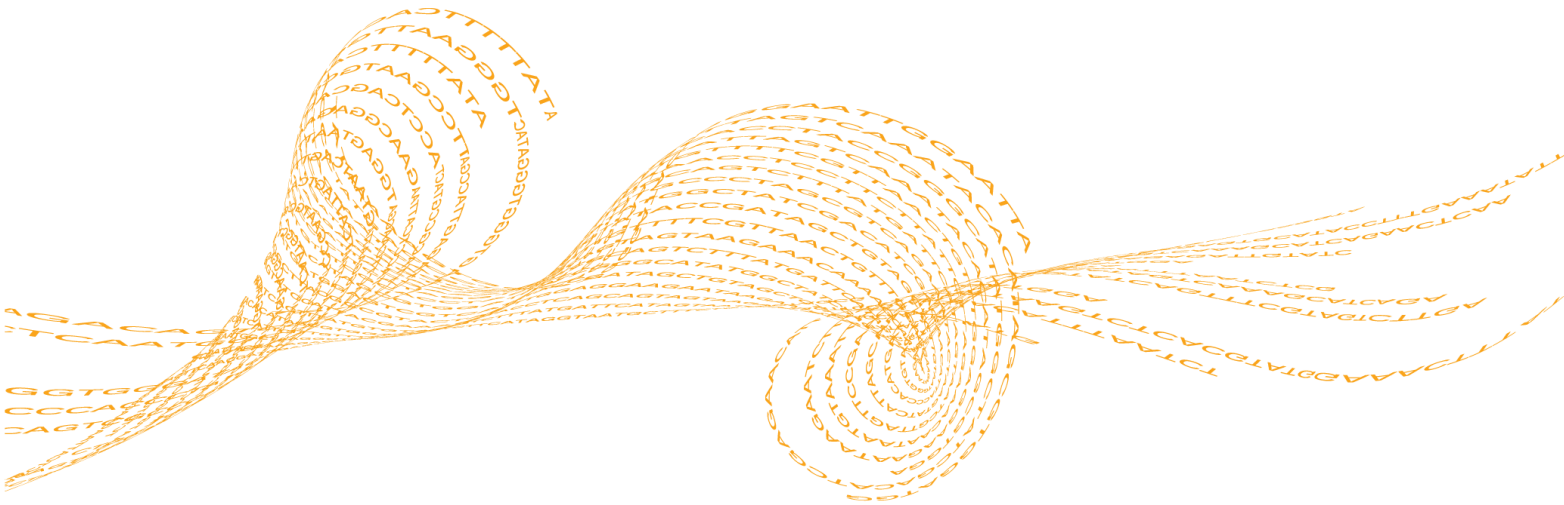
HiSeq® High Output Primer Rehybridization Reference Guide

FOR RESEARCH USE ONLY



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Part # 15050105 Rev. B
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Revision History

Part #	Revision	Date	Description of Change
15050105	B	December 2014	Added the following procedures: • Read 2 primer rehybridization on a TruSeq v3 flow cell • Manual reagent priming
15050105	A	April 2014	Initial release.

Introduction

A rehybridization run on the HiSeq repeats the sequencing primer hybridization step. If run metrics indicate low cluster numbers, low cluster intensities, or other concerns, perform primer rehybridization to rescue the flow cell. Primer rehybridization does not damage clusters on the flow cell.

Sequencing primers are specific to a read. To perform primer rehybridization for certain primers, stop the run before the run progresses beyond the point associated with that primer.

Primer	Perform After	Perform Before
Read 1	Read 1 begins	The last cycle of Read 1
Index 1 Read	Read 1 completes and the first Index Read begins	The last cycle of Index 1 Read
Index 2 Read	Index 1 Read completes and the Index Read begins (single-read flow cells only)*	The last cycle of Index 2 Read
Read 2	Paired-end resynthesis completes and Read 2 begins	The last cycle of Read 2

* Rehybridization of Index 2 Read is not possible on a paired-end flow cell.

NOTE
For TruSeq v3 mode, only Read 2 primer rehybridization is possible on the HiSeq.

After stopping a run, you do not need to remove the flow cell or remove reagents unless a subsequent wash is performed. Prepare rehybridization reagents, manually prime reagents, and set up a rehyb run from the Welcome screen.

After primer rehybridization, sequencing resumes automatically from the point that directly follows the hybridization step. For example, if the Read 2 primer (HP11) was rehybridized, the run resumes automatically at Read 2.

Rehybridized Primer	Run Resumes At
Read 1 (HP10)	Read 1
Index 1 Read (HP12)	Index Read i7
Index 2 Read (HP9) Single-read flow cells only	Index Read i5
Read 2 (HP11 or HP7)	Read 2

TruSeq v3 Read 2 Rehyb

For a TruSeq v3 flow cell, you can perform Read 2 primer rehybridization on the HiSeq using HiSeq Control Software (HCS) v2.0 or later. Read 1 primer rehybridization is performed on the cBot. For instructions, see *Performing Primer Rehybridization on the cBot (part # 15018149)*.

Read 2 rehybridization requires additional reagents and sequencing primers. These reagents are not sold in a separate kit.

Reagent	Volume	Kit
HP3—Denaturation Solution HT2—Wash Buffer	4 ml each	TruSeq PE Cluster Kit v3
HP7—Read 2 Primer Mix (not compatible with most Nextera libraries) HP11—Read 2 Primer Mix (compatible with all library types)	3 ml each	TruSeq Dual Indexing Sequencing Primer Box (PE)

HiSeq Multi-Primer Rehyb Kit v4 Overview

The HiSeq® Multi-Primer Rehyb Kit provides reagents and sequencing primers for performing on-instrument primer rehybridization for Read 1, Index 1 (i7) Read, Index 2 (i5) Read on a single-read flow cell, or Read 2.

Kit Name	Catalog #
HiSeq Multi-Primer Rehyb Kit v4	GD-403-4001

The kit is shipped at -25°C to -15°C. As soon as you receive your kit, promptly store the kit components at the indicated temperature to ensure proper performance.

HiSeq Rehyb Kit v4 Contents

Kit Component	Container	Description
SB2*	250 ml bottle	Incorporation Buffer
FDR	15 ml tube	Fast Denaturation Reagent, High Output (contains formamide)
HP9	15 ml tube	Index 2 (i5) Primer Mix, High Output (used with single-read flow cells only)
HP10	15 ml tube	Read 1 Primer Mix, High Output
HP11	15 ml tube	Read 2 Primer Mix, High Output
HP12	15 ml tube	Index 1 (i7) Primer Mix, High Output

*SB2 can be stored at 2°C to 8°C.

Prepare Reagents

All reagents are loaded onto the instrument during rehyb run setup unless a wash was performed after the sequencing run was stopped. If a wash was performed, reagents are loaded before the manual prime step.

Before loading reagents, use the following instructions to prepare reagents. Reagents for a HiSeq v4 rehyb run are provided in the HiSeq Multi-Primer Rehyb Kit v4. Reagents for a TruSeq v3 Read 2 rehyb are prepared separately.

Prepare Reagents for HiSeq v4 Rehyb

1. Remove SB2 from -25°C to -15°C storage and thaw in a room temperature water bath for about 90 minutes, or until the reagent is thawed.
   If SB2 was stored at 2°C to 8°C, use directly from storage.
2. Invert the SB2 bottle several times to mix and visually inspect to make sure that the reagent is thawed.
3. Remove the following tubes from -25°C to -15°C storage depending on the primer to be rehybridized:
   - **Read 1**—FDR and HP10
   - **Index 1 Read**—FDR and HP12
   - **Index 2 Read** (single-read flow cell)—FDR and HP9
   - **Read 2**—FDR and HP11
4. Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents have thawed.
5. Invert each tube several times to mix.
6. Set aside at room temperature until you are ready to load reagents.

Prepare Reagents for TruSeq v3 Read 2 Rehyb

1. Thaw reagents in a beaker filled with room temperature deionized water for about 20 minutes, or until reagents are thawed.
2. Prepare HP11 and HT2 as follows:
   a. Invert each tube several times to mix.
   b. Centrifuge each reagent at 1,000 rpm for 1 minute.
3. Prepare HP3 as follows:
   a. Invert the tube several times to mix the reagent and then pulse centrifuge.
   b. Transfer 2.85 ml PW1 into a 15 ml Sarstedt conical tube and add 150 µl HP3.
   c. Invert the tube several times to mix.
4. Set aside at room temperature until you are ready to load the reagents.

Manually Prime Reagents

Priming reagents is required when an instrument wash is performed after a sequencing run is stopped for rehybridization. Otherwise, skip priming and proceed with setting up the rehyb run for HiSeq v4 mode or setting rehyb parameters for TruSeq v3 mode.

Use the Check button on the Welcome screen to manually prime paired-end positions before setting up the rehyb run. SBS positions are primed during run set up.

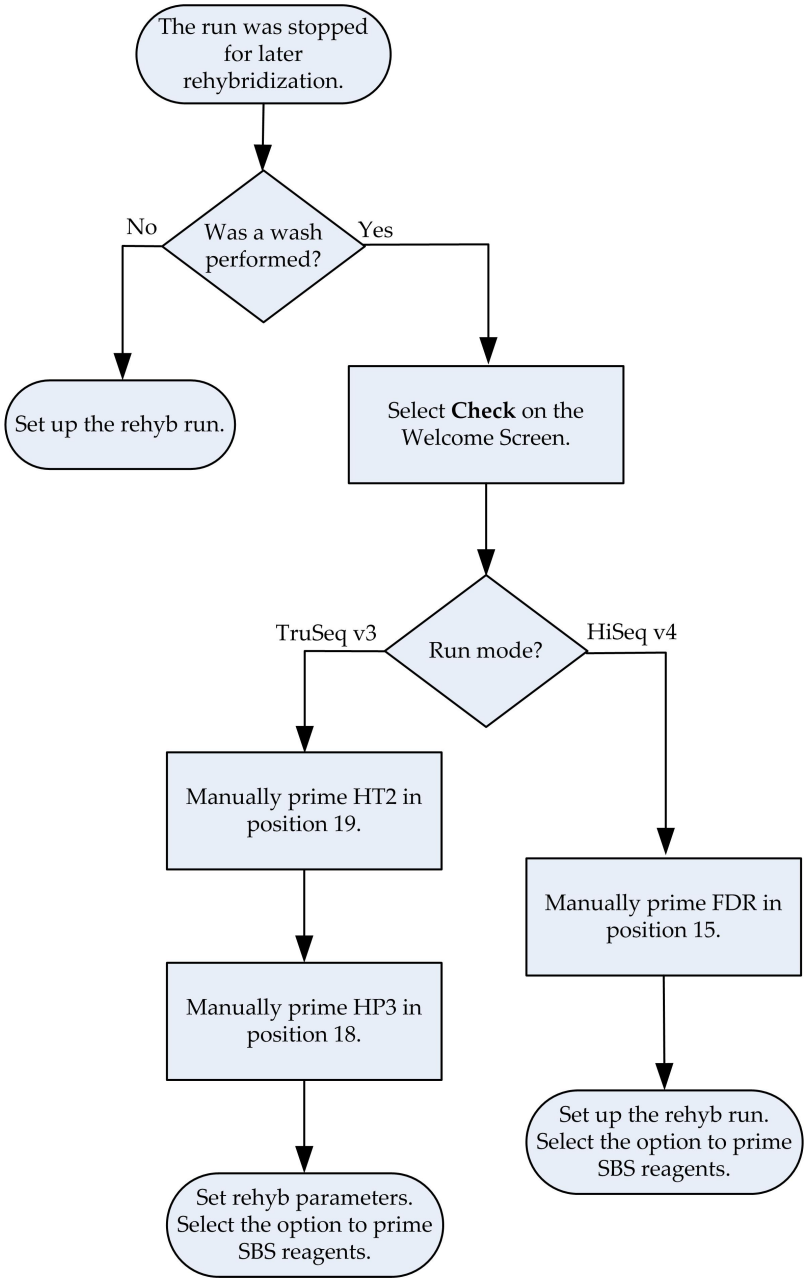
Before beginning the following manual prime steps, load rehyb reagents onto the instrument. For instructions, see *Load HiSeq v4 Rehyb Reagents* on page 10 or *Load TruSeq v3 Reagents* on page 12.

1. From the Welcome screen, select **Check**.
2. Enter the flow cell ID. Make sure that a used wash flow cell is loaded onto the instrument. Do not use the flow cell from the stopped run to prime reagents.
3. Confirm that the **Vacuum Engaged** checkbox is selected, and then select **Next**.
4. [For HiSeq v4 mode] Select position 15 (FDR) from the drop-down list.
5. [For TruSeq v3 mode] Select position 19 (HT2) from the drop-down list.
6. Confirm the following default values:
   - Volume: **250**
   - Aspirate Rate: **250**
   - Dispense Rate: **2000**
7. Select **Pump**.
8. [For TruSeq v3 mode] Select position 18 (HP3) from the drop-down list and repeat steps 6 and 7.
9. Select **Next** to return to the Welcome screen.

Manual Prime Flowchart

The following flowchart illustrates the manual prime procedure and when it is needed.

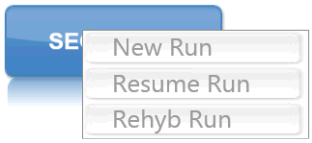
Figure 1 Manual Prime Flowchart



Set Up a Rehyb Run for HiSeq v4

NOTE
If a run is in progress on the adjacent flow cell, check the status of the run. Make sure that the adjacent run is not within the first 5 cycles of Read 1 (template generation) or performing paired-end resynthesis before Read 2.

1 From the Welcome screen, select **Sequence**, and then **Rehyb Run**.



The Rehyb screen opens.

2 From the Rehyb screen, select the run folder associated with the flow cell.

3 Use the Rehyb At drop-down list to select 1 of the following points in the run to perform the rehybridization step:
- **Read 1**—Rehybridizes the Read 1 primer, HP10
- **Index i7**—Rehybridizes the Index 1 (i7) Read primer, HP12
- **Index i5**—Rehybridizes the Index 2 (i5) Read primer, HP9 (single-read flow cells only)
- **Read 2**—Rehybridizes the Read 2 primer, HP11

4 Review the run parameters on the screen, and then select **Next**.

5 From the Reagents screen, enter the rehyb kit ID.
The SBS kit ID, kit size, and number of cycles remaining are automatically populated based on run parameters stored in the run folder.

6 Select **Prime SBS Reagents** to prime SBS reagents only if a wash was performed before the rehyb run. Always perform the priming step with a used wash flow cell.

7 Select **Next**.

Load HiSeq v4 Rehyb Reagents

NOTE
If rehyb reagents were loaded for manual priming, do not reload them. Proceed to *Start the Rehyb Run* on page 11.

1 Using the SB2 provided in the rehyb kit, replenish the SB2 in position 5 on the SBS rack.

NOTE
Depending on how many cycles were performed before the run was stopped, additional SBS reagents might be required. Make sure that remaining SBS reagents are sufficient to repeat the read associated with the primer to be rehybridized. A 250-cycle SBS kit performs up to 275 cycles. A 50-cycle SBS kit performs up to 74 cycles.

2 Using FDR provided in the rehyb kit, replace the FDR in position 15 in the paired-end rack.

NOTE
The new reagent tube contains sufficient FDR reagent to perform primer rehybridization, any subsequent indexing reads, and paired-end resynthesis.

3 Using primers provided in the rehyb kit, replace reagents in the paired-end rack depending on the primer to be rehybridized:

- **Read 1:** Replace the PW1 in position 18 with the tube of HP10.
- **Index 1 (i7):** Replace the tube in position 17 with the fresh tube of HP12.
- **Index 2 (i5):** For single-read flow cells only, replace the tube in position 16 with the fresh tube of HP9.
- **Read 2:** Replace the tube in position 16 with the fresh tube of HP11.

Figure 2 Rehyb Reagent Positions

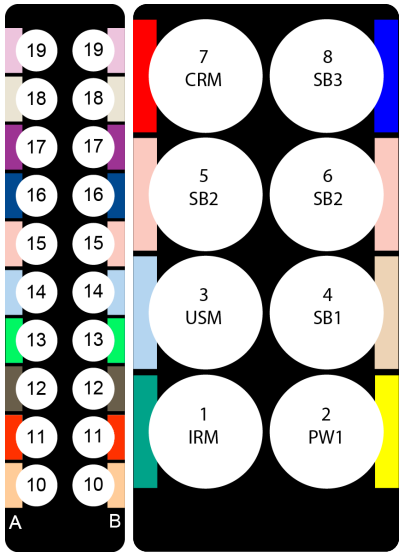


Figure 3 Rehyb Reagent Positions

Position	Read 1	Index 1 (i7)	Index 2 (i5) SR flow cell only	Read 2 PE flow cell
15	FDR	FDR	FDR	FDR
18	HP10	-	-	-
17	-	HP12	-	-
16	-	-	HP9	HP11

NOTE
The tube of HP9 and HP11 are each labeled with a blue color-coded label for position 16. Make sure that you load the correct primer in position 16. Use HP9 for Index 2 on a single-read flow cell only. Use HP11 for Read 2 on a paired-end flow cell.

Start the Rehyb Run

Select **Start**. The instrument performs a rehyb run and then continues the original run based on run parameters stored in the run folder.

For more information, see the user guide for your sequencing instrument:

- *HiSeq 2500 System User Guide (part # 15035786)*
- *HiSeq 2000 System User Guide (part # 15011190)*
- *HiSeq 1500 System User Guide (part # 15035788)*

Set Rehyb Parameters for TruSeq v3

1 From the Welcome screen, select **Sequence** | **Resume Run**. The Resume screen opens.

2 From the Run Folder drop-down list, select the run folder for the affected run.

3 Specify the point to resume the run:
   a Select **Paired End Turnaround** from the Resume At drop-down list.
   b Select **Rehybridization** from the adjacent drop-down list.

4 Select **Next**.

5 When prompted, select **Yes** to reset Real-Time Analysis (RTA) when the run resumes. If RTA is running, it stops and removes any output files generated for Read 2, which includes phasing and matrix estimates. RTA will restart after rehybridization is complete.

6 Select **Prime SBS Reagents** to prime SBS reagents only if a wash was performed before the rehyb run. Always perform the priming step with a used wash flow cell.

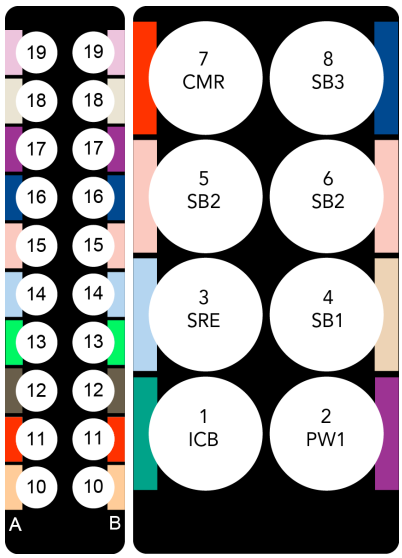
7 Select **Next**.

Load TruSeq v3 Reagents

NOTE
If rehyb reagents were loaded for manual priming, do not reload them. Proceed to *Start the Rehyb Run* on page 11.

1 Using the prepared reagents, replace reagents in the paired-end rack.

Figure 4 Reagent Positions



Position	Reagent
16	HP11
18	HP3 (diluted)
19	HT2

2 Make sure that remaining SBS reagents are sufficient to repeat the read.
Depending on how many Read 2 cycles were performed before the run was stopped, SBS reagents might need replenishing. A 200-cycle SBS kit performs up to 209 cycles. A 50-cycle SBS kit performs up to 58 cycles. If you replenish SBS reagents, thoroughly mix the replenished reagent.

Resume the Sequencing Run

Select **Start**. The instrument performs rehybridization and then continues the original run based on run parameters stored in the run folder.

NOTE
Data from the original Read 2 cycles appears in the analysis charts on the HCS Run Overview screen and in Sequencing Analysis Viewer (SAV) until new data are processed. Expect a delay of several cycles before new data are reported.

For more information, see the user guide for your sequencing instrument:

- *HiSeq 2500 System User Guide (part # 15035786)*
- *HiSeq 2000 System User Guide (part # 15011190)*
- *HiSeq 1500 System User Guide (part # 15035788)*

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then click **Documentation & Literature**.

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