

# Using a PhiX Control for TruSight HLA v2 Sequencing Runs

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The PhiX virus has a small, well-defined genome with a diverse yet balanced representation of nucleotides. The nature of the PhiX genome permits rapid alignment and helps calculate sequencing metrics. Therefore, Illumina recommends a spike-in of 12.5 pM PhiX as an effective positive control for alignment and error rate estimates with TruSight® HLA v2 Sequencing Panel libraries. Perform the following procedure to prepare PhiX controls to be sequenced with TruSight HLA v2 libraries.

For more information about PhiX, see the *Using a PhiX Control for HiSeq® Sequencing Runs* technical note.

## Consumables

- ▶ 10 nM PhiX
- ▶ HT1 (Hybridization Buffer)
- ▶ Freshly prepared 0.2 N NaOH
- ▶ 10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20
- ▶ 1.5 ml microcentrifuge tubes
- ▶ Deionized water

## Preparation

- 1 Prepare a fresh dilution of 0.2 N NaOH from 1 N NaOH.
- 2 Label 3 new 1.5 ml microcentrifuge tubes DPX, IPL, and SHL.
- 3 Denature the TruSight HLA v2 library in the final DHL tube. For the procedure, see the *TruSight HLA v2 Sequencing Panel Reference Guide* (document # 1000000010159).
- 4 Prepare the denatured 20 pM PhiX library as follows.
  - a Add 2 µl PhiX to the DPX tube.
  - b Add 3 µl Tris-HCl with Tween 20.
  - c Add 5 µl of 0.2 N NaOH.
  - d Vortex and then centrifuge briefly.
  - e Incubate at room temperature for 5 minutes.
  - f Add 990 µl HT1 for a final volume of 1000 µl, and then invert to mix.

The DPX tube can be stored at -25°C to -15°C for up to 3 weeks.

## Procedure

- 1 Transfer 375 µl from the DPX tube to the IPL tube.

- 2 Add 225 µl HT1, and then invert to mix.
- 3 Transfer 6 µl from the IPL tube to the SHL tube.
- 4 Add 594 µl denatured TruSight HLA v2 library (DHL tube) to the SHL tube for a final volume of 600 µl. Invert to mix.
- 5 Load the 600 µl from the SHL tube into the thawed cartridge. For more information about loading consumables and setting up a run, see the *MiSeq® System Guide* (document # 15027617).

## Data

The quality control measures provided by adding PhiX to a TruSight HLA v2 library can be viewed in the summary tab of Sequencing Analysis Viewer. For more information, see the *Sequencing Analysis Viewer Software Guide* (document # 15066069).

When using the recommended PhiX spike-in, the percent of total reads aligned to the PhiX genome is 0.5–1.5%. The error rate is 0.3–0.5%. The percent aligned and error rate varies depending on library quality and PhiX lots. If the percent aligned is outside of these ranges, the dilutions of PhiX can be modified. The final target input amount of PhiX in the SHL tube is 22 pg.

For more information, contact Illumina Customer Solutions.

## Supporting Information

### Acronyms

Acronym	Definition
DHL	Diluted HLA Libraries
DPX	Denatured PhiX
IPL	Intermediate PhiX Libraries
SHL	Sequence HLA Libraries

### 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
10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20	Teknova, Catalog #T7724
1 N NaOH	Sigma Molecular Grade 10 N NaOH, Catalog #72068, General lab supplier
Laboratory-grade water	General lab supplier
PhiX	Illumina, Catalog #FC-110-3001

### Equipment

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

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