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 TAG58 program.
- ☐7 Add 15 µl ST2, and then pipette to mix
- ☐8 Place on the preprogrammed thermal cycler and run the TAG60 program.

Clean Up Tagmented DNA

- ☐1 Transfer all supernatant.
- ☐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 Add 22.5 µl RSB, and then mix thoroughly.
- ☐14 Remove from the magnetic stand.
- ☐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] Arrange Index 1 (i7) adapters in columns 1–12.
- ☐2 [Plate] Arrange Index 2 (i5) adapters in rows A–H.
- ☐3 [Plate] Place the plate on the TruSeq Index Plate Fixture.
- ☐4 Add 5 µl of each Index 1 (i7) adapter.
- ☐5 Add 5 µl of each Index 2 (i5) adapter.
- ☐6 Add 20 µl LAM, and then mix thoroughly.
- ☐7 Centrifuge.
- ☐8 Place on the thermal cycler and run the LAM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap 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 Add 17 µl RSB, and then mix thoroughly.
- ☐12 Remove from the magnetic stand.
- ☐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.

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 Add 7.7 µl EHB1, and then mix thoroughly.
- ☐14 Remove from the magnetic stand.
- ☐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 Add 7.7 µl EHB1, and then mix thoroughly.
- ☐13 Remove from the magnetic stand.
- ☐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 Add 27.5 µl RSB and mix.
- ☐10 Remove from the magnetic stand.
- ☐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 plate or cap 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 Add 32 µl RSB and mix.
- ☐12 Remove from the magnetic stand.
- ☐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.

Validate 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 chip, dilute the library 1:10 with RSB.
- ☐3 Run 1 µl using a High Sensitivity DNA chip.

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
LAM	Library Amplification Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST2	Stop Tagment Buffer 2
TD	Tagment DNA Buffer
TDE2	Tagment DNA Enzyme 2