

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: $A_{260}/A_{280} = 1.8-2.0$
- 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.