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

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Protocol status: Working
We use this protocol in our workspace and it is working.

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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

MATERIALS

- 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)
- Ligation Sequencing Kit 1D **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

Troubleshooting

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 ~ XXX bp, spini 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 µL 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 µL of eluate into 1.5 mL Eppendorf LoBind DNA tube.

22 Quantify 1 µL 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 µL of prewarmed 55°C nuclease-free water.

37 Incubate for 2 minutes at 55°C.

38 Pipette off 25 µL of eluate into 1.5 mL Eppendorf LoBind DNA tube.

39 Quantify 1 µL of eluate using the Qubit HS dsDNA kit.

40 Mix together 22.5 µL of end-prepped DNA, 2.5 µL of Native barcode, and 25 µL of Blunt/TA ligase 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 µL of nuclease-free water.

53 Incubate for 2 minutes at RT.

54 Pellet beads on the magnet until clear and colorless.

55 Transfer 26 µL 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 µg).

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 µL 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 µL of eluate into a clean 1.5 mL Eppendorf DNA LoBind tube

77 Place the tube of eluate (library) on ice until required fro library loading

77.1 Use 1 µL of DNA library to Quantify using Qubit HS dsDNA assay kit.