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PathoSense

2025

CONFIDENTIAL

Wet lab flow diagnostics PathoSense

WET LAB V - ONT

Preparation of the library and loading of the flow cell

Timing ~ 45 min

Overview

DNA fragmentation and barcoding

DNA clean-up using AMPure XP beads

Adapter Ligation and loading

DNA fragmentation and barcoding

Reagents

- SQK-RBK114.24 ONT
- Ethanol
- Bovine Serum Albumine (no recombinant!)

Materials

- DNA-Lobind 1.5 mL Eppendorf tubes (3)
- 0.2 mL thin-walled PCR tubes (per sample +1)

- Magnetic rack
- Wide-bore tips

- All surfaces should be cleaned with Hibiscrub, 70% Ethanol, and 10% Bleach in this order of cleaning. Prevent contamination.
- Aerosol-barrier tips should be used throughout the entire procedure.
- Perform all steps in the dedicated sample preparation area.

Preparations

- Pre-heat the **heat block to 50°C**

Protocol

1. Transfer **15 µl sample** in a 0.2 ml thin-walled PCR tube
2. Quick spin the **Fragmentation Mix RB01-24,** one barcode for each sample
3. Add **2.5 µl Fragmentation Mix RB01-24** to each sample (add a different barcode to each sample)
4. **Mix gently** by tapping the tube with your fingers, and spin down
5. Incubate the tube at **30°C for 2 minutes** and then at **80°C for 2 minutes** in a thermocycler

Immediately transfer to ice

DNA clean-up using AMPure XP beads

6. Quickly spin the samples
7. Pool the samples together in a 1.5 mL Eppendorf LoBind tube
8. Resuspend the **AMPure XP beads** by vortexing and quick spin
9. Use wide-bore tips to add an equal volume of resuspended **AMPure XP beads** to the pooled sample (1:1 ratio, 210 µL for 12 samples)
10. **Gently mix** the suspension by tapping the tube with your fingers and **quick spin**
11. **Incubate** the suspension for **5 minutes** at **room temperature**

In the meanwhile prepare the fresh 80% ethanol:

- It is important to prepare **fresh** 80% ethanol each time, since ethanol attracts water when kept too long

- Add **800 µL 100% ethanol** (Molecular grade) and **200 µL DNase/RNase- free water** in a 1.5 mL Eppendorf tube.

12. Place the sample centered into the **magnet holder**, and incubate for **2 minutes**

13. **Remove the supernatant** using a P200 pipette tip

14. Gently **wash the beads** using **500 µL fresh 80% ethanol**, without disturbing them

15. **Remove the ethanol**, and **repeat** the wash step (14)

16. **Remove the ethanol**, and spin the sample down

17. Place the sample in **magnet holder** and **remove the residual of the ethanol** with P20 pipette tip

18. **Incubate** the tubes with open lids for **MAX 5 minutes at 50°C** to evaporate all the ethanol and to dry the beads

    Incubate until a '**dry**' pellet is observed and no more ethanol is visible

19. When a 'dry' pellet is observed **add 13 µL Elution buffer** straight on the pellet (DO NOT PIPET)

20. **Tap the tube** to dissolve the magnetic beads, quick spin

21. Incubate at **room temperature for 2 minutes** to elute the DNA

22. Incubate for **2 more minutes** on the **magnetic rack**

23. Transfer the **13 µL of eluate** to a newly labeled Eppendorf Lobind tube

24. Place the tube once more on **the magnetic rack for 2 minutes**

25. **Transfer 11 µL of the supernatant** to a 0.2 mL thin-walled PCR tube

Library preparation - Adapter Ligation and loading

26. Quick spin the **Rapid Adapter (RA)** and the **Adapter Buffer (ADB)**

27. In a new 1.5 mL Eppendorf DNA LoBind tube, add **1.5 µL Rapid Adapter (RA)** and **3.5 µL Adapter Buffer (ADB)**, mix by tapping with fingers and quick spin

28. Add **1 µL of the RA/ADB mix** to 11 µL of barcoded DNA, mix by tapping with fingers and quick spin

29. Incubate the tube for **5 minutes at 21°C** in the thermocycler

30. Vortex and quick spin **Flow Cell Flush (FCF)** and **Flow Cell Tether (FCT)**

31. Add **30 µL Flow Cell Tether (FCT)** to the tube with **1.17 mL Flow Cell Flush (FCF)**

32. Add **5 µL Bovine Serum Albumine (BSA)** to the tube and put the tube on ice

33. Vortex **Library Beads (LIB)** and **Sequencing Buffer (SB).** Quick spin the sequencing buffer, do not spin the library beads.

34. Add **37.5 µL Sequencing Buffer (SB)** to a new tube

35. Add **25.5 µL Library Beads (LIB)** to the tube using wide bore tips

36. Add **12 µL DNA Library** to the tube

37. Open the **MinION** device lid and slide the flowcell under the clip

38. Perform a **flow cell check**.
    a. Go to the 'start' tab in the MinKNOW software
    b. Click on 'Flow cell check'
    c. Select the correct flow cell position
    d. Click on 'Start'

39. Open the device lid and **open the priming port** by sliding the priming port cover clockwise

40. Set a **P1000 pipette to 800 µl** and insert the tip into the **priming port**

41. Turn the wheel until the dial shows 820-830 µl, to **draw back 20-30 µl**, or until you can see a small volume of buffer entering the pipette tip

42. Load **800 µl of the priming mix** into the flow cell via the priming port, avoiding the introduction of air bubbles

43. Close the priming port and **wait for 5 minutes**

44. **Open the priming port** and gently **lifting the SpotON sample port** cover to make the SpotON sample port accessible

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

46. **Mix the library** gently by shaking it or pipetting it up and down before loading

47. Add **75 µl of sample** to the flow cell via the SpotON sample port in a dropwise fashion

48. Gently **replace the SpotON sample port** cover, making sure the bung enters the SpotON port, **close the priming port** and **close the MinION device lid**

Set up a sequence run on MinKNOW, proceed with

WET LAB VI - RUN SET-UP