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 We use this protocol in our workspace and it is working.

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Modified 1D Native Barcoding genomic DNA protocol from the Temperton Lab (University of Exeter)

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ABSTRACT

Modified 1D Native Barcoding genomic DNA protocol (v. NBE_9006_v103_revO_21Dec2016 for SQK-LSK108) from the Temperton Lab (University of Exeter)

MATERIALS

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- ⊗ Blunt/TA Ligase Master Mix - 50 rxns **New England Biolabs Catalog #M0367S**
- ⊗ NEBNext Ultra II End Repair/dA-Tailing Module - 24 rxns **New England Biolabs Catalog #E7546S**
- ⊗ Ampure XP beads **Beckman Coulter Catalog #A63881**
- ⊗ Native Barcoding Kit 1D (EXP-NBD103) **Contributed by users**
- ⊗ Ligation Sequencing Kit 1D **Contributed by users Catalog #SQK-LSK108**
- ⊗ Qubit® dsDNA HS assay kit, 100 reactions **Life Technologies Catalog #Q32851**
- ⊗ g-TUBE **Covaris Catalog #520079**
- ⊗ DNA LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108051**
- ⊗ Qubit™ dsDNA BR Assay Kit **Thermo Fisher Catalog #Q32853**

Keywords: MinION
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Barcoding genomic DNA
protocol,
NBE_9006_v103_revO_21Dec
2016, Genome sequencing

DNA Fragmentation

- 1 Load 46 μL of genomic DNA into a Covaris g-TUBE.
- 2 Spin the g-TUBE for 1 minute in an Eppendorf 5424 centrifuge.
 - 2.1 To achieve $\sim$ XXX bp, spin the tube at 6000 x g.
- 3 Invert the tube and spin again for 1 minute.
- 4 Transfer volume to sterile 1.5 mL Eppendorf DNA LoBind tubes.
*Prior to the next step be sure to analyze your DNA for size, quantity, and quality.

Library Preparation

- 5 Combine: 45 μL genomic DNA, 7 μL Ultra II End-prep reaction buffer, 3 μL Ultra II End-prep enzyme mix, 5 μL of Nuclease-free water.
- 6 Mix tube gently by flicking, then spin down.

- 7 Transfer volume to a new 0.2 mL PCR tube.
- 8 Incubate for 30 minutes at 20°C and 30 minutes at 65°C.
- 9 Add 60 µL of AmPure XP beads and mix by pipetting.
- 10 Incubate for 5 minutes at room temperature (RT), while flicking occasionally.
- 11 Spin down the sample and pellet on the magnet.
- 12 Pipette off supernatant while on the magnet, avoiding pellet.
- 13 Wash beads with 200 µL of 80% ethanol (EtOH). Do not disturb the pellet. Remove EtOH.
- 14 Repeat step 13

- 15** Spin down the tube and place the tube back in the magnetic rack.
- 16** Remove any residual ethanol.
- 17** Allow beads to briefly dry.
- 18** Remove the tube from the magnetic rack, and resuspend in 25 μ L of prewarmed 55°C nuclease-free water.
- 19** Incubate for 2 minutes at 55°C.
- 20** Pellet beads on magnet until eluate is clear.
- 21** Pipette off 25 μ L of eluate into 1.5 mL Eppendorf LoBind DNA tube.
- 22** Quantify 1 μ L of eluate using Qubit HS dsDNA kit.
- 23** Thaw barcodes at RT.

- 24** Add 22.5 μL of end-prepped DNA, 2.5 μL Native barcode, and 25 μL of Blunt/TA ligase Master Mix.
- 25** Mix gently by flicking tube and spin down.
- 26** Incubate reaction mixture for 10 minutes at RT.
- 27** Add 50 μL of AMPure XP beads to the reaction mixture and mix by pipetting.
- 28** Incubate for 5 minutes at 55°C while occasionally flicking to mix.
- 29** Spin down the sample and pellet on the magnetic rack.
- 30** Wash beads with 200 μL of 80% EtOH. Do not disturb the pellet. Remove EtOH.
- 31** Pipette off supernatant, avoiding pellet.

- 32 Repeat step 31
- 33 Spin down the tube and place the tube back in the magnetic rack.
- 34 Remove any residual EtOH.
- 35 Allow beads to briefly dry.
- 36 Remove the tube from the magnetic rack, and resuspend in 25 μ L of prewarmed 55°C nuclease-free water.
- 37 Incubate for 2 minutes at 55°C.
- 38 Pipette off 25 μ L of eluate into 1.5 mL Eppendorf LoBind DNA tube.
- 39 Quantify 1 μ L of eluate using the Qubit HS dsDNA kit.

Mix together 22.5 μ L of end-prepped DNA, 2.5 μ L of Native barcode, and 25 μ L of Blunt/TA ligase

- 40** Master Mix (in that order).
- 40.1** Mix by gently flicking.
- 41** Spin the tube down.
- 42** Incubate the reaction for 10 minutes at RT.
- 43** Add 50 μL of resuspended AMPure XP beads to the reaction and mix by pipetting.
- 44** Incubate at 55°C for 5 minutes while occasionally flicking to mix.
- 45** Spin down sample(s) and pellet on the magnetic rack.
- 46** Pipette off the supernatant.
- 47** Wash beads with 200 μL of 80% EtOH. Do not disturb the pellet. Remove EtOH.

- 48 Repeat step 47
- 49 Spin down the tube and place the tube back in the magnetic rack.
- 50 Remove any residual EtOH.
- 51 Allow the beads to briefly dry.
- 52 Remove the tube from the magnetic rack, and resuspend in 26 μ L of nuclease-free water.
- 53 Incubate for 2 minutes at RT.
- 54 Pellet beads on the magnet until clear and colorless.
- 55 Transfer 26 μ L into clear, sterile 1.5 mL Eppendorf DNA LoBind tube.

- 56 Quantify 1 μL of eluate using Qubit HS dsDNA kit.
- 57 Pool equimolar amounts of each barcoded sample into a 1.5 mL Eppendorf DNA LoBind tube ($< 2 \mu\text{g}$).
- 58 Quantify 1 μL of eluate using Qubit HS dsDNA kit.
- 59 Dilute pooled sample to 50 μL in Nuclease-free water.

Pooled Library Preparation

- 60 Mix together 50 μL pooled barcoded samples sample, 20 μL barcode Adapter Mix (BAM), 20 μL Blunt/TA Master Mix, 10 μL Quick T4 DNA ligase.
 - 60.1 Mix by flicking between each sequential addition.
- 61 Mix by flicking the tube.
- 62 Spin down the sample.

- 63 Incubate the reaction for 10 minutes at RT.
- 64 Add 40 μL of resuspended AMPure XP beads to the adapter ligation reaction and mix by pipetting.
- 65 Incubate for 5 minutes at RT, occasionally mixing by flicking.
- 66 Place on a magnetic rack and allow beads to pellet.
- 67 Pipette off supernatant.
- 68 Wash beads with 140 μL of ABB buffer.
- 69 Resuspend beads in ABB buffer by flicking the tube.
- 70 Pellet beads on the magnetic rack.
- 71 Remove residual ABB buffer.

- 72** Repeat step 68 - 71
- 73** Remove the tube from the magnetic tube and resuspend pellet in 15 μ L of Elution buffer.
- 74** Incubate for 10 minutes at RT.
- 75** Pellet beads on the magnetic tube rack until the eluate is clear and colorless.
- 76** Pipet up and retain the 15 μ L of eluate into a clean 1.5 mL Eppendorf DNA LoBind tube
- 77** Place the tube of eluate (library) on ice until required for library loading
- 77.1** Use 1 μ L of DNA library to Quantify using Qubit HS dsDNA assay kit.