Metagenomic library preparation

DNA Library Prep – Illumina DNA Prep Kit

Target insert size: ~150 bp

Final library size: ~600 bp (for 2×150 bp reads)

DNA input: 150 ng per sample

DNA Quantification & Preparation

- Quantify DNA using Qubit dsDNA HS Assay
- Check purity: A260/280 = 1.8–2.0
- Dilute 150 ng DNA in 30 µL 10 mM Tris-HCl (pH 7.5–8.5)

Tagmentation

- Prepare Tagmentation Master Mix:
  - 11 µL BLT (bead-linked transposomes)
  - 11 µL TB1 (tagmentation buffer)
- Add 20 µL master mix to 30 µL DNA
- Mix well, seal plate
- Thermocycle: 55°C for 15 min, hold at 10°C

Post-Tagmentation Cleanup

- Add 10 µL TSB (Stop Buffer), pipette to mix
- Thermocycle: 37°C for 15 min, hold at 10°C
- Place on magnetic stand, discard supernatant
- Wash beads twice with 100 µL TWB
- Leave final wash in wells (do not dry)

PCR Amplification (5 Cycles)

- Prepare PCR Master Mix per sample:
  - 22 µL EPM
  - 22 µL Nuclease-free water
- Add 40 µL master mix to beads
- Add 10 µL pre-paired index adapters (i5+i7)
- Thermocycle:
  - 68°C 3 min
  - 98°C 3 min
  - 5 cycles: 98°C 45s, 62°C 30s, 68°C 2 min
  - 68°C 1 min → Hold at 10°C

Library Cleanup (Ampure beads)

- Transfer 45 µL PCR product to new plate
- Add 40 µL water + 45 µL SPB → mix, incubate 5 min
- Transfer 125 µL to new plate with 15 µL SPB → mix, incubate 5 min
- Wash twice with 200 µL 80% EtOH
- Air dry beads for 5 min
- Elute in 32 µL RSB, transfer 30 µL clean library

Library QC & Pooling

- Tapestation for analyzing the size of library
- Qubit for quantification of the final pooled library.