S O P

10.3.5 Rapid sequencing: gDNA barcoding and library preparation

Objectives and scope

This SOP describes the various steps to follow to carry out rapid barcoding of genomic DNA (gDNA) using the Rapid Barcoding Kit 96 (SQK-RBK114.96) for sequencing purposes using the nanopore flow cells.

This SOP is intended for Mini-Lab laboratory technicians.

Principle

During this procedure the genomic DNA previously extracted (SOP 10.3.3) and quantified (SOP 10.3.4), is tagged using barcodes, i.e short DNA sequences (Rapid Barcodes contained in the kit); this process simultaneously attaches a pair of barcodes to the DNA fragments. All the samples are barcoded with the same barcodes and then mixed. After, sequencing adapters, supplied in the kit, are attached to the DNA ends (Diagram 1). These adapters are used for sequencing on the Nanopore Flow Cells. Steps required, timings and stopping options are in Table 1 at the end of the procedure.

Safety and environment

- Wear your PPE for the duration of this technique: lab coat, gloves;
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

Sample

- Type of material:
  - 200ng high molecular weight genomic DNA per sample (SOP 10.3.4)

Equipment

Common Name	*Associated SOP*
Freezer -20°C	SOP-7.7-REFCON
Ice bucket with ice	N/A
Microplate centrifuge	TBD
Thermal cycler or 2 heat blocks	TBD
Vortex	TBD
Vortex Adapter for 24 (1.5–2.0 ml) tubes	TBD
Magnetic rack	TBD
MinION Mk1D	

Reagents & Consumables

*Common Name**	*Storage conditions*
Rapid Barcoding Kit 96 (SQK-RBK114.96)	*-20°C*
MinION Flow Cell	*2-8°C*
Nuclease-free water (e.g. ThermoFisher, AM9937)	*Room temperature*
Denatured ethanol 95%	*Room temperature*
Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)	*-20°C*
Gloves	*N/A*
1.5 ml Eppendorf DNA LoBind tubes	*N/A*
2 ml Eppendorf DNA LoBind tubes	*N/A*
0.2 ml thin-walled PCR tubes	*N/A*
P1000 pipette and tips	*N/A*
P100 pipette and tips	*N/A*

Commenté [1]: estimated quant depends on DNA concentration. Per sample 10 ul input (DNA + mol grade water as diluent) and 400 ul for preparing 80% ethanol, max 1,5 ml

Rapid Barcoding Kit 96 (SQK-RBK114.96) Contents

Commenté [2]: is this the right kit?

Commenté [3]: yes

Name	*Acronym*	*Cap colour*	*N of vials*	*Fill volume per vial (µL)*	*Storage conditions*
Rapid Adapter	**RA**	*Green*	2	15	
Adapter Buffer	**ADB**	*Clear*	1	100	
AMPure XP Beads	**AXP**	*Amber*	3	1,200	
Elution Buffer	**EB**	*Black*	1	1,500	
Sequencing Buffer	**SB**	*Red*	1	1,700	
Library Beads	**LIB**	*Pink*	1	1,800	
Library Solution	**LIS**	*White cap, pink label*	1	1,800	
Flow Cell Flush	**FCF**	*Clear*	1	15,500	
Flow Cell Tether	**FCT**	*Purple*	2	200	
Rapid Barcodes	**RB01-96**	-	3 plates	8 µl per well	

Library Preparation

Before starting

1. Program the thermal cycler: 30°C for 2 minutes, then 80°C for 2 minutes/ OR set two thermal blocks next to each other, one at 30°C and one at 80°C
2. Thaw kit components at room temperature, spin down briefly using a microcentrifuge and mix by pipetting as indicated by the table below:

Commenté [4]: if we don't have one in the MIni-Lab

Commenté [5]: we don't have any experience with heath blocks. I think it will work if you have two heath blocks, 1 on 30 degrees and one on 80. It takes too much time to go from 30-80 degrees. i think heath blocks are better than sand.

Reagent	1. Thaw at room temp	2. Briefly spin down	3. Mix well by pipetting
Rapid Barcodes	Not frozen	✓	✓
Rapid Adapter (RA)	Not frozen	✓	✓
AMPure XP Beads (AXP)	✓	✓	Mix by pipetting or vortexing immediately before use
Elution Buffer (EB)	✓	✓	✓
Adapter Buffer (ADB)	✓	✓	Mix by vortexing

Prepare the DNA in nuclease-free water

3. Transfer 200 ng of genomic DNA per sample into 0.2 ml thin-walled PCR tubes
4. Adjust the volume of each sample to 10 µl with nuclease-free water.
5. Pipette mix the content of the tubes 10-15 times to avoid unwanted shearing
6. Spin down briefly in a microfuge
7. In the 0.2 ml thin-walled PCR tubes, mix the following:

Reagent	Volume per sample
Template DNA (200 ng from previous step)	10 µl
Rapid Barcodes	1.5 µl
Total	**11.5 µl**

8. Ensure the components are well mixed by pipetting and spin down briefly
9. Incubate the tubes at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tubes or plate on ice to cool.
10. Spin down the tubes or plate to collect the liquid at the bottom
11. Pool all barcoded samples, noting the total volume

	Vol per sample	*X4*	*x12*	*x24*	*x48*	*x96*
Tot Volume	11.5µl	46µl	138µl	276µl	552µl	1,104 µl

12. Resuspend the AMPure XPBeads (AXP) by vortexing

Ensure you have sufficient capacity in your reaction tube for all the reagents.
Limit the volume taken forward of pooled barcoded sample to 1,000 µl (i.e. half the capacity of the 2 ml Eppendorf DNA LoBind tube) to ensure feasibility of the next step

13. To the entire pooled barcoded sample from Step 11, add an equal volume of resuspended AMPure XPBeads (AXP) and mix by flicking the tube with your finger
14. Incubate on a mixer (rotator mixer) for 10 minutes at room temperature
15. Prepare at least 2 ml of fresh 80% ethanol in nuclease-free water
16. Spin down the sample and pellet on a magnet. Keep the tube on the magnetic block, and pipette off the supernatant.
17. Keep the tube on the magnet and wash the beads with 1.0 ml of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.
18. Repeat step 17.
19. Briefly spin down and place the tube back on the magnet. Pipet off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking.
20. Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB) per 24 barcodes used.

Note: Always elute your sample in at least 15 µl of Elution Buffer (EB). The elution volume can be adjusted for over 24 barcodes by following the table below:

Commenté [6]: can the denatured ethanon 95% present in the minilab be used for that? I think so

Commenté [7]: yes

	For 01-24 barcodes	*For 48 barcodes*	*For 72 barcodes*	*For 96 barcodes*
Volume of Elution Buffer (EB)	15µl	30µl	45µl	60µl

21. Incubate for 10 minutes at room temperature (between 18 and 23 °C).

22. Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.

23. Remove and retain the full volume of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
    - ❖ Remove and retain the eluate which contains the DNA library in a clean 1.5 ml Eppendorf DNA LoBind tube
    - ❖ Dispose of the pelleted beads

CHECKPOINT:

Quantify 1 µl of eluted sample using a Qubit fluorometer

24. Transfer 11 µl of the sample into a clean 1.5 ml Eppendorf DNA LoBind tube.

25. In a fresh 1.5 ml Eppendorf DNA LoBind tube, dilute the Rapid Adapter (RA) as follows and pipette mix:

Reagent	Volume
Rapid Adapter (RA)	1.5 µl
Adapter Buffer (ADB)	3.5 µl
Total	**5 µl**

26. Add 1 µl of the diluted Rapid Adapter (RA) to the barcoded DNA.

27. Mix gently by flicking the tube and spin down.

28. Incubate the reaction for 5 minutes at room temperature.

    The prepared library is used for loading into the flow cell.

    Store the library on ice until ready to load.

Tip: while this incubation step is taking place you can proceed to the Flow Cell priming and loading (SOP 10.3.6).

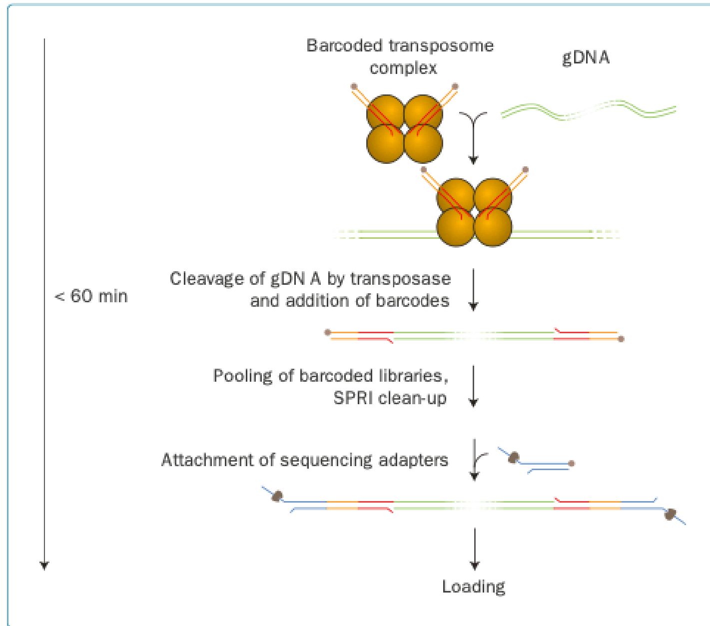

Diagram 1. The process of barcoding and attachment of adapters to genomic DNA.

Library preparation step	Process	Time	Stop option
DNA barcoding	Tagmentation of the DNA using the Rapid Barcoding Kit V14	15 minutes	4°C overnight
Sample pooling and clean-up	Pooling of barcoded libraries and AMPure XP Bead clean-up	25 minutes	4°C overnight
Adapter ligation	Attach the sequencing adapters to the DNA ends	5 minutes	We strongly recommend sequencing your library as soon as it is adapted
Priming and loading the flow cell	Prime the flow cell and load the prepared library for sequencing	5 minutes	

Table 1. Overview of the steps required in the library preparation, including timings and stopping points.

Related documents

- Commercial SOP: Rapid sequencing DNA V14 - barcoding (SQK-RBK114.24 or SQK-RBK114.96) - version V RBK_9176_v114_revR_04Jun2025

Internal SOPs

- SOP-10.3.3. DNA extraction for sequencing
- SOP 10.3.4: DNA quantification with Qubit
- SOP 10.3.6. Oxford Nanopore Sequencing – priming and loading a flow cell
- DOC-6.8-DECHINT: 6.8 Internal waste management