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 the NLT plate.

Item	Volume (µl)
Normalized gDNA	10
TD	25
TDE1	15

- ☐4 Shake at 1800 rpm for 1 minute.
- ☐5 Centrifuge at 280 × g for 1 minute.
- ☐6 Place on the 58°C microheating system with the lid closed for 10 minutes.
- ☐7 Add 15 µl ST.
- ☐8 Shake at 1800 rpm for 1 minute.
- ☐9 Centrifuge at 280 × g for 1 minute.
- ☐10 Incubate at room temperature for 4 minutes.

Clean Up Tagmented DNA

- ☐1 Add 65 µl SPB.
- ☐2 Shake at 1800 rpm for 1 minute.
- ☐3 Incubate at room temperature for 8 minutes.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on a magnetic stand until the liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Wash 2 times with 200 µl 80% EtOH.
- ☐8 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐9 Air-dry on the magnetic stand for 10 minutes.
- ☐10 Remove from the magnetic stand.
- ☐11 Add 22.5 µl RSB.
- ☐12 Shake at 1800 rpm for 1 minute.
- ☐13 Incubate at room temperature for 2 minutes.
- ☐14 Centrifuge at 280 × g for 1 minute.
- ☐15 Place on a magnetic stand until the liquid is clear.
- ☐16 Transfer 20 µl supernatant to the NLA plate.

Amplify Tagmented DNA

- ☐1 Arrange Index 1 (i7) adapters in columns 1–12.
- ☐2 Arrange Index 2 (i5) adapters in rows A–H.
- ☐3 Place the plate on the TruSeq Index Plate Fixture.
- ☐4 Add 5 µl of each Index 1 adapter down each column.
- ☐5 Add 5 µl of each Index 2 adapter across each row.
- ☐6 Add 20 µl NLM.
- ☐7 Shake at 1200 rpm for 1 minute.
- ☐8 Centrifuge at 280 × g for 1 minute.
- ☐9 Place on the thermal cycler and run the NLM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the plate 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 at 280 × g for 1 minute.
- ☐2 Transfer 50 µl supernatant to the NLC plate.
- ☐3 Add 90 µl SPB.
- ☐4 Shake at 1800 rpm for 1 minute.
- ☐5 Incubate at room temperature for 10 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until 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 27 µl RSB.
- ☐13 Shake at 1800 rpm for 1 minute.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 25 µl supernatant to the NIL plate.
- ☐18 Quantify the library using a fluorometric method.

SAFE STOPPING POINT

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

Hybridize Probes

- ☐1 Combine 500 ng of each DNA library. Make sure that each library has a unique index.
  - If the total volume is > 40 µl, concentrate the pooled sample to 40 µl.
  - If the total volume is < 40 µl, increase the volume to 40 µl with RSB.
- ☐2 Add the following to the NEH1 plate.

Item	Volume (µl)
DNA library sample or pool	40
EHB	50
CEX, EEX, or RCO	10

- ☐3 Shake at 1200 rpm for 1 minute.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on the thermal cycler and run the NRC HYB program.
- ☐6 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

- ☐1 Centrifuge at 280 × g for 1 minute.
- ☐2 Transfer all to the NEW1 plate.
- ☐3 Add 250 µl SMB.
- ☐4 Shake at 1200 rpm for 5 minutes.
- ☐5 Incubate at room temperature for 25 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Remove from the magnetic stand.
- ☐10 Wash 2 times with 200 µl EWS.
- ☐11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
- ☐12 Add 23 µl elution premix.
- ☐13 Shake at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 21 µl supernatant to the NEH2 plate.
- ☐18 Add 4 µl ET2.
- ☐19 Shake at 1200 rpm for 1 minute.
- ☐20 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

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

Perform Second Hybridization

☐1 Add the following.

Reagent	Volume (µl)
RSB	15
EHB	50
CEX, EEX, or RCO	10

☐2 Shake at 1200 rpm for 1 minute.
☐3 Centrifuge at 280 × g for 1 minute.
☐4 Place on the thermal cycler and run the NRC HYB program.
☐5 Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

☐1 Centrifuge at 280 × g for 1 minute.
☐2 Transfer all supernatant to the NEW2 plate.
☐3 Add 250 µl SMB.
☐4 Shake at 1200 rpm for 5 minutes.
☐5 Incubate at room temperature for 25 minutes.
☐6 Centrifuge at 280 × g for 1 minute.
☐7 Place on a magnetic stand until liquid is clear.
☐8 Remove and discard all supernatant.
☐9 Remove from the magnetic stand.
☐10 Wash 2 times with 200 µl EWS.
☐11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
☐12 Add 23 µl elution premix.
☐13 Shake at 1800 rpm for 2 minutes.
☐14 Incubate at room temperature for 2 minutes.
☐15 Centrifuge at 280 × g for 1 minute.
☐16 Place on a magnetic stand until liquid is clear.
☐17 Transfer 21 µl supernatant to the NEC1 plate.
☐18 Add 4 µl ET2.
☐19 Shake at 1800 rpm for 1 minute.
☐20 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

☐1 Add 45 µl SPB.
☐2 Shake at 1800 rpm for 1 minute.
☐3 Incubate at room temperature for 10 minutes.
☐4 Centrifuge at 280 × g for 1 minute.
☐5 Place on a magnetic stand until liquid is clear.
☐6 Remove and discard all supernatant.
☐7 Wash 2 times with 200 µl 80% EtOH.
☐8 Use a 20 µl pipette to remove residual EtOH.
☐9 Air-dry for 10 minutes.
☐10 Remove from the magnetic stand.
☐11 Add 27.5 µl RSB.
☐12 Shake at 1800 rpm for 1 minute.
☐13 Incubate at room temperature for 2 minutes.
☐14 Centrifuge at 280 × g for 1 minute.
☐15 Place on a magnetic stand until liquid is clear.
☐16 Transfer 25 µl supernatant to the NEA plate.

SAFE STOPPING POINT

If you are stopping, seal the plate 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 NEM.
☐3 Shake at 1200 rpm for 1 minute.
☐4 Centrifuge at 280 × g for 1 minute.
☐5 Place on the thermal cycler and run the AMP10 or AMP12 program.

SAFE STOPPING POINT

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

Clean Up Amplified Enriched Library

☐1 Centrifuge at 280 × g for 1 minute.
☐2 Transfer 50 µl to the NEC2 plate.
☐3 Add 90 µl SPB.
☐4 Shake at 1800 rpm for 1 minute.
☐5 Incubate at room temperature for 10 minutes.
☐6 Centrifuge at 280 × g for 1 minute.
☐7 Place on a magnetic stand until liquid is clear.
☐8 Remove and discard all supernatant.
☐9 Wash 2 times with 200 µl 80% EtOH.
☐10 Use a 20 µl pipette to remove residual EtOH.
☐11 Air-dry on the magnetic stand for 10 minutes.
☐12 Remove from the magnetic stand.
☐13 Add 32 µl RSB.
☐14 Shake at 1800 rpm for 1 minute.
☐15 Incubate at room temperature for 2 minutes.
☐16 Centrifuge at 280 × g for 1 minute.
☐17 Place on a magnetic stand until liquid is clear.
☐18 Transfer 30 µl supernatant to the NEL plate.

SAFE STOPPING POINT

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

Check Enriched Libraries

☐1 Quantify using a fluorometric method.
☐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
CEX	Coding Exome Oligos
EE1	Enrichment Elution Buffer 1
EEX	Expanded Exome Oligos
EHB	Enrichment Hybridization Buffer
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
HP3	2N NaOH
NEA	Nextera Enrichment Amplification Plate
NEC1	Nextera Enriched Clean Up Plate 1
NEC2	Nextera Enriched Clean Up Plate 2
NEH1	Nextera Enrichment Hyb Plate 1
NEH2	Nextera Enrichment Hyb Plate 2
NEL	Nextera Enrichment Library Plate
NEM	Enrichment Amp Mix
NEW1	Nextera Enrichment Wash Plate 1
NEW2	Nextera Enrichment Wash Plate 2
NIL	Nextera Index Library Plate
NLA	Nextera Library Amplification Plate
NLC	Nextera Library Clean Up Plate
NLM	Library Amp Mix
NLT	Nextera Library Tagment Plate
PPC	PCR Primer Cocktail
RCO	Rapid Capture Oligos
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST	Stop Tagment Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme TDE