LABORATORY OF MOLECULAR PATHOLOGY

MiniSeq denature and dilute guide

Standard manual normalization

Prep:

Prepare reagents

- ☐ 00 Cartridge needs to be thawed a day prior to sequencing. Store at 4 °C.
- ☐ 01 Prepare a fresh **0.1 N dilution** of NaOH from the 10 N stock solution.
  Perform a two-step, ten-fold dilution with water. The stock solution is viscous. Ensure proper mixing. Quick spin.
- ☐ 02 Thaw the **HT1** Hybridization Buffer. Quick vortex and spin. Keep it on ice before use.
- ☐ 03 Thaw the **PhiX** sequencing control. Quick vortex and spin. Keep it on ice before use.
- ☐ 04 Dillute sequencing primers to **50 µM** with water from their 100 µM stock.
- ☐ 05 Denature the sequencing primers by heating them to **95 °C for three minutes** in a cycler with a heated lid to 105 °C.
- ☐ 06 Immediately place the **primers into ice**. Do not allow them to cool slowly.

Library

- ☐ 07 Dilute the pooled and quantified library to **1 nM** using the RSB buffer.
- ☐ 08 Combine **5 µL** of the 1nM library and **5 µL of** the **0.1 N NaOH**.
- ☐ 09 Quick vortex and quick spin.
- ☐ 10 Incubate at room temperature for **5 minutes**.
- ☐ 11 Add **5 µL** of **200 µM Tris**-HCl, pH 7.0
- ☐ 12 Quick vortex and quick spin.
- ☐ 13 Add **985 µL** of chilled **Hybridization Buffer** to the denatured library. Vortex before.
  The total volume is 1 mL at 5 pM concentration.
- ☐ 14 Quick vortex and quick spin.
- ☐ 15 Transfer **140 µL** of the diluted library to a new tube.
- ☐ 16 Add **360 µL** of chilled Hybridization Buffer. Vortex before use.
  The total volume is 500 µL at 1.4 pM concentration.

PhiX control

- ☐ 17 Prepare a fresh 4 nM solution of PhiX from 10 nM stock or use previously diluted PhiX solution if available.
- ☐ 18 Combine **5 µL** of the 4nM PhiX solution and **5 µL of** the **0.1 N NaOH**.
- ☐ 19 Quick vortex and quick spin.

☐ 20 Incubate at room temperature for **5 minutes**.

☐ 21 Add **5 µL** of **200 µM Tris**-HCl, pH 7.0

☐ 22 Quick vortex and quick spin.

☐ 23 Add **985 µL** of chilled **Hybridization Buffer** to the denatured library. Vortex before.
The total volume is 1 mL at 20 pM concentration.

☐ 24 Quick vortex and quick spin.

☐ 25 Transfer **35 µL** of the diluted library to a new tube.

☐ 26 Add **465 µL** of chilled Hybridization Buffer. Vortex before use.
The total volume is 500 µL at 1.4 pM concentration.

Loading the MiniSeq

☐ 27 Combine the library and the PhiX control. Quick vortex and quick spin.
*The PhiX control should be added to the library so that its final concentration is between **5 and 15 %**. E.g. combine 450 µL of the library and 50 µL of the PhiX.*

☐ 28 Pierce the aluminium cover on the cartridge at position **16** using a **clean and empty 1 mL pipette tip**. Use firm downward pressure. When pierced clean the edges.

☐ 29 The resulting mixture of library and PhiX control is to be loaded in position **16** – marked by: *Load library here*.

☐ 30 Positions **24**, **25** and **28** also need to be pierced for the addition of custom primers.
Use clean and empty 1mL for each position. Beware of splashback.

☐ 31 Add the sequencing primers according to the table:

Primer	Position	Volume
Read 1 primer	24	3.3 µL
Read 2 primer	25	3.6 µL
Index 1 primer	28	4.9 µL
Index 2 primer	28	4.9 µL

☐ 32 **Mix** the positions 24, 25 and 28 with a 1mL pipette set to 400 µL by pipetting up and down. Make sure not to introduce an excess of bubbles.

☐ 33 Check the cartridge from below. Knock out any **bubbles** present down in the wells.

☐ 34 Remove the wash cartridge and insert the prepared sequencing cartridge.

☐ 35 Take out a fresh flow cell. Check the flow cell for specks of dust or any other dirt. **Clean the flow cell if necessary**. Be careful not to introduce any more dirt.

☐ 36 Be careful when inserting a new flow cell not to crack or damage it.