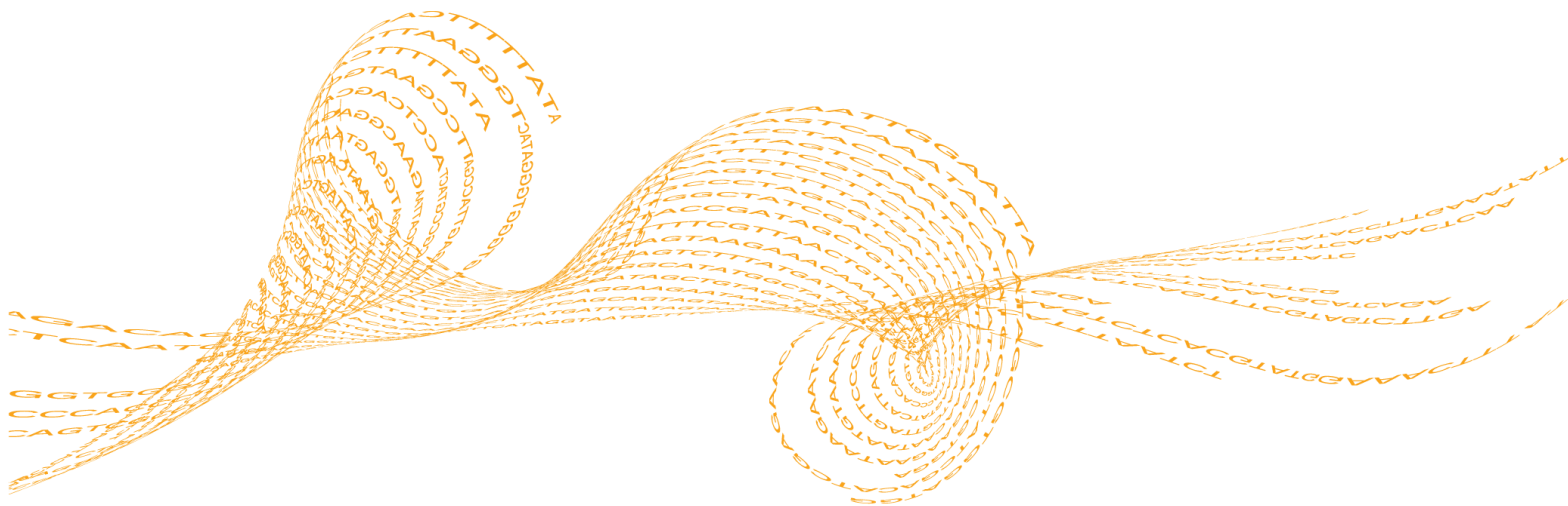


TruSight Tumor 15 Protocol Guide

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

Amplify and Tag Targets	3
Index Targets	4
Clean Up Libraries	5
Check Libraries	6
Pool Libraries	7
Appendix	7
Technical Assistance	8



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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 μ l
 - ▶ 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/ μ l with RNase/DNase-free water in a final volume of 12.5 μ l.
- 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 μ l	47 μ l	94 μ l	141 μ l
TPA or TPB	6.25 μ l	50 μ l	100 μ l	150 μ l
TTE	0.375 μ l	3 μ l	6 μ l	9 μ l

- 4 Pipette to mix.
- 5 Add 10 μ l of each PCR master mix.
 - ▶ Master Mix A—Rows A and C
 - ▶ Master Mix B—Rows B and D
- 6 Add 5 μ l of 2 ng/ μ l 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 \times 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 μ l
 - ▶ 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 μ l of each Index 2 (i5) adapter across rows A and B.
 - b Using a multichannel pipette, add 4 μ l 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 μ l of each Index 2 (i5) adapter across rows C and D.
 - d Using a multichannel pipette, add 4 μ l of each Index 1 (i7) adapter (R725–R736) to each column of rows C and D.
- 6 Add 27 μ l TAM.
- 7 Pipette to mix.
- 8 Apply the seal and centrifuge at 1000 \times 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 $1000 \times g$ 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 $1000 \times g$ 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 $C_1V_1 = C_2V_2$, where C_1 is the result of library quantification, V_1 is 8 μl, and C_2 is 5 ng/μl to calculate the value for V_2 .
 - b Calculate the amount of RSB ($V_2 - 8$ 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 $1000 \times g$ 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**.



Illumina

5200 Illumina Way

San Diego, California 92122 U.S.A.

+1.800.809.ILMN (4566)

+1.858.202.4566 (outside North America)

techsupport@illumina.com

www.illumina.com