TruSeq Genotype N_e Checklist

Quantify and Dilute DNA

- ☐ 1 Quantify DNA using a fluorometric method.
- ☐ 2 Dilute DNA to 25 ng/µl in RS1.
- ☐ 3 Requantify the diluted DNA.
- ☐ 4 In a microcentrifuge tube, dilute the 25 ng/µl DNA in RS1 to a total volume of 4 µl.
- ☐ 5 Add 1 µl SS1 to the 4 µl of 50 ng DNA.

Hybridize Oligo Pool

- ☐ 1 Dilute 2.5 µl CAT with 2.5 µl RS1 per sample well.
- ☐ 2 Pulse vortex to mix, and then centrifuge briefly.
- ☐ 3 Add 5 µl RS1 to one well.
- ☐ 4 Add 5 µl diluted DNA to the remaining wells.
- ☐ 5 Add 5 µl diluted CAT to all wells.
- ☐ 6 Add 15 µl OHS2 to each well. Pipette to mix.
- ☐ 7 If bubbles form, centrifuge the plate at 100 × g for 20 seconds.
- ☐ 8 Place on the thermal cycler and run the HYB program.
- ☐ 9 For 96 samples, combine ELE and ELB as follows.
  - ☐ a Transfer 137 µl ELE to the contents of the ELB tube.
  - ☐ b Flick and invert to mix.
- ☐ 10 Place the ELB/ELE mixture on ice for later use.

Remove Unbound Oligos

- ☐ 1 Add 25 µl SPB. Pipette slowly to mix.
- ☐ 2 Incubate at room temperature for 5 minutes.
- ☐ 3 Place on the magnetic stand until liquid is clear.
- ☐ 4 Remove and discard all supernatant.
- ☐ 5 Wash three times with 80 µl SW1.
- ☐ 6 Use a 20 µl pipette to remove residual SW1.
- ☐ 7 Add 80 µl of 60% EtOH.
- ☐ 8 Incubate at room temperature for 30 seconds.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Use a 20 µl pipette to remove residual EtOH.
- ☐ 11 Air-dry for up to 5 minutes.

Extend and Ligate Bound Oligos

- ☐ 1 Remove the plate from the magnetic stand.
- ☐ 2 Add 22 µl ELB/ELE mixture to each well.
- ☐ 3 Pipette to mix.
- ☐ 4 If bubbles form, centrifuge at 100 × g for 20 seconds.
- ☐ 5 Place on the thermal cycler and run the EXT_LIG program.
- ☐ 6 Combine EDP and EMM as follows.
  - [1 sample] 1.1 µl EDP and 21 µl EMM
  - [96 samples] 106 µl EDP and 2006 µl EMM
- ☐ 7 Pipette the EDP/EMM mixture to mix, and then centrifuge briefly.
- ☐ 8 Place the EDP/EMM mixture on ice for later use.

Amplify Libraries

- ☐ 1 Arrange the Index 1 adapters in columns 1–12.
- ☐ 2 Arrange the Index 2 adapters in rows A–H.
- ☐ 3 Place the HYP plate on a TruSeq Index Plate Fixture.
- ☐ 4 Add 4 µl of each Index 1 adapter down each column.
- ☐ 5 Add 4 µl of each Index 2 adapter across each row.
- ☐ 6 Place the plate on ice or iceless cooler.
- ☐ 7 Add 20 µl EDP/EMM mixture. Pipette to mix.
- ☐ 8 Centrifuge at 280 × g for 1 minute.
- ☐ 9 Place the plate on ice or iceless cooler.
- ☐ 10 Immediately transfer to the post-PCR area.
- ☐ 11 Place on the thermal cycler and run the PCR program for the appropriate number of cycles.

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 Libraries

- ☐ 1 Centrifuge the HYP plate at 280 × g for 1 minute.
- ☐ 2 Add 36 µl SPB to the CLP plate.
- ☐ 3 Place the HYP plate on a magnetic stand until liquid is clear.
- ☐ 4 Transfer 45 µl supernatant from the HYP plate to the CLP plate.
- ☐ 5 Shake the plate at 1800 rpm for 2 minutes.
- ☐ 6 Incubate at room temperature for 5 minutes.
- ☐ 7 Centrifuge at 280 × g for 1 minute.
- ☐ 8 Place on a magnetic stand until liquid is clear.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Wash two times with 200 µl 80% EtOH.
- ☐ 11 Using a 20 µl pipette, remove residual EtOH.
- ☐ 12 Remove from the magnetic stand and air-dry for up to 5 minutes.
- ☐ 13 Add 25 µl RSB.
- ☐ 14 Shake the plate at 1800 rpm for 2 minutes.
- ☐ 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 20 µl purified library from the CLP plate to the LNP plate.
- ☐ 19 From the liquid in the CLP plate, run an aliquot of the samples and control to confirm the PCR product sizes.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 6 months.

Normalize Libraries

- ☐ 1 Add 44 µl LNA1 per library to a new 15 ml conical tube.
- ☐ 2 Use a P1000 pipette to resuspend LNB1.
- ☐ 3 Transfer 8 µl LNB1 per library to the tube of LNA1. Invert to mix.
- ☐ 4 Add 45 µl LNA1/LNB1 to the LNP plate.
- ☐ 5 Shake at 1800 rpm for 30 minutes.
- ☐ 6 Place on a magnetic stand until liquid is clear.
- ☐ 7 Remove and discard all supernatant.
- ☐ 8 Remove from the magnetic stand.
- ☐ 9 Wash two times with 45 µl LNW1. Shake at 1800 rpm for 5 minutes per wash.
- ☐ 10 Use a 20 µl pipette to remove residual LNW1.
- ☐ 11 Remove from the magnetic stand.
- ☐ 12 Add 30 µl fresh 0.1 N NaOH.
- ☐ 13 Shake at 1800 rpm for 5 minutes.
- ☐ 14 Place on a magnetic stand until liquid is clear.
- ☐ 15 Add 30 µl LNS2 to the SGP plate.
- ☐ 16 Transfer 30 µl supernatant from the LNP plate to the SGP plate.
- ☐ 17 Centrifuge at 1000 × 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 30 days.

Pool Libraries

- ☐ 1 Centrifuge at 1000 × g for 1 minute.
- ☐ 2 Transfer 5 µl of each library to an 8-tube strip.
- ☐ 3 Seal the plate and store at -25°C to -15°C.
- ☐ 4 Transfer the contents of the 8-tube strip to the PAL tube. Pipette to mix.
- ☐ 5 Denature and dilute the library pool to the appropriate loading concentration.

SAFE STOPPING POINT

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

Acronyms

Acronym	Definition
CLP	Cleanup Plate
EDP	Enhanced DNA Polymerase
ELB	Extension-Ligation Buffer
ELE	Extension-Ligation Enzyme
EMM	Enhanced Master Mix
CAT	TruSeq Genotype N_e Oligos
HT1	Hybridization Buffer
HYP	Hybridization Plate
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNP	Library Normalization Plate
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
OHS2	Oligo Hybridization for Sequencing Reagent 2
PAL	Pooled Amplicon Library
RS1	Resuspension Solution 1
RSB	Resuspension Buffer
SGP	Storage Plate
SNP	Single Nucleotide Polymorphism
SPB	Sample Purification Beads
SS1	Sample Stabilization Solution 1
SW1	Stringent Wash 1