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Rapid Barcoding Protocol V14

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We use this protocol and it's working

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Abstract

Preparation of DNA for sequencing using the Oxford Nanopore Technologies Rapid Barcoding kit

Materials

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- 100 ng gDNA per sample for >4 samples
- OR 200 ng gDNA per sample for <_4 samples
- Rapid Barcoding Kit 24 or 96 V14 comprising:
- Rapid Barcodes (RB01-24) or Rapid Barcode Plate (RB01-96)
- Rapid Adapter (RA)
- Adapter Buffer(ADB)
- AMPure XP Beads (AXP)
- Elution Buffer (EB)
- Flow Cell Flush (FCF)
- Flow Cell Tether (FCT)
- Library Solution (LIS)
- Library Beads (LIB)
- Sequencing Buffer (SB)

Consumables

- 0.2 ml thin-walledPCR tubes
- PCR plate 96 LoBind with heat seals
- 1.5 ml Eppendorf DNA LoBind tubes
- 2 ml Eppendorf DNA LoBind tubes
- Nuclease-free water
- Freshly bprepared 80% ethanol in nuclease-free water
- Qubit Assay Tubes (Invitrogen, Q32856)
- Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)
- SpotON Flow Cell
- Bovine Serum Albumin (BSA) (50 mg/ml)

Equipment

- Ice bucket with ice
- Timer
- Thermal cycler
- Microcentrifugeplate
- Magnetic rack
- Gentle rotator mixer
- P1000, 200, 100, 20, 10, 2 pipette and tips
- Multichannelpipette
- Qubit fluorometer (or equivalent for QC check)
- MinION Device

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

	Reagent	1. Thaw at room temperature	2. Briefly spin down	3. Mix well by pipetting
	Rapid barcodes	Not frozen	Yes	Yes
	Rapid adaptor (RA)	Not frozen	Yes	Yes
	AMPure Beads	Yes	Yes	Mix by vortexing
	Elution Buffer (EB)	Yes	Yes	Yes
	Adaptor Buffer (ADB)	Yes	Yes	Mix by vortexing

2 Prepare the DNA in nuclease-free water.
Note: Sample input quantity will vary depending on the number of samples you wish to barcode and sequence.

2.1 a. Transfer your gDNA into 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind:

2.2 b. For >4 samples transfer 100 ng genomic DNA per sample into each tube/well

2.3 c. For ≤4 samples transfer 200 ng genomic DNA per sample into each tube/well

2.4 d. Adjust the volume to 10 µl with nuclease-free water

2.5 e. Pipette mix the tubes for 10-15 times to avoid unwanted shearing

2.6 f. Spin down briefly in a microfuge

3 In the 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind, mix the following:

	Reagent	Volume per sample (uL)
	Template DNA	10
	Rapid barcodes (one for each sample)	1
	Total	11

4 Ensure the components are thoroughly mixed by pipetting and spin down briefly.

5 Incubate the tubes or plate at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tubes or plate on ice to cool.

6 Spin down the tubes or plate to collect the liquid at the bottom.

7 Pool all barcoded samples in a clean 5 ml Eppendorf DNA LoBind tube, noting the total volume.

	Volume per sample (uL)	For 12 samples (uL)	For 24 samples (uL)	For 48 samples (uL)	For 96 samples (uL)	F
	11	132	264	528	1056	

8 Resuspend the AMPure XP Beads (AXP) by vortexing.

9 To the entire pooled barcoded sample, add an equal volume of resuspended AMPure XP Beads (AXP) and mix by flicking the tube.

10 Incubate on a Hula mixer (rotator mixer) for 5 minutes at room temperature.

11 Prepare at least 3 ml of fresh 80% ethanol in nuclease-free water.

12 Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.

13 Keep the tube on the magnet and wash the beads with 1.5 ml of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.

14 Repeat the previous step.

15 Briefly spin down and place the tube back on the magnet. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellet to the point of cracking.

16 Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB). Incubate for 10 minutes at room temperature.

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

18 Remove and retain 15 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.

19 Dispose of the pelleted beads

20 Quantify 1 µl of eluted sample using a Qubit fluorometer.

21 Transfer 11 µl of the sample into a clean 1.5 ml Eppendorf DNA LoBind tube.
Note: We recommend transferring a maximum of 800 ng of the DNA library.
If necessary, take forward only the necessary volume for 800 ng of DNA library and make up the rest of the volume to 11 µl using Elution Buffer (EB).

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

	Reagent	Volume (uL)
	Rapid adapter (RA)	1.5
	Adapter Buffer (ADB)	3.5
	Total	5

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

24 Mix gently by flicking the tube, and spin down.

25 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.

26 Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and one tube of Flow Cell Flush (FCF) at room temperature before mixing by vortexing. Then spin down and store on ice.

27 To prepare the flow cell priming mix with BSA, combine the following reagents and pipette mix at room temperature: Note: The vials of Flow Cell Flush (FCF) in kit SQK-RBK114.24 and SQK-RBK114.96 have different formats. Please ensure you are using the correct volume when preparing your flow cell priming mix.

If using SQK-RBK114.24: The reagents can be added directly to the single-use tube of Flow Cell Flush (FCF).

If using SQK-RBK114.96: Prepare the reagents in a suitable tube.

	Reagents	Volume per flow cell (uL)
	Flow cell Flush (FCF)	1170
	Bovine Serum Albumin (BSA) at 50 mg/mL	5
	Flow Cell Tether (FCT)	30
	Total volume	1205

28 Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.

29 Slide the flow cell priming port cover clockwise to open the priming port.

Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

30 After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

30.1 Set a P1000 pipette to 200 µl

30.2 Insert the tip into the priming port

30.3 Turn the wheel until the dial shows 220-230 ul, to draw back 20-30 ul, or until you can see a small volume of buffer entering the pipette tip

30.4 Note: Visually check that there is continuous buffer from the priming port across the sensor array.

31 Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. During this time, prepare the library for loading by following the steps below.

32 Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

33 In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

	Reagent	Volume per flow cell (uL)
	Sequencing Buffer (SB)	37.5
	Library Beads (LIB)	25.5
	DNA Library	12
	Total	75

34 Complete the flow cell priming:

34.1 Gently lift the SpotON sample port cover to make the SpotON sample port accessible.

34.2 Load 200 µl of the priming mix into the flow cell priming port (not the SpotON sample port), avoiding the introduction of air bubbles.

35 Mix the prepared library gently by pipetting up and down just prior to loading.

36 Add 75 µl of the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.

37 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.

Protocol references

Based on the Oxford Nanopore Technologies Rapid Barcoding Kit 24 V14 (SQK-RB114.24) protocol