

Read 1 Primer Rehyb on a TruSeq v3 Flow Cell

For Research Use Only. Not for use in diagnostic procedures.

Read 1 primer rehybridization repeats the Read 1 sequencing primer hybridization step on the cBot 2 or cBot. If run metrics indicate low cluster numbers, low cluster intensities, or other concerns, perform primer rehybridization to save the flow cell.

| Rehybridization Kit Name | Catalog # |
|--|-------------|
| TruSeq® v2 cBot™ Multi-Primer Re-Hybridization Kit | GD-304-2001 |

Rehybridization Kit Contents

The cBot reagent plate provided in the kit contains 4 rows of foil-sealed 8-tube strips filled with hybridization reagents.

- ▶ Row 1—HT1 (Hybridization Buffer)
- ▶ Row 7—HT2 (Wash Buffer)
- ▶ Row 10—HP5 (0.1 N NaOH)
- ▶ Row 11—HP6 (Read 1 Sequencing Primer)

Additional cBot Consumables

- ▶ **HiSeq flow cell**—cBot Manifold, catalog # SY-401-2015
- ▶ **Dual-index sequencing primer**—Primer rehybridization with a flow cell containing dual-indexed Nextera libraries requires HP10 provided in the TruSeq Dual Index Sequencing Primer Box, catalog # FC-121-1003 (SR) or catalog # PE-121-1003 (PE).
- ▶ **Custom primers**—Custom sequencing primers, if necessary for your experiment.

Thaw the cBot Reagent Plate

- 1 Remove the cBot reagent plate from -25°C to -15°C storage.
- 2 Thaw in a room temperature water bath for 30 minutes.

Prepare the cBot Reagent Plate

- 1 Invert the cBot reagent plate to mix.
- 2 Vortex to dislodge trapped air bubbles.
- 3 Tap on a hard surface or pulse centrifuge to collect droplets at the bottom of the tubes.
- 4 Remove the clear plastic cover, and make sure that the tube strips are securely seated.
- 5 **Promptly** proceed to setting up the cBot.

Prepare HP10 for Nextera Libraries

- 1 Remove HP10 from -25°C to -15°C storage.
- 2 Thaw in a room temperature water bath for 20 minutes. Use deionized water.
- 3 Add 150 µl HP10 to each tube of a 0.2 ml 8-tube strip.
- 4 Set aside on ice.

Rehybridize Primers on the cBot

- 1 Select from the following recipes:
 - ▶ Without custom primers—**Repeat_Hyb_v#**
 - ▶ With custom primers or HP10—**Repeat_TubeStripHyb_v#**

| Flow Cell | Recipe Version |
|-------------------|----------------|
| TruSeq v3 (HiSeq) | v8 recipes |

- 2 Remove the red foil seal from row 10 (HP5).
- 3 Follow the software prompts to load the cBot rehybridization reagent plate, the flow cell to be rehybridized, and the manifold.
- 4 If you are using Nextera libraries, load the 8-tube strip of HP10 in the Primer row when prompted by the cBot software.
- 5 After passing the pre-run check, select **Start**. Primer rehybridization takes approximately 20 minutes.

Set Up a New Run

- 1 When rehybridization is complete, remove the rehybridized flow cell from the cBot.
- 2 Set up a new sequencing run. For instructions, see the system guide for your sequencing instrument.

Technical Assistance

For questions, visit the Illumina support page at support.illumina.com or log in to your MyIllumina account for access to support bulletins. If you do not find the information you need, contact Illumina Technical Support.

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