Illumina DNA PCR-Free Library Prep Low Input Checklist

Tagment Genomic DNA

- ☐ 1 For each reaction, combine the following volumes to prepare diluted HP3:
  - HP3 (6 µl)
  - Nuclease-free water (54 µl)
- ☐ 2 For each reaction, combine the following volumes to prepare 80% EtOH:
  - EtOH (400 µl)
  - Nuclease-free water (100 µl)
- ☐ 3 Save the following TAG program on the thermal cycler:
  - Choose the preheat lid option and set to 100°C
  - Set the reaction volume to 50 µl
  - 41°C for 5 minutes
- ☐ 4 Save the following ELM program on the thermal cycler:
  - Choose the preheat lid option and set to 100°C
  - Set the reaction volume to 50 µl
  - 37°C for 5 minutes
  - 50°C for 5 minutes
- ☐ 5 Label a new 96-well PCR plate LP1.
- ☐ 6 Add 2–30 µl DNA to each well, so that the total input amount is within the desired range (25-99 ng).
- ☐ 7 If sample volume is < 30 µl, bring the total volume to 30 µl using RSB.
- ☐ 8 Vortex BLT-PF vigorously to resuspend.
- ☐ 9 If beads adhere to the sides or top of the tube, centrifuge at 280 x g for 3 seconds, and then pipette to resuspend.
- ☐ 10 For each reaction, ***combine*** the following volumes in a 1.5 ml tube to prepare the Tagmentation Master Mix:
  - BLT-PF (11 µl)
  - TB1 (11 µl)
- ☐ 11 Pipette to mix.
- ☐ 12 Add 20 µl master mix to each well containing DNA.
- ☐ 13 Pipette to mix, and then seal.
- ☐ 14 Place on the thermal cycler and run the TAG program.
- ☐ 15 Proceed Immediately to *Post Tagmentation Cleanup*

Post Tagmentation Cleanup

- ☐ 1 Add 10 µl ST2.
- ☐ 2 Pipette to mix.
- ☐ 3 Incubate at room temperature for 2 minutes.
- ☐ 4 Place on the magnetic stand until liquid is clear.
- ☐ 5 Remove and discard all supernatant.
- ☐ 6 Remove from the magnetic stand.
- ☐ 7 Add 150 µl TWB.
- ☐ 8 Pipette to mix.
- ☐ 9 Place on the magnetic stand until liquid is clear.

Ligate Indexes

- ☐ 1 Remove and discard residual supernatant from each well.
- ☐ 2 Remove from the magnetic stand.
- ☐ 3 Add 45 µl ELM.
- ☐ 4 Pipette to mix.
- ☐ 5 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.
- ☐ 6 Add 5 µl index adapters.
- ☐ 7 Pipette to mix, and then seal.
- ☐ 8 Place on the thermal cycler and run the ELM program.
- ☐ 9 Place on the magnetic stand until liquid is clear.
- ☐ 10 Remove and discard all supernatant.
- ☐ 11 Remove from the magnetic stand.
- ☐ 12 Add 75 µl TWB.
- ☐ 13 Pipette to mix.
- ☐ 14 Place on the magnetic stand until liquid is clear.
- ☐ 15 Remove and discard all supernatant.
- ☐ 16 Seal, and then centrifuge at 280 x g for 10 seconds.
- ☐ 17 Place on the magnetic stand.
- ☐ 18 Without disturbing the bead pellet, remove and discard residual supernatant from each well.
- ☐ 19 Remove from the magnetic stand.
- ☐ 20 Add 45 µl diluted HP3.
- ☐ 21 Pipette to mix.
- ☐ 22 Incubate at room temperature for 2 minutes.
- ☐ 23 Proceed immediately to *Clean Up Libraries*.

Clean Up Libraries

- ☐ 1 Add 81 µl IPB to each well containing sample with BLT-PF beads.
- ☐ 2 Pipette to mix.
- ☐ 3 Incubate at room temperature for 5 minutes.
- ☐ 4 Place on the magnetic stand until liquid is clear.
- ☐ 5 Remove and discard all supernatant.
- ☐ 6 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.
- ☐ 7 Wash beads a **second** time.
- ☐ 8 Apply Microseal 'B', and then centrifuge 280 x g for 10 seconds.
- ☐ 9 Place on the magnetic stand until liquid is clear.
- ☐ 10 Remove residual EtOH.
- ☐ 11 Discard unused 80% EtOH.
- ☐ 12 Air-dry on the magnetic stand (~2 minutes).
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Add 15 µl RSB.
- ☐ 15 Pipette to mix.
- ☐ 16 Incubate at room temperature for 2 minutes.
- ☐ 17 Centrifuge 280 x g for 10 seconds.
- ☐ 18 Place on the magnetic stand until liquid is clear.
- ☐ 19 Transfer 14 µl supernatant to a new PCR plate.
- ☐ 20 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 Analyze 2 µl of each library using KAPA qPCR library quantification kit.
- ☐ 2 Use 450 bp as the library length.
- ☐ 3 In a 1.5 or 1.7 ml microcentrifuge tubes, combine libraries equimolarly to between 0.75-1nM final concentration.
- ☐ 4 Vortex to mix, and then centrifuge at 280 x g for 1 minute.

Dilute Libraries to the Starting Concentration and Sequence

- ☐ 1 Use the molarity value determined by the KAPA qPCR protocol to calculate the volumes of RSB and library pool to dilute libraries.

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

- ☐ 2 Dilute the pool to the starting concentration.
- ☐ 3 Prepare the VP10 custom sequencing primer.
- ☐ 4 See the *NovaSeq Denature and Dilute Libraries Guide (document # 1000000106351)* to dilute to the final loading concentration.