Tagment Genomic DNA

- ☐ 1 Quantify gDNA using a fluorometric method.
- ☐ 2 Dilute gDNA in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl.
- ☐ 3 Add the following to a new plate or to a new tube.
  - TD (25 µl)
  - Normalized gDNA (10 µl)
  - TDE2 (15 µl)
- ☐ 4 Mix thoroughly.
- ☐ 5 Centrifuge.
- ☐ 6 Place on the preprogrammed thermal cycler and run the tagmentation program. When the sample reaches 10°C, ***immediately*** proceed to step 7 because the transposome is still active.
- ☐ 7 Add 15 µl ST, and then pipette to mix
- ☐ 8 Place on the preprogrammed thermal cycler and run the TAG60 program.

Clean Up Tagmented DNA

- ☐ 1 Transfer total sample volume .
- ☐ 2 Add 52 µl SPB, and then mix thoroughly.
- ☐ 3 Incubate at room temperature for 5 minutes.
- ☐ 4 Place on a magnetic stand until the liquid is clear.
- ☐ 5 Transfer 98 µl supernatant.
- ☐ 6 Add 137 µl SPB, and then mix thoroughly.
- ☐ 7 Incubate at room temperature for 5 minutes.
- ☐ 8 Place on a magnetic stand until the liquid is clear.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Wash 2 times with 200 µl 80% EtOH.
- ☐ 11 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐ 12 Air-dry on the magnetic stand for 5 minutes.
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Add 22.5 µl RSB, and then mix thoroughly.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Centrifuge.
- ☐ 17 Place on a magnetic stand until the liquid is clear.
- ☐ 18 Transfer 20 µl supernatant.

Amplify Tagmented DNA

- ☐ 1 [Plate] Remove the Index Adapter Plate seal.
- ☐ 2 [Tube] Arrange Index 1 (i7) adapters in columns 1–12.
- ☐ 3 [Tube] Arrange Index 2 (i5) adapters in rows A–H.
- ☐ 4 [Tube] Place the plate on the TruSeq Index Plate Fixture.
- ☐ 5 [Plate] Using a multichannel pipette, add 10µL to each sample well.
- ☐ 6 [Tube] Add 5 µl of each Index 1 (i7) adapter.
- ☐ 7 [Tube] Add 5 µl of each Index 2 (i5) adapter.
- ☐ 8 Add 20 µl LAM, and then mix thoroughly.
- ☐ 9 Centrifuge.
- ☐ 10 Place on the thermal cycler and run the LAM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the platecap the tube and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Amplified DNA

- ☐ 1 Centrifuge.
- ☐ 2 Transfer 50 µl total volume.
- ☐ 3 Add 90 µl SPB, and then mix thoroughly.
- ☐ 4 Incubate at room temperature for 5 minutes.
- ☐ 5 Centrifuge.
- ☐ 6 Place on a magnetic stand until liquid is clear.
- ☐ 7 Remove and discard all supernatant.
- ☐ 8 Wash 2 times with 200 µl 80% EtOH.
- ☐ 9 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐ 10 Air-dry on the magnetic stand for 5 minutes.
- ☐ 11 Remove from the magnetic stand.
- ☐ 12 Add 17 µl RSB, and then mix thoroughly.
- ☐ 13 Incubate at room temperature for 2 minutes.
- ☐ 14 Centrifuge.
- ☐ 15 Place on a magnetic stand until liquid is clear.
- ☐ 16 Transfer 15 µl supernatant.
- ☐ 17 Quantify the library using a fluorometric method.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 14 days.

If performing the Illumina-IDT Exome Enrichment Workflow, do not proceed with the Illumina protocol as documented in the remainder of this guide, switch to the IDT xGen hybridization protocol. For more information, see the *Hybridization capture of DNA libraries using xGen Lockdown Probes and Reagents* protocol guide found on the Integrated DNA Technologies website.

If you are following the Nextera DNA Exome Workflow using the Nextera Exome Kit, continue with the sections that follow.

Hybridize Probes

- ☐ 1 Combine 500 ng of each DNA library, making sure that each library has a unique index.
  - If the total volume is > 30 µl, concentrate the pooled sample to 30 µl.
  - If the total volume is < 30 µl, increase the volume to 30 µl with RSB.
- ☐ 2 Add the following to a new plate or to a new tube.
  - DNA library sample or pool (30 µl)
  - BLR (10 µl)
  - CEX (10 µl)
- ☐ 3 Mix thoroughly.
- ☐ 4 Centrifuge.
- ☐ 5 Add 125 µl SPB, and then mix thoroughly.
- ☐ 6 Incubate at room temperature for 10 minutes.
- ☐ 7 Centrifuge.
- ☐ 8 Place on a magnetic stand until the liquid is clear.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Wash 2 times with 200 µl 80% EtOH.
- ☐ 11 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐ 12 Air-dry on the magnetic stand for 10 minutes.
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Add 7.7 µl EHB1, and then mix thoroughly.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Centrifuge.
- ☐ 17 Place on a magnetic stand until the liquid is clear.
- ☐ 18 Transfer 7.5 µl supernatant .
- ☐ 19 Add 2.5 µl EHB2, and then mix thoroughly.
- ☐ 20 Centrifuge.
- ☐ 21 Place on the thermal cycler and run the TRE HYB program.

Capture Hybridized Probes

- ☐ 1 Centrifuge.
- ☐ 2 Transfer all (~10 µl).
- ☐ 3 Add 250 µl SMB and mix.
- ☐ 4 Incubate at room temperature for 25 minutes.
- ☐ 5 Centrifuge.
- ☐ 6 Place on a magnetic stand until liquid is clear.
- ☐ 7 Remove and discard all supernatant.
- ☐ 8 Remove from the magnetic stand.
- ☐ 9 Add 200 µl EEW and mix.
- ☐ 10 Incubate at 50°C as follows.
  - [Plate] Place on the microheating system for 30 minutes.
  - [Tube] Place on the heat block for 30 minutes.
- ☐ 11 Place on a magnetic stand until liquid is clear.
- ☐ 12 Remove and discard all supernatant.
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Repeat steps 9–13 for a total of 2 washes.
- ☐ 15 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
- ☐ 16 Add 23 µl elution premix and mix.
- ☐ 17 Incubate at room temperature for 2 minutes.
- ☐ 18 Centrifuge.
- ☐ 19 Place on a magnetic stand until liquid is clear.
- ☐ 20 Transfer 21 µl supernatant.
- ☐ 21 Add 4 µl ET2 and mix.
- ☐ 22 Add 5 µl RSB and mix.
- ☐ 23 Centrifuge.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Perform Second Hybridization

- ☐ 1 Add the following.
  - BLR (10 µl)
  - CEX (10 µl)
- ☐ 2 Mix thoroughly.
- ☐ 3 Centrifuge.
- ☐ 4 Add 125 µl SPB, and then mix thoroughly.
- ☐ 5 Incubate at room temperature for 10 minutes.
- ☐ 6 Centrifuge.
- ☐ 7 Place on a magnetic stand until the liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Wash 2 times with 200 µl 80% EtOH.
- ☐ 10 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐ 11 Air-dry on the magnetic stand for 10 minutes.
- ☐ 12 Remove from the magnetic stand.
- ☐ 13 Add 7.7 µl EHB1, and then mix thoroughly.
- ☐ 14 Incubate at room temperature for 2 minutes.
- ☐ 15 Centrifuge.
- ☐ 16 Place on a magnetic stand until the liquid is clear.
- ☐ 17 Transfer 7.5 µl supernatant .
- ☐ 18 Add 2.5 µl EHB2, and then mix thoroughly.
- ☐ 19 Centrifuge.
- ☐ 20 Place on the thermal cycler and run the TRE HYB program.

Perform Second Capture

- ☐ 1 Centrifuge.
- ☐ 2 Transfer 10 µl supernatant.
- ☐ 3 Add 250 µl SMB and mix.
- ☐ 4 Incubate at room temperature for 25 minutes.
- ☐ 5 Centrifuge.
- ☐ 6 Place on a magnetic stand until liquid is clear.
- ☐ 7 Remove and discard all supernatant.
- ☐ 8 Remove from the magnetic stand.
- ☐ 9 Add 200 µl EEW and mix.
- ☐ 10 Incubate at 50°C as follows.
  - [Plate] Place on the microheating system for 30 minutes.
  - [Tube] Place on the heat block for 30 minutes.
- ☐ 11 Place on a magnetic stand until liquid is clear.
- ☐ 12 Remove and discard all supernatant.
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Repeat steps 9–13 for a total of 2 washes.
- ☐ 15 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
- ☐ 16 Add 23 µl elution premix and mix.
- ☐ 17 Incubate at room temperature for 2 minutes.
- ☐ 18 Centrifuge.
- ☐ 19 Place on a magnetic stand until liquid is clear.
- ☐ 20 Transfer 21 µl supernatant.
- ☐ 21 Add 4 µl ET2 and mix.
- ☐ 22 Centrifuge.

Clean Up Captured Library

- ☐ 1 Add 45 µl SPB and mix.
- ☐ 2 Incubate at room temperature for 5 minutes.
- ☐ 3 Centrifuge.
- ☐ 4 Place on a magnetic stand until liquid is clear.
- ☐ 5 Remove and discard all supernatant.
- ☐ 6 Wash 2 times with 200 µl 80% EtOH.
- ☐ 7 Use a 20 µl pipette to remove residual EtOH.
- ☐ 8 Air-dry until dry.
- ☐ 9 Remove from the magnetic stand.
- ☐ 10 Add 27.5 µl RSB and mix.
- ☐ 11 Incubate at room temperature for 2 minutes.
- ☐ 12 Centrifuge.
- ☐ 13 Place on a magnetic stand until liquid is clear.
- ☐ 14 Transfer 25 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Amplify Enriched Library

- ☐ 1 Add 5 µl PPC.
- ☐ 2 Add 20 µl EAM and mix.
- ☐ 3 Centrifuge.
- ☐ 4 Place on the thermal cycler and run the AMP10 program.

SAFE STOPPING POINT

If you are stopping, seal the platecap the tube and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Amplified Enriched Library

- ☐ 1 Centrifuge.
- ☐ 2 Transfer 50 µl.
- ☐ 3 Add 50 µl SPB and mix.
- ☐ 4 Incubate at room temperature for 5 minutes.
- ☐ 5 Centrifuge.
- ☐ 6 Place on a magnetic stand until liquid is clear.
- ☐ 7 Remove and discard all supernatant.
- ☐ 8 Wash 2 times with 200 µl 80% EtOH.
- ☐ 9 Use a 20 µl pipette to remove residual EtOH.
- ☐ 10 Air-dry until dry.
- ☐ 11 Remove from the magnetic stand.
- ☐ 12 Add 32 µl RSB and mix.
- ☐ 13 Incubate at room temperature for 2 minutes.
- ☐ 14 Centrifuge.
- ☐ 15 Place on a magnetic stand until liquid is clear.
- ☐ 16 Transfer 30 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Check Enriched Libraries

- ☐ 1 Quantify using the Qubit dsDNA BR Assay Kit.
- ☐ 2 If the concentration is higher than the quantitative range for the High Sensitivity DNA kit, dilute the library 1:10 with RSB.
- ☐ 3 Run 1 µl

Acronyms

Acronym	Definition
BLR	Blocker
CEX	Coding Exome Oligos
EAM	Enrichment Amplification Mix
EE1	Enrichment Elution Buffer 1
EEW	Enhanced Enrichment Wash Solution
EHB1	Enrichment Hybridization Buffer 1
EHB2	Enrichment Hybridization Buffer 2
ET2	Elute Target Buffer 2
HP3	2N NaOH
IEM	Illumina Experiment Manager
LAM	Library Amplification Mix
LRM	Local Run Manager
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST	Stop Tagment Buffer
TD	Tagment DNA Buffer
TDE2	Tagment DNA Enzyme 2