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 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 If using gDNA input, do as follows.
  - ☐ a Label a new 96-well PCR plate LP1.
  - ☐ b Add 2–25 µl DNA to each well, so that the total input amount is within the desired range (100-299 or 300-2000 ng).
  - ☐ c If sample volume is < 25 µl, bring the total volume to 25 µl using RSB.
- ☐ 6 Vortex BLT-PF vigorously to resuspend.
- ☐ 7 If beads adhere to the sides or top of the tube, briefly spin down and then pipette to resuspend.
- ☐ 8 **[gDNA input]** Add 15 µl BLT-PF.
- ☐ 9 **[Blood/Saliva input]** Add 10 µl BLT-PF to each well containing 30 µl extracted DNA.
- ☐ 10 Pipette to mix.
- ☐ 11 Add 10 µl TB1.
- ☐ 12 Pipette to mix, and then seal.
- ☐ 13 Place on the thermal cycler and run the TAG program.
- ☐ 14 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 Vortex IPB.
- ☐ 2 Add 36 µl IPB to each well containing sample with BLT-PF beads.
- ☐ 3 Pipette to mix.
- ☐ 4 Incubate at room temperature for 2 minutes.
- ☐ 5 Place on the magnetic stand until liquid is clear.
- ☐ 6 Label a new 96-well PCR plate LP2.
- ☐ 7 Vortex and then invert IPB until fully resuspended.
- ☐ 8 Add 42 µl IPB to each well of LP2.
- ☐ 9 Without disturbing the bead pellet, **transfer** 76 µl supernatant to the LP2.
- ☐ 10 Pipette to mix until all beads are in solution.
- ☐ 11 Remove LP1 from the magnetic stand, and then discard.
- ☐ 12 Incubate LP2 at room temperature for 2 minutes.
- ☐ 13 Place on the magnetic stand until liquid is clear.
- ☐ 14 Without disturbing the bead pellet, remove and discard all supernatant.
- ☐ 15 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.
- ☐ 16 Wash beads a **second** time.
- ☐ 17 Apply Microseal 'B' and then centrifuge 280 x g for 10 seconds.
- ☐ 18 Place on the magnetic stand, and then wait 10 seconds.
- ☐ 19 Remove residual EtOH .
- ☐ 20 Discard unused 80% EtOH.

- ☐ 21 Air-dry on the magnetic stand (~2 minutes).
- ☐ 22 Remove from the magnetic stand.
- ☐ 23 Add 22 µl RSB.
- ☐ 24 Pipette to mix.
- ☐ 25 Incubate at room temperature for 2 minutes.
- ☐ 26 Centrifuge 280 x g for 10 seconds.
- ☐ 27 Place on the magnetic stand until liquid is clear.
- ☐ 28 Label a new PCR plate FLP.
- ☐ 29 Transfer 20 µl supernatant to FLP.
- ☐ 30 Proceed 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 For DNA inputs ≥ 300 ng, do as follows.
  - ☐ a For each sequencing library pool, combine 9 µl of each library in a 1.5 or 1.7 ml microcentrifuge tube.
  - ☐ b Vortex to mix, and then centrifuge at 280 x g for 1 minute.
  - ☐ c Quantify the library pool using the Qubit ssDNA (single-stranded) assay kit or a KAPA qPCR Library Quantification Kit.
- ☐ 2 For DNA inputs 100-299 ng, do as follows.
  - ☐ a Analyze 2 µl of each library using KAPA qPCR library quantification kit.
  - ☐ b Use 450 bp as the library length.
  - ☐ c In a 1.5 or 1.7 ml microcentrifuge tubes, combine libraries equimolarly to between 0.75-1nM final concentration.
  - ☐ d Vortex to mix, and then centrifuge at 280 x g for 1 minute.
- ☐ 3 Proceed immediately to *Dilute Libraries to the Starting Concentration and Sequence*.

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.

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

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

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

- ☐ 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.

Perform Whole Blood Lysis

- ☐ 1 For each reaction, combine the following volumes to prepare 80% EtOH:
  - EtOH (960 µl)
  - Nuclease-free water (240 µl)
- ☐ 2 Heat the ThermoMixer C with a 2 ml heat block to 56°C.
- ☐ 3 Label a 15 ml tube Lysis Master Mix.
- ☐ 4 For each sample, combine the following volumes in a 15 ml tube to prepare Lysis Master Mix.
  - MLB (30 µl)
  - PK1 (2 µl)
  - Nuclease-free water (248 µl)
- ☐ 5 Vortex or invert the master mix thoroughly to mix, and then centrifuge.
- ☐ 6 For each sample, add 280 µl master mix to a new 2 ml tube.
- ☐ 7 Invert each EDTA tube 5 times to mix.
- ☐ 8 Add 20 µl blood to each 2 ml tube.
- ☐ 9 Pipette to mix.
- ☐ 10 Vortex briefly, and then centrifuge.
- ☐ 11 Incubate and shake at 1000 rpm for 15 minutes in ThermoMixer C.
- ☐ 12 Centrifuge briefly.
- ☐ 13 Make sure the liquid is brown. If the liquid is not brown, shake at 1000 rpm for 5 minutes.
- ☐ 14 Vortex IPB, and then invert until fully resuspended.
- ☐ 15 Add 135 µl IPB to each tube.
- ☐ 16 Vortex for 15 seconds, and then pipette to mix.
- ☐ 17 Incubate at room temperature for 5 minutes.
- ☐ 18 Place tube on the magnetic stand and wait 5 minutes.
- ☐ 19 Without disturbing the bead pellet, remove and discard supernatant.
- ☐ 20 Make sure all lysis material is removed.
- ☐ 21 Wash beads as follows.
  - ☐ a Add 500 µl fresh 80% EtOH.
  - ☐ b Wait 30 seconds.
  - ☐ c Remove and discard all supernatant.
- ☐ 22 Wash beads a **second** time.
- ☐ 23 Remove all residual EtOH.
- ☐ 24 Discard unused 80% EtOH.
- ☐ 25 Air-dry on the magnetic stand for 5 minutes.
- ☐ 26 Remove from the magnetic stand.
- ☐ 27 Add 35 µl RSB to each tube.
- ☐ 28 Vortex or pipette to mix.
- ☐ 29 Incubate at room temperature for 2 minutes.
- ☐ 30 Place on the magnetic stand until liquid is clear (≥ 2 minutes).
- ☐ 31 Label a new PCR plate LP1.
- ☐ 32 Transfer as follows.
  - If you are not quantifying before beginning library prep, transfer 30 µl supernatant from each tube to the LP1 plate.
  - If you are quantifying before beginning library prep, transfer 32 µl from each tube to the LP1 plate, and then follow the quantification recommendations for your input.
- ☐ 33 If not stopping, proceed to *Tagment Genomic DNA* on page 1.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days.

Perform Dried Blood Spots Lysis

- ☐ 1 For each reaction, combine the following volumes to prepare 80% EtOH:
  - EtOH (960 µl)
  - Nuclease-free water (240 µl)
- ☐ 2 Preheat a 2.0 ml adapter block to 56°C using the ThermoMixer.
- ☐ 3 Label a 15 ml tube Lysis Master Mix.
- ☐ 4 For each sample, combine the following volumes in a 15 ml tube to prepare Lysis Master Mix.
  - 10x Lysis Buffer (30 µl)
  - PK1 (2 µl)
  - Nuclease-free water (268 µl)
- ☐ 5 Vortex or invert the master mix thoroughly to mix, and then centrifuge.
- ☐ 6 For each sample, add 300 µl master mix to a new 2 ml tube.
- ☐ 7 Add 6 x 3 mm^2 punches to a each 2 ml tube.
- ☐ 8 Vortex briefly, and then centrifuge.
- ☐ 9 Incubate and shake at 1000 rpm for 15 minutes in ThermoMixer C.
- ☐ 10 Centrifuge briefly.
- ☐ 11 Make sure the liquid is brown. If the liquid is not brown, shake at 1000 rpm for 5 minutes.
- ☐ 12 Without removing the punches, transfer all supernatant from each tube to a new 2 ml tube.
- ☐ 13 Vortex IPB, and then invert until fully resuspended.
- ☐ 14 Add 135 µl IPB to each sample tube.
- ☐ 15 Vortex for 15 seconds, and then pipette to mix.
- ☐ 16 Incubate at room temperature for 5 minutes.
- ☐ 17 Place on the magnetic stand and wait 5 minutes.
- ☐ 18 Without disturbing the bead pellet, remove and discard supernatant.
- ☐ 19 Make sure all lysis material is removed.
- ☐ 20 Wash beads as follows.
  - ☐ a Add 500 µl fresh 80% EtOH.
  - ☐ b Wait 30 seconds.
  - ☐ c Remove and discard all supernatant.
- ☐ 21 Wash beads a **second** time.
- ☐ 22 Remove all residual EtOH.
- ☐ 23 Discard unused 80% EtOH.
- ☐ 24 Air-dry on the magnetic stand for 5 minutes.
- ☐ 25 Remove from the magnetic stand.
- ☐ 26 Add 35 µl RSB to each tube.
- ☐ 27 Vortex or pipette to mix.
- ☐ 28 Incubate at room temperature for 2 minutes.
- ☐ 29 Place on the magnetic stand until liquid is clear (≥ 2 minutes).
- ☐ 30 Label a new PCR plate LP1.
- ☐ 31 Transfer as follows.
  - If you are not quantifying before beginning library prep, transfer 30 µl supernatant from each tube to the LP1 plate.
  - If you are quantifying before beginning library prep, transfer 32 µl from each tube to the LP1 plate, and then follow the quantification recommendations for your input.
- ☐ 32 If not stopping, proceed to *Tagment Genomic DNA* on page 1.

Perform Saliva Lysis

- ☐ 1 Follow manufacturer's instructions for saliva extraction.
- ☐ 2 For each reaction, combine the following volumes to prepare 80% EtOH:
  - EtOH (960 µl)
  - Nuclease-free water (240 µl)
- ☐ 3 For each sample, add 250 µl nuclease-free water to a new 2 ml tube.
- ☐ 4 Invert each heat-treated saliva collection tube 5 times to mix.
- ☐ 5 Add 50 µl saliva from the collection tube to each 2 ml tube.
- ☐ 6 Pipette to mix.
- ☐ 7 Vortex briefly, and then centrifuge.
- ☐ 8 Vortex IPB, and then invert until fully resuspended.
- ☐ 9 Add 135 µl IPB to each sample tube.
- ☐ 10 Vortex for 15 seconds, and then centrifuge.
- ☐ 11 Incubate at room temperature for 5 minutes.
- ☐ 12 Place on the magnetic stand and wait 5 minutes.
- ☐ 13 Without disturbing the bead pellet, remove and discard supernatant.
- ☐ 14 Make sure all lysis material is removed.
- ☐ 15 Wash beads as follows.
  - ☐ a Add 500 µl fresh 80% EtOH.
  - ☐ b Wait 30 seconds.
  - ☐ c Remove and discard all supernatant.
- ☐ 16 Wash beads a **second** time.
- ☐ 17 Remove all residual EtOH.
- ☐ 18 Discard unused 80% EtOH.
- ☐ 19 Air-dry on the magnetic stand for 5 minutes.
- ☐ 20 Remove from the magnetic stand.
- ☐ 21 Add 35 µl RSB to each tube.

- ☐ 22 Vortex or pipette to mix.
- ☐ 23 Incubate at room temperature for 2 minutes.
- ☐ 24 Place on the magnetic stand until liquid is clear (≥ 2 minutes).
- ☐ 25 Label a new PCR plate LP1.
- ☐ 26 Transfer as follows.
  - If you are not quantifying before beginning library prep, transfer 30 µl supernatant from each tube to the LP1 plate.
  - If you are quantifying before beginning library prep, transfer 32 µl from each tube to the LP1 plate, and then follow the quantification recommendations for your input.
- ☐ 27 If not stopping, proceed to *Tagment Genomic DNA* on page 1.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days.