in this kit and protocol is not interchangeable with other sequencing adapters.

Rapid PCR Barcoding Kit 24 V14 (SQK-RPB114.24) contents

For automated applications, we recommend using the SQK-RPB114.24 kit in plate format with Rapid Barcode Primers at 1 μ M concentration.

Plate format with Rapid Barcode Primers at 1 μ M



RA :Rapid Adapter
 SB :Sequencing Buffer
 LIB :Library Beads
 LIS :Library Solution
 EB :Elution Buffer
 AX P :AMPure XP Beads

FCF :Flow Cell Flush
 FCT :Flow Cell Tether
 EDTA :EDTA
 FRM :Fragmentation Mix
 ADB :Adapter Buffer
 RPB : Rapid Barcode Primers 01-24

Name	Acronym	No. of vials	Cap colour	Fill volume per vial (µl)
Fragmentation Mix	FRM	1	Brown	160
Rapid Adapter	RA	1	Green	15
Adapter Buffer	ADB	1	Clear	100
AMPure XP Beads	AXP	3	Amber	1,200
Elution Buffer	EB	2	Black	500
EDTA	EDTA	1	Blue	700
Sequencing Buffer	SB	1	Red	700
Library Beads	LIB	1	Pink	600
Library Solution	LIS	1	White cap, pink label	600
Flow Cell Flush	FCF	1 bottle	Clear cap, light blue label	8,000
Flow Cell Tether	FCT	1	Purple	200
Rapid Barcode Primers (01–24) at 1 µM	RPB	-	2 plates, 3 sets of primer barcodes per plate	15 µl per well

Note: This product contains AMPure XP reagent manufactured by Beckman Coulter Inc. and can be stored at -20°C with the kit, without detriment to reagent stability.

Rapid Barcode Primers

Component	Sequence
Rapid Barcode Primer 01	AAGAAAGTTGTCGGTGTCTTTGTG
Rapid Barcode Primer 02	TCGATTCCGTTTGTAGTCGTCTGT
Rapid Barcode Primer 03	GAGTCTTGTGTCCCAGTTACCAGG
Rapid Barcode Primer 04	TTCGGATTCTATCGTGTTTCCCTA
Rapid Barcode Primer 05	CTTGTCAGGGTTTGTGTAACTT
Rapid Barcode Primer 06	TTCTCGCAAAGGCAGAAAGTAGTC
Rapid Barcode Primer 07	GTGTTACCGTGGAATGAATCCTT
Rapid Barcode Primer 08	TTCAGGGAACAAACCAAGTTACGT
Rapid Barcode Primer 09	AACTAGGCACAGCGAGTCTTGTT
Rapid Barcode Primer 10	AAGCGTTGAAACCTTTGTCCTCTC
Rapid Barcode Primer 11	GTTTCATCTATCGGAGGGAATGGA
Rapid Barcode Primer 12	GTTGAGTTACAAAGCACCGATCAG
Rapid Barcode Primer 13	AGAACGACTTCCATACTCGTGTGA
Rapid Barcode Primer 14	AACGAGTCTCTTGGGACCCATAGA
Rapid Barcode Primer 15	AGGTCTACCTCGCTAACACCACTG
Rapid Barcode Primer 16	CGTCAACTGACAGTGGTTCGTACT
Rapid Barcode Primer 17	ACCCTCCAGGAAAGTACCTCTGAT
Rapid Barcode Primer 18	CCAAACCAACAACCTAGATAGGC

Component	Sequence
Rapid Barcode Primer 19	GTTCTCGTGCAGTGTCAAGAGAT
Rapid Barcode Primer 20	TTGCGTCCTGTTACGAGAACTCAT
Rapid Barcode Primer 21	GAGCCTCTCATTGTCCGTTCTCTA
Rapid Barcode Primer 22	ACCACTGCCATGTATCAAAGTACG
Rapid Barcode Primer 23	CTTACTACCCAGTGAACCTCCTCG
Rapid Barcode Primer 24	GCATAGTTCTGCATGATGGGTTAG

3. ElysION SQK-RPB114.24 sample sheet setup

Sample sheet information

The sample sheet assigns a barcode to each sample, allowing you to pick a range from the 96-well plate, and for a sample ID to be tracked from input to results.

For more information on setting up a sample sheet, refer to the [ElysION MinION user guide](#).

1 Set up your sample sheet as a CSV file with the following information:

Required field	User input	Definition / field info
v1		Version field, no input required outside of field
assay	rpb:vX.x	Assay ID and version
library_id	User-defined	Identification for the DNA library
created_by	User-defined	Identification of operator setting up sample sheet
created_at	User-defined	Date and time of creation. Follow the format outlined in the ElysION MinION user guide .
sample_count	User-defined	Number of samples processed in run (1 to 24 samples)
well_id	A1-H3	Positions in 96-well plate being used (1 to 24 samples) Samples must start from position A1 in the 96-well plate and run consecutively column-wise.
barcode	barcode01–barcode24	Rapid barcode primers from SQK-RPB114.24 being used in library preparation. Up to 24 barcodes are available in the sequencing kit. These should be used sequentially (e.g. Barcodes 01–12 or Barcodes 13–24).
sample_type	User-defined	Description or characteristics of sample input
barcode_set	set01, set02, set03	Each rapid barcode primer plate contains 3 sets of 24 barcodes. To select the first set (columns 01 to 03), enter set01 , for the second set (columns 05 to 07), enter set02 , or for the third set (columns 09 to 11), enter set03 .
sample_id	User-defined from LIMS system (lims-sampleid-12345)	Identification for each sample input (e.g. from LIMS system)

Required field	User input	Definition / field info
sample_volume	User-defined	Input sample volume to be automatically normalised by the robot to the required input volume using nuclease-free water.

Below is an example of a sample sheet CSV file:

	A	B	C	D	E	F
1	v1					
2						
3	assay	rpb:v1.0				
4	library_id	RPB				
5	created_by	labuser01				
6	created_at	2026-04-09T14:07:14+01:00				
7	sample_count	24				
8						
9	well_id	barcode	sample_type	barcode_set	sample_id	sample_volume
10	A1	barcode01	sample	set01	1	6
11	B1	barcode02	sample	set01	2	6
12	C1	barcode03	sample	set01	3	6
13	D1	barcode04	sample	set01	4	6

4. ElysION run setup

The automated library preparation and sequencing method using the ElysION device can be followed using the on-screen display on the device.

Ensure you have all the correct hardware components, software packages, and workflows installed to carry out this method.

For more information on the device and the processes, refer to the [ElysION MinION user guide](#).

- 1 Thaw the kit components at room temperature, spin down briefly using a microfuge and mix by pipetting as indicated in the Table below:**

Reagent	1. Thaw at room temperature	2. Briefly spin down	3. Mix well by pipetting
Rapid Barcode Primers in plate (RPB01–24 at 1 µM)	✓	✓	-
Fragmentation Mix (FRM)	Not frozen	✓	✓
Rapid Adapter (RA)	Not frozen	✓	✓
LongAmp Hot Start Taq 2X Master Mix	✓	✓	✓
AMPure XP Beads (AXP)	✓	✓	Mix by pipetting or vortexing immediately before use
EDTA (EDTA)	✓	✓	✓
Elution Buffer (EB)	✓	✓	✓
Adapter Buffer (ADB)	✓	✓	Mix by vortexing
Flow Cell Flush (FCF)	✓	✓	✓
Flow Cell Tether (FCT)	✓	✓	✓
Sequencing Buffer (SB)	✓	✓	✓
Library Beads (LIB)	✓	✓	Mix by pipetting or vortexing immediately before use
Bovine Serum Albumin (BSA)	-✓	✓	✓
Wash Mix (WMX)	Not frozen	✓	✓
Wash Diluent (DIL)	✓	✓	Mix by vortexing
Storage Buffer (S)	✓	✓	Mix by vortexing



The wells of the barcoding plate are intended for single use only. Ensure your barcode well is sealed before use. Do not reuse the barcode wells.

2 Switch on the ElysION device and its computer following the user guide.

The Background Services application (BotQL) should start up automatically in the background when starting up the device.

3 Ensure the Background Services application is running.

If the UI is unable to connect to the instrument, open the **Services** application using the Windows search bar. Scroll down to the **BotQL** application which should show as running. If the application is not running, press **Start**. To restart the application, press **Restart**. Wait a few seconds before reopening the UI.

4 Open the ElysION UI application and wait for a few seconds for the UI to connect to the liquid handler.



Ensure that the ElysION deck is clear of any labware before performing automated checks.

Failure to ensure the deck is clear can lead to errors in checks or damage to the equipment.

5 Close the door to the robot and select "Initialise" on the display.

The ElysION device will perform automated checks.

Robot initialisation must complete before proceeding with the library preparation method.

Method variables

Refer to the [ElysION MinION user guide](#) for more details.

If you wish to alter the default variables for your assay, select the **System** tab on the bottom left of the screen, then **Method configurator**. Then select the method that you wish to modify.

The variables for the Rapid PCR Barcoding protocol are detailed below:

Variable name	Default parameter	Parameter range	Description
Fragmentation Time - Step 1 (unit = seconds)	120 s	Min = 60 s Max = 240 s	Allows you to adjust the length of the 30°C hold on the On-Deck Thermal Cycler (ODTC) during fragmentation.
Fragmentation Time - Step 2 (unit = seconds)	120 s	Min = 60 s Max = 240 s	Allows you to adjust the length of the 80°C hold on the ODTC during fragmentation.
Number of PCR Cycles	14	Min = 1 Max = 40	Allows you to adjust the number of PCR cycles.
Pause for QC post PCR	0 = off	0 = off 1 = on	Enabling this feature will instruct the ElysION to pause post PCR, allowing normalisation of samples.
AMPure XP Bead Clean-up Ratio	0.6X	0.4X, 0.6X, 0.8X, 1.0X, 1.2X	Allows you to adjust the ratio of AXP bead volume to sample volume during the clean-up step. For example, for the default value of 0.6, 60 µl of beads will be added to 100 µl of pooled samples. For a value of 1.0, 100 µl of beads will be added to 100 µl of pooled samples.
Bead Binding Time (unit = minutes)	5 min	Min = 2 min Max = 30 min	Allows you to adjust the bead binding time.
Bead Elution Time (unit = minutes)	15 min	Min = 2 min Max = 30 min	Allows you to adjust the bead elution time before pelleting on the magnet.
Pause for QC pre Flow Cell Loading	0 = off	0 = off 1 = on	Enabling this feature will instruct the ElysION to pause prior to the final eluate collection, allowing sample normalisation to a desired concentration, before automated flow cell loading.

Recommendations for the "Pause for QC" variable when enabled are provided at the end of this section.

6 Select "Run method" on the on-screen display.

7 Select the method "Rapid PCR Barcoding Kit" on the on-screen display.

If you have created a modified assay, it will display as: **Rapid PCR Barcoding Kit.[version number]**

Click **Next** to proceed.



If you require data analysis to be automatically performed after sequencing, select the "Metagenomics" assay.

Then click **Next** to proceed.

8 Select the MinION Mk1D position that you wish to load and insert a MinION/GridION Flow Cell into the correct location on the deck as shown on the on-screen display.

Instructions for inserting a flow cell onto the ElysION device can be found in the [ElysION MinION user guide](#).

9 Select "Run checks" on the on-screen display.

You may check three flow cells simultaneously on the ElysION deck if you wish to set up interleaving runs. However, any unused flow cells should be returned to the fridge after checking, until required for the subsequent library preparation.

Refer to the end of this section for interleaving run setup.

Note: If your flow cell does not reach the required number of pores for your run, insert a new flow cell and repeat the flow cell check before proceeding with the run setup.

10 Select "Import" on the on-screen display to upload your sample sheet.

Once completed, click **Next** to proceed.

11 Prepare the reagents in accordance with the reagent preparation page on the on-screen display.

Once completed, click **Next** to proceed.

12 Once your flow cell check has successfully completed, set up your sequencing run conditions on the on-screen display.

1. Select the sequencing time limit, or stop the run when the flow cell pores are depleted.
2. Select a data target.
3. Select whether to perform:
 - A flow cell flush to return the used flow cell to Oxford Nanopore.
 - Or a flow cell wash using the Flow Cell Wash Kit (EXP-WSH004) to reuse your flow cell.
4. Select whether write out to a POD5 file format is turned on or off.

Volumes for reagents and guidelines for flow cell wash or flush setup will be provided on the deck loading page. If you wish to perform a flow cell wash for reuse, it is recommended to thaw the wash reagents at the same time as the library preparation reagents.

For more information on the run conditions, refer to the [ElysION MinION user guide](#).



Reagents and consumables requirements

Consider the following when preparing and loading your reagents and consumables into the ElysION device to mitigate risk of workflow failure and equipment damage:

- Correct consumables are used with each reagent.
- Spin down all samples and reagents, making sure they do not contain bubbles.
- Tip boxes are inserted correctly into the nest positions and sit flat against the deck.
- Plates are flat in their deck position and sit within the barriers on the deck, and tubes are fully inserted into the rack.

13 Set up the ElysION deck according to the deck layout outlined in the UI of the on-screen display.

The position of the tip boxes and reagents will change depending on the sample count and run setup. Follow the instructions on the on-screen display correctly for your run.

14 Insert two empty waste bins into the disposable waste drawer below the deck.

- 15 Close the door of the ElysION device.
- 16 Click "Begin run" to start the automated library preparation and sequencing.
- 17 Data analysis will automatically be carried out by the ElysION device, if the "Metagenomics" assay was selected.

The analysis pipeline is [wf-metagenomics](#).

For more information on how to access your sequencing data and the analysis outputs, consult the [ElysION MinION user guide](#).

Pause for QC post PCR

If "Pause for QC post PCR" is enabled via the Method configurator during the run setup, the robot will pause for sample normalisation post PCR, with the PCR plate on position B3. Quantify 1 µl of barcoded prep using a Qubit fluorometer (or equivalent) for QC check.



Pause for QC post PCR sample normalisation

- We recommend normalising your samples so that 800 ng total DNA is pooled in an equimolar ratio by the robot.
- 11.5 µl is pooled per sample by the robot from at least 20 µl in the PCR plate.
- Normalise your samples to a concentration which when pooled (11.5 µl per sample) by the robot, results in 800 ng total DNA. There should be at least 20 µl of each normalised sample present in the same well location of a new PCR plate to account for the required dead volume.
- Spin down the PCR plate to ensure the samples do not contain bubbles and then place the plate in position B3 on the ElysION.
- To resume library preparation, press **Continue** on the on-screen display if using the 'Overview' page, or press **Resume** if using the 'Run Details' page.

Example for 8 samples:

$800 \text{ ng}/8 = 100 \text{ ng per sample per } 11.5 \text{ }\mu\text{l pooled by robot}$

$100 \text{ ng}/11.5 \text{ }\mu\text{l} = \sim 8.7 \text{ ng}/\mu\text{l}$

- Add a minimum of 20 µl of each sample at ~8.7 ng/µl to the same well location of a new PCR plate.

Pause for QC pre Flow Cell Loading

If "Pause for QC pre Flow Cell Loading" is enabled via the Method configurator during the run setup, the robot will pause prior to the final eluate collection, with the next step shown as "Pause for QC".



Pause for QC pre Flow Cell Loading

To QC your samples pre flow cell loading, we recommend the following:

- Quantify 1 µl of eluted sample using a Qubit fluorometer. The eluted sample is present in well A1 of the DeepWell plate (DWP) in position B1 on the deck.
- Make up 17.6 µl of 10–50 fmol eluate in well A1 of a new DWP using Elution Buffer (EB). Then spin down the plate to ensure the sample does not contain bubbles and place the plate in position B1 on the deck.
- If you have less than the minimum ng mass of DNA required to generate the minimum fmol amount of DNA in 17.6 µl, return the original DWP with the remaining 16.6 µl of library in EB to position B1 on the deck.
- To resume library preparation, press **Continue** on the on-screen display if using the 'Overview' page, or press **Resume** if using the 'Run Details' page.

Interleaving run setup

Once the first library preparation has completed and started sequencing, to set up a second run, select a new "Run Method" in the UI. Follow the on-screen instructions to unload any reagents and consumables that are not required from the deck and complete the deck setup for the new run.



Once your runs have completed, unload the labware according to the deck clearing instructions, empty the waste bins, and discard or store in the freezer any unused reagents.

5. Issues during the automated library preparation

ElysION device troubleshooting

For commonly encountered issues, refer to the [ElysION MinION user guide](#).

For in-depth troubleshooting, refer to the [ElysION Operating manual](#) provided with your device.

For additional customer support, contact the Oxford Nanopore support channel (support@nanoporetech.com).

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