TruSight HLA v2 Sequencing Panel Checklist

Generate HLA PCR Amplicons

- ☐ 1 Add 5 µl of each HLA primer to the LRP plates.
- ☐ 2 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
- ☐ 3 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

- ☐ 4 Add 40 µl PCR master mix. Pipette to mix.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 Place the LRP1 plate on a thermal cycler and run the PCR1 program.
- ☐ 7 Place the LRP2 plate on a thermal cycler and run the PCR2 program.
- ☐ 8 Centrifuge the plates at 280 × g for 2 minutes.
- ☐ 9 Label a new midi plate LRC.
- ☐ 10 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

- ☐ 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 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

- ☐ 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

- ☐ 1 [Option 1] Using a thermal cycler:
  - ☐ a Using a prealiquoted PCR 8-tube strip, add 10 µl HTM to the TAG plate, and then pipette to mix.
  - ☐ b Transfer 40 µl to the TAG plate.
  - ☐ c Place on a thermal cycler (58°C) for 12 minutes.
  - ☐ d Spin the plate briefly.
  - ☐ e 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 Spin the plate briefly.
  - ☐ e 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

- ☐ 1 Pool tagmentation products from the NTC plate into row A of the TPP plate.
- ☐ 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 Place on a magnetic stand and wait for the liquid to clear (~2 minutes).
- ☐ 14 Transfer 20 µl supernatant to the NPP plate.
  - 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

- ☐ 1 Arrange the Nextera XT Index Kit, 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.
- ☐ 4 Using a multichannel pipette, add 5 µl of each Index 2 (i5) adapter to columns 1–6.
- ☐ 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

- ☐ 1 Transfer 45 µl of the PCR reactions 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 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

- ☐ 1 Transfer 7 µl from each well to the PHL tube.
- ☐ 2 Quantify the library.
- ☐ 3 Determine the library volume to denature:
  $Y = 15/x$
  - X is the library concentration (ng/µl)
  - Y is the library volume (µl)
- ☐ 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, and then invert to mix.
- ☐ 10 Load 600 µl denatured library 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