TruSight HLA v2 Sequencing Panel

Protocol Guide

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

ILLUMINA PROPRIETARY

Document # 1000000010160 v00
May 2016

This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

© 2016 Illumina, Inc. All rights reserved.

Illumina, **24sure**, **BaseSpace**, **BeadArray**, **BlueFish**, **BlueFuse**, **BlueGnome**, **cBot**, **CSPro**, **CytoChip**, **DesignStudio**, **Epicentre**, **ForenSeq**, **Genetic Energy**, **GenomeStudio**, **GoldenGate**, **HiScan**, **HiSeq**, **HiSeq X**, **Infinium**, **iScan**, **iSelect**, **MiniSeq**, **MiSeq**, **MiSeqDx**, **MiSeq FGx**, **NeoPrep**, **NextBio**, **Nextera**, **NextSeq**, **Powered by Illumina**, **SureMDA**, **TruGenome**, **TruSeq**, **TruSight**, **Understand Your Genome**, **UYG**, **VeraCode**, **verifi**, **VeriSeq**, the pumpkin orange color, and the streaming bases design are trademarks of Illumina, Inc. and/or its affiliate(s) in the U.S. and/or other countries. All other names, logos, and other trademarks are the property of their respective owners.

For Research Use Only– not for any clinical or therapeutic use in humans or animals.
This product includes 2800M Control DNA manufactured by Promega Corporation for distribution by Illumina, Inc.

Promega

Generate HLA PCR Amplicons

Preparation

1. Label 2 new 96-well PCR plates LRP1 and LRP2.
2. Save the following programs as PCR1 and PCR2 on a thermal cycler with a heated lid (95°C to 100°C).

PCR1: LRP1 Plate	PCR2: LRP2 Plate

▸ 94°C for 3 minutes
▸ 30 cycles of:
  ▸ 94°C for 30 seconds
  ▸ 60°C for 2 minutes
  ▸ 68°C for 15 minutes
▸ 68°C for 10 minutes
▸ Hold at 10°C
	▸ 94°C for 3 minutes
	▸ 10 cycles of:
	  ▸ 94°C for 30 seconds
	  ▸ 55°C for 2 minutes
	  ▸ 72°C for 15 minutes
	▸ 20 cycles of:
	  ▸ 94°C for 30 seconds
	  ▸ 60°C for 2 minutes
	  ▸ 72°C for 15 minutes
	▸ 72°C for 10 minutes
	▸ Hold at 10°C

Procedure

1. Quantify and normalize DNA to 10 ng/µl in a volume of at least 40 µl in water or 10 mM Tris-HC1.
2. Add 5 µl of each HLA primer to the LRP plates, as follows.
   - HLA-A—LRP1 row A
   - HLA-B.2—LRP1 row B
   - HLA-C—LRP1 row C
   - DPA1—LRP1 row D
   - DPB1—LRP1 row E
   - DQA1—LRP1 row F
   - DRB.2—LRP1 row G
   - DQB1—LRP2 row D (to decrease evaporation risk)
3. Add 5 µl of 10 ng/µl template DNA to the LRP plates, as follows.
   - Samples 1–12—LRP1 rows A–G
   - Samples 1–12—LRP2 row D
4. Combine the following reagents in a 15 ml conical tube to create PCR master mix.

PCR Component	Per Well	Per 12 Samples
HPM (HLA PCR Mix)	25 µl	2640 µl
MasterAmp Extra-Long DNA Polymerase	2 µl	212 µl
PCR-grade water	13 µl	1373 µl

5. Add 40 µl PCR master mix. Pipette to mix.
6. Centrifuge at 280 × g for 1 minute.
7. Place the LRP1 plate on a thermal cycler and run the PCR1 program.

8 Place the LRP2 plate on a thermal cycler and run the PCR2 program.

9 Centrifuge the plates at 280 × g for 2 minutes.

10 Label a new midi plate LRC.

11 Transfer 45 µl of samples from the 2 LRP plates to a single LRC plate, as follows.
- LRP1 rows A–G to LRC rows A–G
- LRP2 row D to LRC row H

SAFE STOPPING POINT

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

Clean Up HLA PCR Amplicons

Preparation

1 Label a new midi plate LRB.

Procedure

1 Add 31.5 µl SPB to the LRC Plate.
2 Shake at 1800 rpm for 2 minutes.
3 Incubate at room temperature for 2 minutes.
4 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
5 Remove and discard all supernatant.
6 Wash 2 times with 200 µl 80% EtOH.
7 Use a 20 µl pipette to remove residual EtOH.
8 Air-dry on the magnetic stand for 2 minutes.
9 Add 30 µl RSB.
10 Shake at 1800 rpm for 2 minutes.
11 Incubate at room temperature for 2 minutes.
12 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
13 Transfer 20 µl supernatant from the LRC plate to the LRB plate.

SAFE STOPPING POINT

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

Normalize HLA PCR Amplicons

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.

For more information, see the SDS for this kit, at support.illumina.com/sds.ilmn.

Procedure

1. Add 4.4 ml LNA1 to a new 15 ml conical tube.
2. Pipette to mix LNB1.
3. Transfer 400 µl LNB1 to the 15 ml conical tube that contains LNA1.
4. Invert the tube to mix.
5. Add 45 µl LNB1/LNA1 mixture to the LRB plate.
6. Shake at 1800 rpm for 30 minutes.
7. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
8. Remove and discard all supernatant.
9. Add 45 µl RSB.
10. Shake at 1800 rpm for 5 minutes.
11. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
12. Remove and discard all supernatant.
13. Remove residual liquid from each well.
14. Add 40 µl HTB.
15. Shake at 1800 rpm for 5 minutes.

Tagment HLA PCR Amplicons

Preparation

1. [Option 1] Preheat a thermal cycler without a heated lid to 58°C.
2. [Option 1] Label a new 96-well PCR plate TAG.
3. [Option 2] Preheat a TruTemp microheating system to 58°C.
4. Label a new PCR plate NTC.

Procedure

1. **[Option 1]** Using a thermal cycler:
   a. Transfer 40 µl from the LRB plate to the TAG plate.
   b. Using a prealiquoted PCR 8-tube strip, add 10 µl HTM to the TAG plate, and then pipette to mix.
   c. Place on a thermal cycler (58°C) for 12 minutes.
   d. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
2. **[Option 2]** Using a TruTemp microheating system:
   a. Using a prealiquoted PCR 8-tube strip, add 10 µl HTM to the LRB plate.
   b. Shake for 1 minute at 1600 rpm.
   c. Place on a TruTemp microheating system set to 58°C for 12 minutes.
   d. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
3. Transfer 50 µl from each well of the TAG plate to the NTC plate.

Pool and Clean Up Tagmentation Reaction

Preparation

1. Label a new midi plate TPP.
2. Label a new PCR plate NPP.

Procedure

1. Pool tagmentation products from the NTC plate into row A of the TPP plate, as follows.
   - HLA-A (10 µl)
   - HLA-B.2 (10 µl)
   - HLA-C (10 µl)
   - DPA1 (10 µl)
   - DPB1 (10 µl)
   - DQA1 (10 µl)
   - DRB.2 (**20 µl**)
   - DQB1 (10 µl)
2. Add 63 µl SPB to row A of the TPP plate.
3. Shake at 1800 rpm for 2 minutes.
4. Incubate at room temperature for 2 minutes.
5. Place on a magnetic stand and wait until the liquid is clear (~5 minutes).
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 on the magnetic stand for 2 minutes.
10. Add 22.5 µl RSB.
11. Shake at 1800 rpm for 2 minutes.
12. Incubate at room temperature for 2 minutes.
13. Centrifuge at 280 × g for 2 minutes.
14. Place on a magnetic stand and wait for the liquid to clear (~2 minutes).
15. Transfer 20 µl supernatant to the NPP plate, as follows.
    - Samples 1–6—Row A, columns 1–6
    - Samples 7–12—Row B, columns 1–6

SAFE STOPPING POINT

If you are stopping, seal the plates and store at -25°C to -15°C for up to 1 day.

Amplify PCR

Preparation

1. Save the following program as IndexAmp on a thermal cycler with a heated lid (100°C).
   - 72°C for 3 minutes
   - 98°C for 30 seconds
   - 10 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 5 minutes
   - 72°C for 5 minutes
   - Hold at 10°C (overnight maximum)

Procedure

1. Arrange the Nextera XT Index Kit in the TruSeq Index Plate Fixture, as follows.
   - Index 1 (i7) adapters in columns 1–6.
   - Index 2 (i5) adapters in rows A and B.
2. Place the NPP plate on a TruSeq Index Plate Fixture.
3. Using a multichannel pipette, add 5 µl of each Index 1 (i7) adapter to row A and B. Replace the cap on each i7 adapter tube with a new orange cap.
4. Using a multichannel pipette, add 5 µl of each Index 2 (i5) adapter to columns 1–6. Replace the cap on each i5 adapter tube with a new white cap.
5. Add 20 µl NLM. Pipette to mix.
6. Centrifuge at 280 × g at 20°C for 1 minute.
7. Place the plate on the thermal cycler and run the IndexAmp 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 PCR

Preparation

1 Label a new midi plate NPC.

2 Label a new PCR plate HLP.

Procedure

1 Transfer 45 µl of the PCR reactions from the NPP plate to the NPC plate.

2 Add 31.5 µl SPB.

3 Shake at 1800 rpm for 2 minutes.

4 Incubate at room temperature for 2 minutes.

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

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 on the magnetic stand for 2 minutes.

10 Add 32.5 µl RSB.

11 Shake at 1800 rpm for 2 minutes.

12 Incubate at room temperature for 2 minutes.

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

14 Transfer 30 µl supernatant from the NPC plate to the HLP plate.

SAFE STOPPING POINT

If you are stopping, seal the plates and store at 2°C to 8°C for up to 7 days.

Pool Final Libraries for MiSeq Sequencing

Preparation

1. Label a new 1.5 ml Eppendorf tube PHL.
2. Label a new 1.5 ml Eppendorf tube DHL.

Procedure

1. Transfer 7 µl from each well of the HLP plate to the PHL tube.
2. Quantify the library with the Qubit BR assay or a fluorometric assay.
3. Determine the library volume to denature using the following formula:
   Y = 15/x
   - X is the library concentration (ng/µl) as determined by the fluorometric assay
   - Y is the library volume (µl) to dilute and denature
4. Transfer the volume determined by Y to the DHL tube.
5. Dilute with RSB to a final volume of 10 µl.
6. Add 10 µl 0.1 N NaOH.
7. Vortex and then centrifuge briefly to mix.
8. Incubate at room temperature for 5 minutes.
9. Add 980 µl HT1 for a final volume of 1000 µl, and then invert to mix.
10. Load 600 µl denatured library from the DHL tube onto the thawed reagent cartridge.

Acronyms

Acronym	Definition
DHL	Diluted HLA Libraries
HLP	HLA Library Plate
HPM	HLA PCR Mix
HTB	HLA Tagmentation Buffer
HTM	HLA Tagmentation Mix
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LRB	Long Range Bead Based Normalization 2
LRC	Long Range Clean Up
LRP	Long Range PCR
NLM	Nextera Library Amplification Mix
NPC	Nextera PCR Clean Up
NPP	Nextera PCR Plate
NTC	Nextera Tagmentation Clean Up
PHL	Pool HLA Libraries
RSB	Resuspension Buffer
SPB	Sample Purification Beads
TPP	Tagmentation Pooling Plate

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