

# HiSeq® Rapid Primer Rehybridization Reference Guide

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## Revision History

Part #	Revision	Date	Description of Change
15059379	B	December 2014	Added instructions for manually priming reagents before rehybridization. Added information about the TruSeq Rapid Rehybridization Kit.
15059379	A	November 2014	Initial release.

## Introduction

A rehybridization run on the HiSeq 2500 or HiSeq 1500 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.

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)	Read 2

## Rapid Rehyb Kits Overview

The rapid rehybridization kits provide 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 Rapid Rehybridization Kit	GD-404-1001
TruSeq Rapid Rehybridization Kit	GD-402-2001

The kits are shipped at -25°C to -15°C. When you receive your kit, promptly store the kit components at the indicated temperature to ensure proper performance.

### Rapid Rehyb Kit Contents

Kit Component	Container	Description
FDR	15 ml tube	Fast Denaturation Reagent
HP9	15 ml tube	Index 2 (i5) Primer Mix (use with single-read flow cells only)
HP10	15 ml tube	Read 1 Primer Mix
HP11	15 ml tube	Read 2 Primer Mix
HP12	15 ml tube	Index 1 (i7) Primer Mix
USB	250 ml bottle	Universal Sequencing Buffer



#### WARNING

**This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).**

## Prepare Rehyb Reagents

- 1 Remove USB 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 USB was stored at 2°C to 8°C, use directly from storage.
- 2 Invert the USB 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.

## Manually Prime Reagents

Manually 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.

Use the **Check** button on the Welcome screen to perform the manual prime before setting up the rehyb run. SBS positions must be primed twice.

Before beginning the following manual prime steps, load rehyb reagents onto the instrument. For instructions, see *Load Rehyb Reagents* on page 9.

- 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 Select position 15 (FDR) from the drop-down list.
- 5 Confirm the following default values:
  - Volume: **250**
  - Aspirate Rate: **1500**
  - Dispense Rate: **2000**
- 6 Select **Pump**.
- 7 Select position 1 (IMT or IMM) from the drop-down list. The reagent in position 1 depends on the SBS kit you are using.
- 8 Select **Pump**.
- 9 When pumping is complete, select **Pump** again.
- 10 Repeat steps 7–9 for each of the SBS reagent positions listed in the following table.

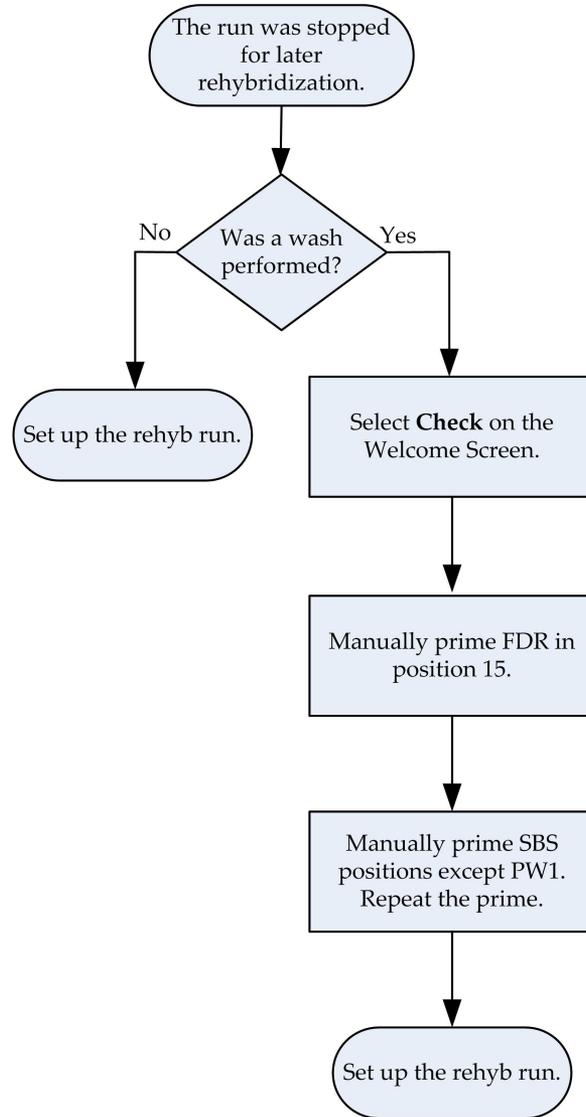
Position	HiSeq Rapid SBS v2 Reagent	TruSeq Rapid SBS Reagent
3	USM	SRM
5	USB	USB
6	USB	USB
7	CRM	CRM
8	CWM	-

- 11 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



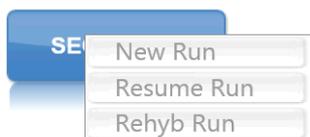
## Set Up a Rehyb Run



### 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 **Next**.

## Load 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 USB provided in the rehyb kit, replenish the USB in positions 5 and 6 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 500-cycle SBS kit performs up to 525 cycles. A 200-cycle SBS kit performs up to 225 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 tube in position 18 with the fresh 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 HiSeq Rehyb Reagent Positions

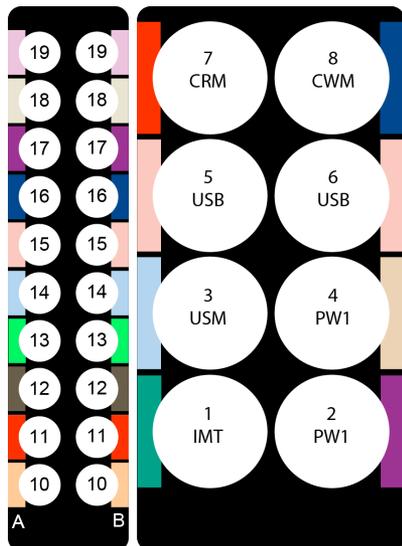
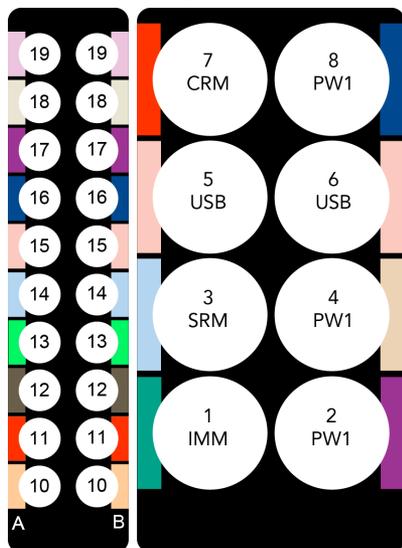


Figure 3 TruSeq Rehyb Reagent Positions



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 1500 System User Guide (part # 15035788)*

## Notes

## Technical Assistance

For technical assistance, contact Illumina Technical Support.

**Table 1** Illumina General Contact Information

<b>Website</b>	www.illumina.com
<b>Email</b>	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](http://support.illumina.com/sds.html).

### Product Documentation

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



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