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Sequencing TruSight HLA v2 Libraries on a MiniSeq System

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TruSight® HLA v2 libraries can be sequenced using several Illumina platforms and kit configurations to accommodate customer needs. Although the *TruSight HLA v2 Sequencing Panel Reference Guide (document # 1000000010159)* recommends sequencing libraries on a MiSeq® System, altering the loading protocol of the final libraries allows for sequencing on a MiniSeq™ System. This guide provides the steps necessary to sequence TruSight HLA v2 libraries on a MiniSeq System.

Denature Libraries

To prepare TruSight HLA v2 libraries, see the *TruSight HLA v2 Sequencing Panel Reference Guide (Document # 1000000010159)*. Follow the procedure up to the beginning of the Pool Final Libraries for MiSeq Sequencing procedure. After quantifying the library in the PHL tube with the Qubit BR assay or a fluorometric assay, use the following protocol to prepare the libraries for sequencing on a MiniSeq System.

Consumables

- MiniSeq Reagent Kit
  - MiniSeq Mid Output Reagent Kit (for 12–48 samples) or
  - MiniSeq High Output Reagent Kit (for 49–144 samples)
- HT1 (Hybridization Buffer)—From MiniSeq Reagent Kit
- RSB (Resuspension Buffer)—From TruSight HLA v2 Sequencing Panel Kit
- Freshly prepared 0.1 N NaOH
- 1.5 ml microcentrifuge tubes
- Deionized water

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
MiniSeq reagent cartridge	-25°C to -15°C	Thaw in a room temperature water bath for 3 hours
HT1	-25°C to -15°C	Thaw at room temperature. Set aside at 2°C to 8°C.

2 Prepare a fresh dilution of 0.1 N NaOH from 2 N NaOH.

3 Label a new 1.5 ml Eppendorf tube IHL.

4 Label a new 1.5 ml Eppendorf tube DHL.

Procedure

1 Determine the library volume to denature using the formula **Y = 15/x**.
  - X—Library concentration (ng/µl) as determined by the fluorometric assay
  - Y—Library volume (µl) to dilute and denature

2 Transfer the volume determined by Y to the IHL tube.
If Y > 10 µl in the calculation, the library yield might be too low. Contact Illumina Technical Support.

3 Dilute with RSB to a final volume of 10 µl.

4 Add 10 µl 0.1 N NaOH.

5 Vortex and then centrifuge briefly to mix.

6 Incubate at room temperature for 5 minutes.

7 Transfer 3 µl to the DHL tube.
Less volume of denatured library is used to sequence on a MiniSeq than a MiSeq. This dilution protocol is a general guideline to achieve satisfactory cluster density. If necessary, adjust the volumes.

8 Add 997 µl HT1 for a final volume of 1000 µl, and then invert to mix.

9 Load 600 µl denatured library from the DHL tube onto the thawed reagent cartridge.
See the *MiniSeq System Guide (document # 1000000002695)* for information about loading consumables and setting up a run.

Local Run Manager/FASTQ File Generation

Use Local Run Manager to set up your MiniSeq run and the Generate FASTQ Analysis Module to generate FASTQ files after sequencing.

For more information, see the *Local Run Manager Software Guide (document # 1000000002702)* and the *Local Run Manager Generate FASTQ Analysis Module Workflow Guide (document # 1000000003344)*.

Specify Run Settings

When specifying run settings, select the following options:

- **Library Kit**—The Nextera kit used during Amplify PCR (Nextera XT Index Kit or Nextera XT Index Kit v2)
- **Index Reads**—2
- **Read Type**—Paired End
- **Number of Cycles**—Enter the number of cycles needed (eg, 151 × 8 × 8 ×151)

Specify Samples for the Run

Specify samples using 1 of the following methods:

- Enter samples manually
- Import samples using the TruSight HLA v2 MiniSeq Sample Sheet Template

Data Quality

For more information about performance parameters, see the MiniSeq System Specification Sheet. Many of the preferred run metrics are similar to the MiSeq, but there are some key differences due to the notable changes in sequencing chemistry and the platforms. The following table highlights the values that lead to the best results.

Metric	Target Range
Cluster Density (K/mm2)	140–260
Cluster PF %	≥ 75
R1 Phasing %	≤ 0.3
R1 Prephasing %	≤ 0.3
% Q30 R1	≥ 80
R2 Phasing %	≤ 0.4
R2 Prephasing %	≤ 0.3
% Q30 R2	≥ 60

Supporting Information

Acronyms

Acronym	Definition
PHL	Pooled HLA Libraries
IHL	Intermediate HLA Libraries
DHL	Diluted HLA Libraries
RSB	Resuspension Buffer

Consumables

Consumables	Supplier/Description
20 µl pipette filter tips	General lab supplier
20 µl single channel pipette	General lab supplier
1000 µl pipette filter tips	General lab supplier
1000 µl single channel pipette	General lab supplier
1.5 ml microcentrifuge tubes	General lab supplier
2 N NaOH	Sigma Molecular Grade 10 N NaOH, Catalog #72068, General lab supplier
Laboratory-grade water	General lab supplier

Equipment

Equipment	Supplier/Description
Microplate centrifuge	General lab supplier
Vortexer	General lab supplier

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