

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 |
|--|---|
| <ul style="list-style-type: none"> • 94°C for 3 minutes • 35 cycles of: <ul style="list-style-type: none"> – 94°C for 30 seconds – 60°C for 2 minutes – 68°C for 15 minutes • 68°C for 10 minutes • Hold at 10°C | <ul style="list-style-type: none"> • 94°C for 3 minutes • 10 cycles of: <ul style="list-style-type: none"> – 94°C for 30 seconds – 55°C for 2 minutes – 72°C for 15 minutes • 25 cycles of: <ul style="list-style-type: none"> – 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 $280 \times g$ 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 μ l 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 μ l 80% EtOH.
- 7 Use a 20 μ l pipette to remove residual EtOH.
- 8 Air-dry on the magnetic stand for 5 minutes.
- 9 Add 30 μ l 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 μ l 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 μ l is required for each plate.

- 4 Invert the tube to mix.
- 5 Add 45 μ l 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 μ l 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 μ l 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 μ l from the LRB plates to the TAG plates.
 - b Add 10 μ l 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 μ l 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 μ l 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 μ l 80% EtOH.
- 7 Use a 20 μ l pipette to remove residual EtOH.
- 8 Air-dry on the magnetic stand for 5 minutes.
- 9 Add 22.5 μ l RSB.
- 10 Shake at 1800 rpm for 2 minutes.
- 11 Incubate at room temperature for 2 minutes.
- 12 Centrifuge at $280 \times g$ for 2 minutes.
- 13 Place on a magnetic stand and wait for the liquid to clear (~2 minutes).
- 14 Transfer 20 μ l 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 $280 \times g$ 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 μ l 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 μ l 80% EtOH.
- 8 Use a 20 μ l pipette to remove residual EtOH.
- 9 Air-dry on the magnetic stand for 5 minutes.
- 10 Add 32.5 μ l 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 μ l 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 μ l 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/ μ l) as determined by the fluorometric assay
 - Y is the library volume (μ l) 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 μ l.
- 6 Add 10 μ l 0.1 N NaOH.
- 7 Vortex and then centrifuge briefly to mix.
- 8 Incubate at room temperature for 5 minutes.
- 9 Add 980 μ l HT1 for a final volume of 1000 μ l, and then invert to mix.
- 10 Load 600 μ l 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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