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Read 1 Primer Rehyb on a HiSeq X 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 #
HiSeq X™ cBot™ Multi-Primer Rehybridization Kit v2	GD-305-2001

Rehybridization Kit Contents and Storage

The rehybridization kit provides a cBot reagent plate. Four rows are filled with hybridization reagents:

- Row 1—HT1 (Hybridization Buffer)
- Row 5—LDR1 (Low Bias Denaturation Reagent)
- Row 7—HT2 (Wash Buffer)
- Row 11—HP10 (Read 1 Sequencing Primer)

Store the rehybridization kit contents at -25°C to -15°C.

Additional cBot Consumables

A new cBot manifold is required for primer rehybridization (sold separately). Use cBot Manifold, Illumina catalog # SY-401-2015.

Sequencing Reagents

The same set of reagents can be used to start a new run after rehybridization only when the run is stopped immediately after the first base report. If additional cycles are performed in Read 1, a new set of sequencing reagents is required.

Stop a Run on the HiSeq X

1. Stop the sequencing run.
2. Remove reagent bottles and tubes from the instrument and cover them with parafilm. Store at 2°C to 8°C until you can start a new run after primer rehybridization. Otherwise, prepare a fresh set of sequencing reagents.
3. Remove the flow cell from the HiSeq X.
4. Store in the storage tube with buffer at 2°C to 8°C until the cBot reagents have thawed.

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.
5. ***Promptly*** proceed to setting up the cBot.

Rehybridize Primers on the cBot

1. Perform a cBot instrument wash.
2. Select the recipe **HiSeq_X_Cluster_Kit_v2_cBot_Rehyb_recipe_v2.0**.
3. Remove the flow cell from storage and clean as follows.
   a. Rinse with laboratory-grade water.
   b. Dry with a lens cleaning tissue.
   c. Wipe each side with an alcohol wipe.
   d. Dry with a lens cleaning tissue.
4. Follow the software prompts to load the cBot reagent plate, the flow cell to be rehybridized, and the manifold.
5. After passing the pre-run check, select **Start**.
   Primer rehybridization takes approximately 20 minutes.
6. When primer rehybridization is complete, remove the rehybridized flow cell from the cBot.
7. Set aside the flow cell in the storage tube with buffer at 2°C to 8°C.

Set Up a New Run on the HiSeq X

1. Perform a water wash for the SBS reagent positions only. The wash takes about 40 minutes.
2. Set up a new run on the HiSeq X.
3. When prompted to load reagents, do 1 of the following:
   - Reload reagents stored from the original run.
   - Load a fresh set of reagents.

   NOTE
   Make sure that reagents are freshly inverted to mix.
4. Remove the flow cell from storage and clean as follows.

a Rinse with laboratory-grade water.
b Dry with a lens cleaning tissue.
c Wipe each side with an alcohol wipe.
d Dry with a lens cleaning tissue.

5 When prompted, load the rehybridized flow cell.

6 Start the new run and monitor run metrics.

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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