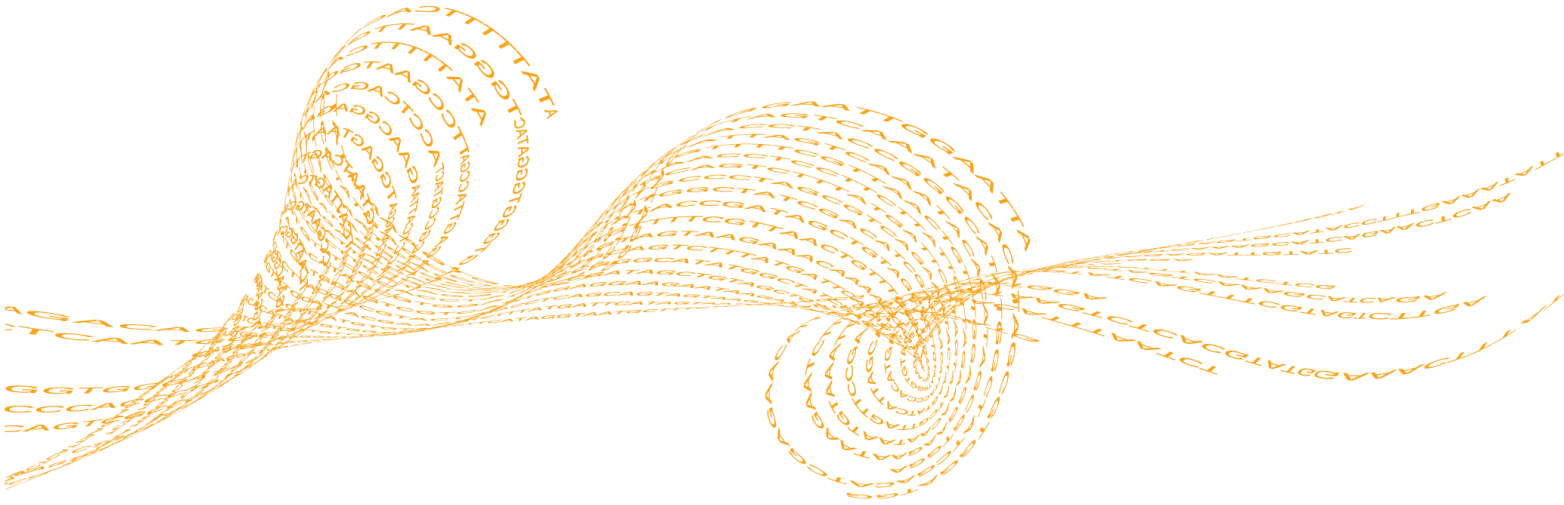
# TruSight Tumor 15

# Protocol Guide

For Research Use Only. Not for use in diagnostic procedures.







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# Amplify and Tag Targets

## Preparation

1. Label a 1.7 ml microcentrifuge tube Master Mix A. Label a second 1.7 ml microcentrifuge tube Master Mix B.
2. Save the following program as TST15 PCR1 on a thermal cycler with a heated lid.
   - Choose the preheated lid option and set to 102°C
   - Set the reaction volume to 15 µl
   - 98°C for 3 minutes
   - 16 cycles of:
     - 96°C for 45 seconds
     - 70°C for 1 minute
     - 54°C for 3 minutes*
     - 72°C for 15 seconds*
   - 72°C for 5 minutes
   - Hold at 10°C

   *The TST15 PCR1 program requires specific ramp rate settings. See *Thermal Cyclers* on page 8.

## Procedure

1. Quantify the sample DNA using a fluorometric method.
2. Dilute each sample DNA to 2 ng/µl with RNase/DNase-free water 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 8 Samples | Per 16 Samples | Per 24 Samples |
|---|---|---|---|---|
| TTM | 5.875 µl | 47 µl | 94 µl | 141 µl |
| TPA or TPB | 6.25 µl | 50 µl | 100 µl | 150 µl |
| TTE | 0.375 µl | 3 µl | 6 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. Apply the seal and 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

## Preparation

1 Save the following program as TST15 PCR2 on a thermal cycler with a heated lid.
- Choose the preheated lid option and set to 102°C
- Set the reaction volume to 50 µl
- 98°C for 30 seconds
- 17 cycles of:
  - 98°C for 20 seconds
  - 60°C for 30 seconds
  - 72°C for 45 seconds
- 72°C for 5 minutes
- Hold at 10°C

## Procedure

1 Arrange Index 1 (i7) adapters in the top row of the TruSeq Index Plate Fixture.

2 Arrange Index 2 (i5) adapters in rows A–B of the TruSeq Index Plate Fixture.

3 Place the plate on the TruSeq Index Plate Fixture.

4 For samples 1–12, perform the following.

a Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter across rows A and B.

b Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter (R701–R709, R711–R712, R749) to each column of rows A and B.

5 For samples 13–24, perform the following.

a Move Index 2 (i5) adapters to rows C–D.

b Replace Index 1 (i7) adapters in the top row with the second set (R725–R736).

c Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter across rows C and D.

d Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter (R725–R736) to each column of rows C and D.

6 Add 27 µl TAM.

7 Pipette to mix.

8 Apply the seal and centrifuge at 1000 × g for 1 minute.

9 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 7 days.

# Clean Up Libraries

## Preparation

1 Label a new 96-well PCR plate PLP (Purified Library Plate).

## Procedure

1 Centrifuge at 1000 × g for 1 minute.

2 Add 40 µl SPB to each well of a new midi plate.

3 Transfer 45 µl supernatant from the PCR plate to the corresponding well of the midi plate.

4 Apply the seal and shake at 1800 rpm for 5 minutes.

5 Incubate at room temperature for 5 minutes.

6 Place on a magnetic stand and wait until the beads bind to the magnet (~2 minutes).

7 Remove and discard all supernatant from each well.

8 Wash 2 times with 200 µl 80% EtOH.

9 Using a 20 µl pipette, remove residual 80% EtOH from each well.

10 Air-dry on the magnetic stand for 5 minutes.

11 Add 32 µl RSB to each well.

12 Apply the seal and shake at 1800 rpm for 2 minutes. If the beads are not resuspended, pipette to mix or repeat shake at 1800 rpm for 2 minutes.

13 Incubate at room temperature for 2 minutes.

14 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

15 Transfer 30 µl supernatant from each well to the corresponding well of the PLP plate.

16 Apply the seal and 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 2 months.

# Check Libraries

## Preparation

1 Label a new 96-well PCR plate NLP (Normalized Library Plate).

## Procedure

1 Quantify the library using a fluorometric method.

2 Calculate the volume of RSB required to adjust the library concentration to 5 ng/µl as follows.

   a Use the formula C1V1 = C2V2, where C1 is the result of library quantification, V1 is 8 µl, and C2 is 5 ng/µl to calculate the value for V2.
   b Calculate the amount of RSB (V2 - 8 µl) required to adjust the concentration of each library to 5 ng/µl.

3 Add the required volume of RSB to the NLP plate. If a library is ≥ 100 ng/µl, transfer the RSB to a 1.7 ml tube.

4 Transfer 8 µl of each library to the NLP plate or 1.7 ml tube.

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

## Preparation

1. Label a new 1.7 ml microcentrifuge tube PNL.
2. Label a new 1.7 ml microcentrifuge tube DNL.
3. To prepare for the sequencing run, begin thawing reagents.

## Procedure

1. Apply the seal and centrifuge the NLP plate at 1000 × g for 1 minute.
2. Transfer 4 µl of each library from the NLP plate 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. Denature and dilute pooled libraries to the loading concentration for the sequencing instrument you are using.

# Appendix

## Thermal Cyclers

| Thermal Cycler | TST PCR1 Estimated Run Time | TST PCR1 54°C and 72°C Cycle Steps Ramp Rate* | |
|---|---|---|---|
| Bio-Rad C1000 Thermal Cycler | 3:08:02 | Down | 0.1 °C/s |
| | | Up | 0.1 °C/s |
| Bio-Rad S1000 Thermal Cycler | 3:51:52 | Down | 0.1 °C/s |
| | | Up | 0.1 °C/s |
| Applied Biosystems GeneAmp PCR System 9700 | 2:55:00 | Down | 7% |
| | | Up | 7% |
| Applied Biosystems Veriti Thermal Cycler | 3:06:00 | Down | 4.5% |
| | | Up | 4.0% |
| Eppendorf Mastercycler ep Gradient | 3:14:00 | Down | 6% |
| | | Up | 4% |
| Eppendorf Mastercycler ep Gradient-S | 2:50:00 | Down | 2% |
| | | Up | 2% |

*All other TST PCR1 steps and all TST PCR2 steps require the default or maximum thermal cycler ramp rates.

## 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 |

| Acronym | Definition |
|---|---|
| TPA | TruSight Tumor Primer Mix A |
| TPB | TruSight Tumor Primer Mix B |
| TTE | TruSight Tumor Targeting Enzyme |
| TTM | TruSight Tumor Targeting Mix |

Notes

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

| | |
|---|---|
| **Website** | www.illumina.com |
| **Email** | techsupport@illumina.com |

Table 2 Illumina Customer Support Telephone Numbers

| Region | Contact Number | Region | Contact Number |
|---|---|---|---|
| North America | 1.800.809.4566 | Japan | 0800.111.5011 |
| Australia | 1.800.775.688 | Netherlands | 0800.0223859 |
| Austria | 0800.296575 | New Zealand | 0800.451.650 |
| Belgium | 0800.81102 | Norway | 800.16836 |
| China | 400.635.9898 | Singapore | 1.800.579.2745 |
| Denmark | 80882346 | Spain | 900.812168 |
| Finland | 0800.918363 | Sweden | 020790181 |
| France | 0800.911850 | Switzerland | 0800.563118 |
| Germany | 0800.180.8994 | Taiwan | 00806651752 |
| Hong Kong | 800960230 | United Kingdom | 0800.917.0041 |
| Ireland | 1.800.812949 | Other countries | +44.1799.534000 |
| Italy | 800.874909 | | |

**Safety data sheets (SDSs)**—Available on the Illumina website at support.illumina.com/sds.html.

**Product documentation**—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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