# Illumina DNA PCR-Free Library Prep Hybex Checklist



## Tagment Genomic DNA

- ☐ 1 For each reaction, combine the following volumes:
  - HP3 (6 µl)
  - Nuclease-free water (54 µl)
- ☐ 2 For each reaction, combine the following volumes:
  - EtOH (400 µl)
  - Nuclease-free water (100 µl)
- ☐ 3 Preheat Hybex to 45°C.
- ☐ 4 Label a new 96-well MIDI plate LP1.
- ☐ 5 Add 2–25 µl DNA to each well, so that the total input amount is within the desired range.
- ☐ 6 If sample volume is < 25 µl, bring the total volume to 25 µl using RSB.
- ☐ 7 Add 10 µl TB1.
- ☐ 8 Vortex BLT vigorously to resuspend. Repeat as necessary.
- ☐ 9 Add 15 µl BLT.
- ☐ 10 Seal and shake at 1800 rpm for 1 minute.
- ☐ 11 Incubate in pre-heated Hybex for 8 minutes. Proceed immediately to *Post Tagmentation Cleanup*

## Post Tagmentation Cleanup

- ☐ 1 Add 10 µl ST2.
- ☐ 2 Seal and then shake at 1800 rpm for 1 minute.
- ☐ 3 Place on the magnetic stand until liquid is clear.
- ☐ 4 Without disturbing the bead pellet, remove and discard all supernatant.
- ☐ 5 Add 150 µl TWB.
- ☐ 6 Seal and shake at 1800 rpm for 1 minute.
- ☐ 7 Place on the magnetic stand until liquid is clear.

## Ligate Indexes

- ☐ 1 Remove and discard all supernatant.
- ☐ 2 Without disturbing the bead pellet, use a 20 µl pipette to remove and discard residual TWB from each well.
- ☐ 3 Add 45 µl ELM.
- ☐ 4 Pierce the foil seal covering the index adapter plate as follows.
  - **[< 96 samples]** Pierce the wells you intend to use. Use a new pipette tip for each well.
  - **[96 samples]** Align a new semi-skirted 96-well PCR plate over the index adapter plate and slowly press down to puncture all 96 wells. Discard the PCR plate.
- ☐ 5 Add 5 µl index adapters.
- ☐ 6 Seal and shake at 1800 rpm for 1 minute.
- ☐ 7 Incubate in the preheated Hybex for 8 minutes.
- ☐ 8 Place on the magnetic stand until liquid is clear.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Add 75 µl TWB.
- ☐ 11 Seal and shake at 1800 rpm for 1 minute.
- ☐ 12 Place on the magnetic stand until liquid is clear.
- ☐ 13 Remove and discard all supernatant.
- ☐ 14 Without disturbing the bead pellet, use a 20 µl pipette to remove and discard residual TWB from each well.
- ☐ 15 Add 45 µl diluted HP3.
- ☐ 16 Seal and shake at 1800 rpm for 1 minute.

## Clean Up Libraries

- ☐ 1 Vortex IPB, and then invert until fully resuspended.
- ☐ 2 Add 36 µl IPB to each well containing BLT-PF beads.
- ☐ 3 Seal and shake at 1800 rpm for 1 minute.
- ☐ 4 Incubate at room temperature for 2 minutes.
- ☐ 5 Place on the magnetic stand until liquid is clear.
- ☐ 6 Label a new 96-well MIDI plate LP2.
- ☐ 7 Add 42 µl IPB to each well of LP2.
- ☐ 8 Without disturbing the bead pellet, **transfer** 76 µl supernatant from each well of LP1 to the corresponding well of the LP2.
- ☐ 9 Seal and shake at 1800 rpm for 1 minute.
- ☐ 10 Discard LP1.
- ☐ 11 Incubate LP2 at room temperature for 2 minutes.
- ☐ 12 Place on the magnetic stand until liquid is clear.
- ☐ 13 Without disturbing the bead pellet, remove and discard all supernatant.
- ☐ 14 Wash beads as follows.
  - ☐ a Keep on magnetic stand and add 180 µl fresh 80% ethanol.
  - ☐ b Wait 30 seconds.
  - ☐ c Remove and discard all supernatant.
- ☐ 15 Wash beads a **second** time.
- ☐ 16 Using a 20 µl pipette, remove residual EtOH .
- ☐ 17 Discard unused 80% EtOH.
- ☐ 18 Air-dry on the magnetic stand (~2 minutes).
- ☐ 19 Add 22 µl RSB.
- ☐ 20 Seal and shake at 1800 rpm for 1 minute.
- ☐ 21 Incubate at room temperature for 2 minutes.
- ☐ 22 Place on the magnetic stand until liquid is clear.
- ☐ 23 Label a new PCR plate FLP.
- ☐ 24 Transfer 20 µl supernatant to FLP.
- ☐ 25 Proceed immediately to *Quantify and Pool Libraries*.

**SAFE STOPPING POINT**

If you are stopping, seal the plate with Microseal 'B' or Microseal 'F' and store at -25°C to -15°C for up to 7 days.

## Quantify and Pool Libraries

- ☐ 1 Combine 9 µl of each library in a 1.5 or 1.7 ml microcentrifuge tube.
- ☐ 2 Vortex to mix, and then centrifuge at 280 x g for 1 minute.
- ☐ 3 Quantify the library pool:
  - Analyze 2 µl pooled library using the Qubit ssDNA (single-stranded) assay kit or a KAPA qPCR Library Quantification Kit.

## Dilute Libraries to the Starting Concentration and Sequence

☐ 1 When using a qPCR method, use the molarity value determined by the KAPA qPCR protocol to calculate the volumes of RSB and library pool needed to dilute libraries to the starting concentration for your system. Use 450 bp as the average library size and 660 g/mol as the DNA mass. This equation will output the double stranded DNA equivalent Illumina recommends diluting libraries by a 1:10000x dilution when using the KAPA method.

☐ 2 When using Qubit method, calculate the molarity value of the pooled libraries using the formula below.

- The formula uses 450 bp as the average library size and 660 g/mol as the DNA mass. This equation will output the double stranded DNA equivalent.

$$Molarity\ (nM) = Yield\ (\frac{ng}{ul}) \times 3.36$$

| Sequencing System | Qubit ssDNA Quantification | |
|---|---|---|
| | Starting Concentration (nM) | Final Loading Concentration (pM) |
| NovaSeq 6000 standard workflow | 2–3 | 400–600 |
| NovaSeq 6000 Xp workflow | 1.5–2 | 300–400 |

| Sequencing System | KAPA qPCR Quantification | |
|---|---|---|
| | Starting Concentration (nM) | Final Loading Concentration (pM) |
| NovaSeq 6000 standard workflow | 1–1.5 | 200–300 |
| NovaSeq 6000 Xp workflow | 0.75–1 | 150–200 |

☐ 3 Dilute the pool to the starting concentration.

☐ 4 Prepare the VP10 custom sequencing primer.

☐ 5 See the *NovaSeq Denature and Dilute Libraries Guide (document # 1000000106351)* to dilute to the final loading concentration.