Amplify and Tag Targets

☐ 1 Quantify the sample DNA.
☐ 2 Dilute each sample DNA to 2 ng/µl in a final volume of 12.5 µl.
☐ 3 Combine the following reagents in separate microcentrifuge tubes to create PCR master mixes for TPA and TPB.

PCR Component	Per Well	Per 24 Samples
TTM	5.875 µl	141 µl
TPA or TPB	6.25 µl	150 µl
TTE	0.375 µl	9 µl

☐ 4 Pipette to mix.
☐ 5 Add 10 µl of each PCR master mix.
- Master Mix A—Rows A and C
- Master Mix B—Rows B and D

☐ 6 Add 5 µl of 2 ng/µl DNA.
- Samples 1–12—Rows A and B
- Samples 13–24—Rows C and D

☐ 7 Pipette to mix.
☐ 8 Centrifuge at 1000 × g for 1 minute.
☐ 9 Immediately place on a thermal cycler and run the TST15 PCR1 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, leave on the thermal cycler overnight.

Index Targets

☐ 1 Arrange Index 1 (i7) adapters in the top row.
☐ 2 Arrange Index 2 (i5) adapters in rows A-B so Mix A and Mix B are given unique i5/i7 index combinations. For example A501 in row A for Mix A, and A502 in row B for Mix B.
☐ 3 Record index adapters used for each sample for sequencing.
☐ 4 Place the plate on the TruSeq Index Plate Fixture.
☐ 5 For samples 1–12, add 4 µl of each Index 2 (i5) adapter across rows A and B.
☐ 6 For samples 1–12, add 4 µl of each Index 1 (i7) adapter (R701–R709, R711–R712, R749) to each column of rows A and B.
☐ 7 For samples 13–24, add 4 µl of each Index 2 (i5) adapter across rows C and D.
☐ 8 For samples 13–24, add 4 µl of each Index 1 (i7) adapter (R725–R736) to each column of rows C and D.
☐ 9 Add 27 µl TAM.
☐ 10 Pipette to mix.
☐ 11 Centrifuge at 1000 × g for 1 minute.
☐ 12 Immediately place on a thermal cycler and run the TST15 PCR2 program.

SAFE STOPPING POINT

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

Clean Up Libraries

☐ 1 Centrifuge at 1000 × g for 1 minute.
☐ 2 Add 40 µl SPB of a new midi plate.
☐ 3 Transfer 45 µl supernatant from the PCR plate to the midi plate.
☐ 4 Shake at 1800 rpm for 5 minutes.
☐ 5 Incubate at room temperature for 5 minutes.
☐ 6 Place on a magnetic stand until beads bind to the magnet.
☐ 7 Remove and discard all supernatant.
☐ 8 Wash two 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 32 µl RSB.
☐ 12 Shake at 1800 rpm for 2 minutes.
☐ 13 Incubate at room temperature for 2 minutes.
☐ 14 Place on a magnetic stand until liquid is clear.
☐ 15 Transfer 30 µl supernatant to the PLP plate.
☐ 16 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the PCR plate and store at -25°C to -15°C for up to 2 months.

Check Libraries

- ☐ 1 Quantify the library
- ☐ 2 Calculate the volume of RSB required to adjust the library concentration to 5 ng/µl.
- ☐ 3 Add the required volume of RSB to the NLP plate.
- ☐ 4 Transfer 8 µl of each library to the NLP plate.
- ☐ 5 Run an aliquot of each normalized library on either of the following methods:
  - 15 µl on a 2% agarose gel
  - 1 µl on a Bioanalyzer using a DNA 1000 chip

SAFE STOPPING POINT

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

Pool Libraries

- ☐ 1 Centrifuge the NLP plate at 1000 × g for 1 minute.
- ☐ 2 Transfer 4 µl of each library to the PNL tube.
- ☐ 3 Vortex to mix, and then centrifuge briefly.
- ☐ 4 Add 41 µl RSB to the DNL tube.
- ☐ 5 Transfer 9 µl from the PNL tube to the DNL tube.
- ☐ 6 Vortex to mix, and then centrifuge briefly to create a 4 nM pooled library.

Acronyms

Acronym	Definition
DAL	Denatured Amplicon Libraries
DNL	Diluted Normalized Libraries
HP3	2N NaOH
HT1	Hybridization Buffer
NLP	Normalized Library Plate
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
RSB	Resuspension Buffer
SPB	Sample Purification Beads
TAM	TruSight Tumor Amplification Mix
TPA	TruSight Tumor Primer Mix A
TPB	TruSight Tumor Primer Mix B
TTE	TruSight Tumor Targeting Enzyme
TTM	TruSight Tumor Targeting Mix