LABORATORY OF MOLECULAR PATHOLOGY

WGS library preparation

Using NEBNext Ultra II FS DNA Library Prep Kit for Illumina

Prep:

Fragmentation and end-repair

- ☐ 00 Thaw all the reagents. Quick vortex and quick spin. **Keep them on ice**.
  Bring magnetic beads to room temperature, at least 30°C prior usage.
  Prepare fresh 80% ethanol. *You will need 0.5 mL per sample.*
- ☐ 01 Check the Ultra II FS Reaction Buffer, make sure there is **no precipitation**.
- ☐ 02 **Vortex** the Ultra II FS Enzyme Mix for 5 seconds before using and keep it on ice.
- ☐ 03 Dilute your DNA sample to a total of **26 µL** with water. Ensure at **least 300 ng** of starting material – purified genomic DNA.
- ☐ 04 Combine the 26 µL of DNA with **7 µL Reaction Buffer** and **2 µL of Enzyme Mix** to have a total volume of 35 µL. Quick vortex and quick spin.
- ☐ 05 Incubate at **37 °C for 17 minutes, followed by 30 minutes at 65 °C** in a cycler with a heated lid to 75 °C. Hold at 4 °C afterwards.

Adaptor ligation

- ☐ 06 Combine the 35 µL reaction mixture with **30 µL Ligation Master Mix**.
  Add **1 µL Ligation Enhancer** and **2.5 µL of Illumina Adaptor** for a total volume of 68.5 µL.
  Ensure proper mixing. The ligation master mix is very viscous. Pipette accordingly.
- ☐ 07 Incubate at **20 °C for 15 minutes**.
- ☐ 08 Add **3 µL of USER Enzyme** to the ligation mixture.
- ☐ 09 Mix well and incubate at **37 °C for 15 minutes** with heated lid at 50 °C.

Size selection

- ☐ 10 Add **28.5 µL of 0.1× TE** to the mixture for a total volume of 100 µL.
- ☐ 11 Vortex the magnetic beads vigorously to properly resuspend them.
- ☐ 12 Add **40 µL of the magnetic beads** to the sample for a total volume of 140 µL. Mix the sample properly – vortex on high and follow with a very quick spin.
- ☐ 13 Incubate at room temperature for **5 minutes**.
- ☐ 14 Put the samples **on the magnetic** stand and incubate another **5 minutes** to separate the beads from the supernatant.
- ☐ 15 Transfer the supernatant into a new tube. **Do not discard the supernatant**. Discard the magnetic beads (those contain large DNA fragments).

☐ 16 Add **20 µL** of fresh resuspended **magnetic beads**. Mix the sample properly.

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

☐ 18 Put the samples **on the magnetic** stand and incubate for another **5 minutes**.

☐ 19 Remove and discard the supernatant. **Do not disturb or discard the beads**.

☐ 20 Add **200 µL** of **fresh 80% ethanol**. Incubate for 30 seconds. Remove the supernatant and do not disturb the beads. Keep the samples on the magnet.

☐ 21 **Repeat step 20 for a second wash** – add 200 µL of ethanol, wait 30 seconds and remove the ethanol. Do not disturb the beads.

☐ 22 Quickly spin the samples to collect residual ethanol. Put the samples on the magnet and remove the rest of the ethanol with a 10 µL pipette.

☐ 23 Air dry the beads if necessary. **Do not overdry the beads**. *The beads need to be dark brown and glossy. When the beads turn light brown and start to crack, it is too late.*

☐ 24 Remove the samples from the magnet and add **17 µL of 0.1× TE buffer**.

☐ 25 Mix well – vortex followed by a quick spin.

☐ 26 Incubate at RT for **5 minutes**. *Off the magnet.*

☐ 27 Put the samples **on the magnet** and incubate them for at least **2 minutes**.

☐ 28 Transfer **15 µL** of the supernatant to a new tube. Do not disturb the beads.

PCR enrichment

☐ 29 Combine the PCR reaction: 15 µL of the sample, **25 µL of Q5 Master Mix** and **5 µL of i7 primer** and **5 µL of i5 primer** for a total reaction volume of 50 µL.
Quick vortex and quick spin.
The i7 and i5 primers identify the sample. The combination of i5 and i7 must be unique for every sample. Watch out for barcode overlap with other samples and libraries.

☐ 30 Run the following PCR programme:

Amplification programme	Temperature	Time	
Initial denaturation	98 °C	30 seconds	
Denaturation	98 °C	10 s	**8×**
Annealing+Extension	65 °C	75 s	
Final extension	65 °C	5 min	
	4 °C	Hold	

PCR cleanup

- ☐ 31 Vortex the magnetic beads to resuspend them.
- ☐ 32 **Add 45 µL beads** to the 50 µL of the PCR mixture for a total volume of 95 µL
- ☐ 33 Incubate at room temperature for **5 minutes**.
- ☐ 34 Put the samples **on the magnetic** stand and incubate for another **5 minutes**.
- ☐ 35 Remove and discard the supernatant. **Do not disturb or discard the beads**.
- ☐ 36 Add **200 µL** of **fresh 80% ethanol**. Incubate for 30 seconds. Remove the supernatant and do not disturb the beads. Keep the samples on the magnet.
- ☐ 37 **Repeat step 20 for a second wash** – add 200 µL of ethanol, wait 30 seconds and remove the ethanol. Do not disturb the beads.
- ☐ 38 Quickly spin the samples to collect residual ethanol. Put the samples on the magnet and remove the rest of the ethanol with a 10 µL pipette.
- ☐ 39 Air dry the beads if necessary. **Do not overdry the beads**.
- ☐ 40 Remove the samples from the magnet and add **33 µL of 0.1× TE buffer**.
- ☐ 41 Mix well – vortex followed by a quick spin.
- ☐ 42 Incubate at RT for **5 minutes**. *Off the magnet.*
- ☐ 43 Put the samples **on the magnet** and incubate them for at least **2 minutes**.
- ☐ 44 Transfer **30 µL** of the supernatant to a new tube. Do not disturb the beads.

- ☐ 45 Measure the concentration using Qubit.
- ☐ 46 Asses library quality using FragmentAnalyzer.