TruSight HLA Sequencing Panel

Protocol Guide

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Generate HLA PCR Amplicons

Preparation

1 Label 3 new semi-skirted 96-well PCR plates LRP1, LRP2, and LRP3.

2 Save the following programs as PCR1 and PCR2 on a thermal cycler with a heated lid (95°C to 101°C).

PCR1: LRP1 and LRP2 Plates	PCR2: LRP3 Plate
• 94°C for 3 minutes • 35 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 • 25 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 DNA.

2 Add 5 µl of each HLA primer to the LRP plates, as follows.
- HLA-A—LRP1 and LRP2 row A
- HLA-B—LRP1 and LRP2 row B
- HLA-C—LRP1 and LRP2 row C
- DPA1—LRP1 and LRP2 row D
- DPB1—LRP1 and LRP2 row E
- DQA1—LRP1 and LRP2 row F
- DRB1—LRP1 and LRP2 row G
- DQB1—LRP3 rows A and B

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—LRP3 row A
- Samples 13–24—LRP2 rows A–G
- Samples 13–24—LRP3 row B

4 Combine the following reagents in a 15 ml conical tube to create PCR master mix.

PCR Component	Per Well	Per 24 Samples
HPM (HLA PCR Mix)	25 µl	5040 µl
MasterAmp Extra-Long DNA Polymerase	2 µl	403.2 µl
PCR-grade water	13 µl	2620.8 µl

5 Add 40 µl PCR master mix. Pipette to mix.

6 Centrifuge at 280 × g for 2 minutes.

7 Place the LRP1 and LRP2 plates on a thermal cycler and run the PCR1 program.

8 Place the LRP3 plate on a thermal cycler and run the PCR2 program.

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

10 Label 2 new midi plates LRC1 and LRC2.

11 Transfer samples from 3 LRP plates to 2 LRC plates, as follows.
- LRP1 rows A–G to LRC1 rows A–G
- LRP3 row A to LRC1 row H
- LRP2 rows A–G to LRC2 rows A–G
- LRP3 row B to LRC2 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 2 new midi plates LRB1 and LRB2.

Procedure

1 Add 45 µl SPB to the LRC1 and LRC2 plates.
2 Shake at 1800 rpm for 2 minutes.
3 Incubate at room temperature for 5 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 5 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 plates to the LRB plates.

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

Procedure

1 Add 8.8 ml LNA1 to a new 15 ml conical tube.

NOTE
A volume of 4.4 ml is required for each plate.

2 Pipette to further resuspend LNB1.

3 Transfer 1.6 ml LNB1 to the 15 ml conical tube that contains LNA1.

NOTE
A volume of 800 µl is required for each plate.

4 Invert the tube to mix.

5 Add 45 µl LNB1/LNA1 mixture to the LRB plates.

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 Add 40 µl HTB.

14 Shake at 1800 rpm for 5 minutes.

NOTE
Proceed ***immediately*** to the next step.

Tagment HLA PCR Amplicons

Preparation

1. [Option 1] Preheat a thermal cycler to 58°C.
2. [Option 1] Label 2 new 96-well PCR plates TAG1 and TAG2.
3. [Option 2] Preheat a TruTemp microheating system to 58°C.
4. Label 2 new midi plates NTC1 and NTC2.

Procedure

1. Calculate the total volume of HTM, including 10% extra. Divide the volume equally in a PCR 8-tube strip.
2. **[Option 1]** Using a thermal cycler:
   a. Transfer 40 µl from the LRB plates to the TAG plates.
   b. Add 10 µl HTM to the TAG plates, 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).
3. **[Option 2]** Using a TruTemp microheating system:
   a. Add 10 µl HTM to the LRB plates.
   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).
4. Transfer all supernatant from each tagmentation reaction to the NTC plates.

NOTE
Proceed ***immediately*** to the next step.

Clean Up Tagmentation Reaction

Preparation

1 Label 2 new semi-skirted PCR plates NPP1 and NPP2.

Procedure

1 Add 25 µl SPB to the NTC plates.
2 Shake at 1800 rpm for 2 minutes.
3 Incubate at room temperature for 5 minutes.
4 Place on a 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 5 minutes.
9 Add 22.5 µl RSB.
10 Shake at 1800 rpm for 2 minutes.
11 Incubate at room temperature for 2 minutes.
12 Centrifuge at 280 × g for 2 minutes.
13 Place on a magnetic stand and wait for the liquid to clear (~2 minutes).
14 Transfer 20 µl supernatant to the NPP plates.

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.
- 72°C for 3 minutes
- 98°C for 30 seconds
- 12 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 first Nextera XT Index Kit in the TruSeq Index Plate Fixture, as follows.
- Index 1 (i7) adapters in columns 1–12.
- Index 2 (i5) adapters in rows A–H.

2 Place the NPP1 plate on a TruSeq Index Plate Fixture.

3 Using a multichannel pipette, add 5 µl of each Index 1 (i7) adapter to each row. 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 each column. 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 Repeat steps 1–6 for the NPP2 plate using a different Nextera XT Index Kit.

8 Place both plates 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 2 new midi plates NPC1 and NPC2.

2 Label 2 new PCR plates HLP1 and HLP2.

Procedure

1 Transfer the PCR reactions from the NPP plates to the NPC plates.

2 Add 25 µl SPB.

3 Shake at 1800 rpm for 2 minutes.

4 Incubate at room temperature for 5 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 5 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 plates to the HLP plates.

SAFE STOPPING POINT

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

Library Pooling and 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 5 µl from each well of the HLP plates 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:
-0.67(X) + 11.9 = Y
- 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 deionized water 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.

NOTE
See the *MiSeq System Guide (part # 15027617)* for information about loading consumables and setting up a run.

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

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	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

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

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Illumina
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