

HiSeq System

Custom Primers Guide

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Introduction

You can use custom sequencing primers for a HiSeq 4000 or HiSeq 3000 run, a TruSeq™ v3 or Rapid Run on the Illumina® HiSeq® 2500 HiSeq® or HiSeq 1500, and a TruSeq v3 run on the HiSeq® 2000. These runs require up to 3 additional steps during setup:

- ▶ Prepare and add each custom primer to an 8-tube strip or 15 ml tube.
- ▶ Load custom primer onto the cBot 2 or cBot.
- ▶ Replace Illumina primer with custom primer on the HiSeq reagent rack.

All other steps follow the run setup described in the system guide for your instrument.



NOTE

Using a custom primer for Index 2 Read on a paired-end flow cell is not supported.

Custom Primers and PhiX

When custom primers are used for a run, the custom primers are loaded in place of Illumina primers for Read 1 and Read 2. Therefore, Illumina primers are not used for the sequencing run.

If Illumina primers are not used for Read 1 or Read 2, the optional Illumina PhiX control is *not* sequenced. To use the PhiX control with custom primers, contact Illumina Technical Support for guidance.



NOTE

Sequencing data from the PhiX control is not generated for index reads, regardless of which indexing primer is used.

Compatible Primer Combinations for HiSeq 3000/4000

Due to the primer design and dual-indexing workflow on the HiSeq 3000 and HiSeq 4000, combinations of custom primers and Illumina primers are limited.

- ▶ If you use a custom Read 1 primer, you have to use a custom Index 2 primer.
- ▶ If you use a Read 1 primer, you have to use a custom Index 1 primer.

For an overview of the indexing workflow, see *Overview of Indexed Sequencing on the NextSeq, MiSeq, and HiSeq Platforms (document # 15057455)*.

Prepare Custom Primers for a HiSeq 3000/4000 Run

Custom primer for Read 1 is loaded onto the cBot. Custom primers for Read 2, Index 1, and Index 2 are loaded onto the HiSeq.

Make sure that you have sufficient HT1 before proceeding. Diluting custom primers might require more than the 15 ml HT1 provided in your cluster kit.



NOTE

Illumina primers can be used as a diluent, but compatibility is not guaranteed.

cBot Recipes for Custom Primers

When using custom primers for Read 1, select a recipe that directs the sippers to pull from the custom primer position.

Flow Cell	Recipe Name
HiSeq 3000/4000 PE Flow Cell	HiSeq_3000_4000_PE_Exclusion_Amp_v1_custom_primer
HiSeq 3000/4000 SR Flow Cell	HiSeq_3000_4000_SR_Exclusion_Amp_v1_custom_primer

Prepare Custom Primers

- 1 If frozen, thaw and inspect each custom primer.
- 2 Use HT1 to dilute custom primers to result in the following concentrations:
 - ▶ **Read 1**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Read 2**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Index 1**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Index 2**—Dilute to 5 ml at final concentration of 0.5 μ M
 Alternatively, dilute each custom primer with the corresponding Illumina primer.
- 3 Dispense custom primers as follows:
 - ▶ **Read 1 custom primer**—Dispense 150 μ l custom primer into each tube of an 8-tube strip for use on the cBot.
 - ▶ **For Read 2, Index 1, and Index 2 custom primers**—Dispense 5 ml each custom primer into separate 15 ml conical tubes for use on the HiSeq.
- 4 Set aside on ice.
- 5 When you are ready to load reagents onto the cBot, place the 8-tube strip containing Read 1 custom primer into the primer row of the tube strip holder.

Primer Positions on the HiSeq 3000/4000

Custom primers for Index 1, Index 2 (single read only), and Read 2 are loaded onto the paired-end reagent rack of the HiSeq 4000 or HiSeq 3000. Load reagents as described in the system guide for your sequencing instrument, replacing Illumina primer with a 15 ml conical tube containing at least 5 ml custom primer.

Illumina Primer	Position
HP11—Read 2 primer	16
HP14—Index 1 and Index 2 primer (on a paired-end flow cell) HP14—Index 1 primer (single index run and single-read flow cell)	17

Prepare Custom Primers for a HiSeq v4 Run

Custom primer for Read 1 is loaded onto the cBot. Custom primers for Read 2, Index 1, and Index 2 are loaded onto the HiSeq.

Make sure that you have sufficient HT1 before proceeding. Diluting custom primers might require more than the 15 ml HT1 provided in your cluster kit.



NOTE

Illumina primers can be used as a diluent, but compatibility is not guaranteed.

cBot Recipes for Custom Primers

When using custom primers for Read 1, select a recipe that directs the sippers to pull from the custom primer position.

Flow Cell	Recipe Name
HiSeq v4 Flow Cell	SR_HiSeq_Cluster_Kit_v4_TubeStripHyb_v9.0 PE_HiSeq_Cluster_Kit_v4_TubeStripHyb_v9.0

Prepare Custom Primers

- 1 If frozen, thaw and inspect each custom primer.
- 2 Use HT1 to dilute custom primers to result in the following concentrations:
 - ▶ **Read 1**—Dilute to 5ml at final concentration of 0.5 μ M
 - ▶ **Read 2**—Dilute to 5ml at final concentration of 0.5 μ M
 - ▶ **Index 1**—Dilute to 5ml at final concentration of 0.5 μ M
 - ▶ **Index 2**—Dilute to 5ml at final concentration of 0.5 μ M
 Alternatively, dilute each custom primer with the corresponding Illumina primer.
- 3 Dispense custom primers as follows:
 - ▶ **Read 1 custom primer**—Dispense 150 μ l custom primer into each tube of an 8-tube strip for use on the cBot.
 - ▶ **For Read 2, Index 1, and Index 2 custom primers**—Dispense 5 ml each custom primer into separate 15 ml conical tubes for use on the HiSeq.
- 4 Set aside on ice.
- 5 When you are ready to load reagents onto the cBot, place the 8-tube strip containing Read 1 custom primer into the primer row of the tube strip holder.

Primer Positions on the HiSeq

Custom primers for Index 1, Index 2 (single read only), and Read 2 are loaded onto the paired-end reagent rack of the HiSeq. Load reagents as described in the system guide for your sequencing instrument, replacing Illumina primer with a 15 ml conical tube containing at least 5 ml custom primer.

Illumina Primer	Position
HP8—Index 1 primer	17
HP7—Read 2 primer (on a paired-end flow cell) HP9—Index 2 primer (on a single-read flow cell)	16

Prepare Custom Primers for a TruSeq v3 Run

Custom primer for Read 1 is loaded onto the cBot. Custom primers for Read 2, Index 1, and Index 2 are loaded onto the HiSeq.

Make sure that you have sufficient HT1 before proceeding. Diluting custom primers might require more than the 15 ml HT1 provided in your cluster kit.



NOTE

Illumina primers can be used as a diluent, but compatibility is not guaranteed.

cBot Recipes for Custom Primers

When using custom primers for Read 1, select a recipe that directs the sippers to pull from the custom primer position.

Flow Cell	Recipe Name
TruSeq v3 Flow Cell	SR_Amp_Lin_Block_TubeStripHyb_v8.0 PE_Amp_Lin_Block_TubeStripHyb_v8.0

Prepare Custom Primers

- 1 If frozen, thaw and inspect each custom primer.
- 2 Use HT1 to dilute custom primers to result in the following concentrations:
 - ▶ **Read 1**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Read 2**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Index 1**—Dilute to 5 ml at final concentration of 0.5 μ M
 - ▶ **Index 2**—Dilute to 5 ml at final concentration of 0.5 μ M
 Alternatively, dilute each custom primer with the corresponding Illumina primer.
- 3 Dispense custom primers as follows:
 - ▶ **Read 1 custom primer**—Dispense 150 μ l custom primer into each tube of an 8-tube strip for use on the cBot.
 - ▶ **For Read 2, Index 1, and Index 2 custom primers**—Dispense 5 ml each custom primer into separate 15 ml conical tubes for use on the HiSeq.
- 4 Set aside on ice.
- 5 When you are ready to load reagents onto the cBot, place the 8-tube strip containing Read 1 custom primer into the primer row of the tube strip holder.

Primer Positions on the HiSeq

Custom primers for Index 1, Index 2, and Read 2 are loaded onto the paired-end reagent rack of the HiSeq. Load reagents as described in the system guide for your sequencing instrument, replacing Illumina primer with a 15 ml conical tube containing at least 5 ml custom primer.

Illumina Primer	Position
HP8—Index 1 primer	17
HP7—Read 2 primer (on a paired-end flow cell) HP9—Index 2 primer (on a single-read flow cell)	16

Prepare Custom Primers for a Rapid Run

Custom primers for a Rapid Run are loaded onto the HiSeq 2500 or HiSeq 1500 at the beginning of the run.

Make sure that you have sufficient HT1 before proceeding. Diluting custom primers might require more than the 15 ml HT1 provided in the cluster kit.



NOTE

Illumina primers can be used as a diluent, but compatibility is not guaranteed.

Prepare Custom Primers

- 1 If frozen, thaw and inspect each custom primer.
- 2 Use HT1 to dilute each custom primer to result 2 ml at 0.5 μ M. Alternatively, dilute each custom primer with the corresponding Illumina primer.
- 3 Dispense 2 ml each custom primer into separate 15 ml conical tubes.
- 4 Set aside on ice.

Primer Positions on the HiSeq

All custom primers are loaded onto the paired-end reagent rack of the HiSeq. Load reagents as described in the system guide for your sequencing instrument, replacing Illumina primer with a 15 ml conical tube containing at least 5 ml custom primer.

Illumina Primer	Primer Position #
HP10–Read 1 primer	18
HP14–Index 1 primer	17
HP11–Read 2 primer (on a paired-end flow cell) HP9–Index 2 primer (on a single-read flow cell)	16

Revision History

Document	Date	Description of Change
Document # 15061846 v03	October 2017	Added a new section - Compatible Primer Combinations for HiSeq 3000/4000 under the Introduction. Changed Illumina primers HP12 to HP14 on primer position 17 for a HiSeq 3000/4000 run and a Rapid Run on the HiSeq.
Document # 15061846 v02	January 2017	Added a new section - Prepare Custom Primers for a HiSeq 3000/4000 Run and added a modified cBot recipe.
Document # 15061846 v01	January 2016	Included cBot 2 as a compatible cluster instrument. Instructions for preparing custom primers for cBot apply to cBot 2 and cBot. Added the <i>cBot 2 System Guide (document # 15065681)</i> to the list of system guides.
Part # 15061846 Rev. B	June 2015	Added information about using an Illumina PhiX control with custom primers, which requires guidance from Illumina Technical Support.
Part # 15061846 Rev. A	October 2014	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

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



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