

NovaSeq

Custom Primers Guide

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Introduction

Using custom primers for a run on an Illumina® NovaSeq™ Sequencing System requires 2 additional steps during run setup:

- ▶ Prepare and add the appropriate volume of each custom primer to the custom primer positions of the cluster cartridge.
- ▶ Specify the use of custom primers on the Run Setup screen.

All other steps follow the run setup workflow as described in the system guide for your NovaSeq System.

Custom primers are supported for Read 1, Read 2, and the Index 1 Read. Due to the primers grafted onto the paired-end flow cell, custom primer for Index Read 2 is not supported.

Custom Primers and PhiX

When custom primers are used for Read 1 or Read 2, the software directs the instrument to pull from reservoirs 5 and 6. 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

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

Primer Positions on the Cluster Cartridge

You can use a combination of Illumina primers and custom primers in the same run. Depending on the combination specified, the software pulls the primer from the appropriate reservoir.

For example, if a custom primer is used for Read 2 but not Read 1, the software pulls the Read 1 primer from the Illumina primer position (#24). The Read 2 primer is pulled from the custom primer position (#6).

Custom Primer	Custom Primer Position
Custom Read 1 primer	5
Custom Read 2 primer	6
Custom Index 1 primer	7

Illumina Primer	Illumina Primer Position
Illumina Read 1 primer	24
Illumina Read 2 primer	13
Illumina Index 1 primer	23

The software does not pull from multiple reservoirs for 1 primer. For example, the software cannot pull from position #5 and position #24 for a combination of custom and Illumina Read 1 primer.

Compatible Primer Combinations

The primer design and dual-indexing workflow on the NovaSeq System limit the combinations of custom primers and Illumina primers. If you use a custom Read 2 primer, you must use a custom Index 1 primer.

For an overview of the indexing workflow, see the *Indexed Sequencing Overview Guide (document # 15057455)*.

Combination	Read 1 Primer	Index 1 Primer	Read 2 Primer
All Illumina	Illumina Read 1	Illumina Index 1	Illumina Read 2
Custom Read 1	Custom Read 1	Illumina Index 1	Illumina Read 2
Custom Read 2	Illumina Read 1	Custom Index 1	Custom Read 2
All custom	Custom Read 1	Custom Index 1	Custom Read 2

Prepare and Add Custom Primers

Custom primers are prepared using HT1 and then added to the cluster cartridge. Make sure that the cluster cartridge is thawed and inspected before proceeding.

HT1 is not provided in the NovaSeq 6000 Reagent Kit, but can be purchased separately: HT1 Buffer, Illumina catalog # 20015892.

Prepare Custom Primers

- 1 If frozen, thaw each custom primer to be used.
- 2 Use HT1 to dilute custom primers to yield the following volumes at 0.3 μM final concentration:

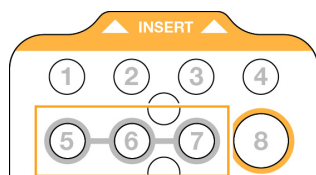
Mode	Custom Primer	Volume (ml)
S4	Read 1	3.5
	Read 2	3.5
	Index 1	5.0
S1/S2	Read 1	2.0
	Read 2	2.0
	Index 1	3.5

Add Custom Primers to the Cluster Cartridge

- 1 Use a low-lint lab tissue to wipe clean the foil seal covering each custom primer position.

Position #	Custom Primer
5	Custom Read 1 primer
6	Custom Read 2 primer
7	Custom Index 1 primer

Figure 1 Custom Primer Positions



- 2 Using a clean pipette tip, pierce the foil seal covering each custom primer position.
- 3 Add the following volumes of custom primer to the appropriate position on the cluster cartridge. Avoid touching the foil seal as you dispense the primer.

Mode	Position #	Custom Primer	Volume (ml)
S4	5	Custom Read 1 primer	3.5
	6	Custom Read 2 primer	3.5
	7	Custom Index 1 primer	5.0
S1/S2	5	Custom Read 1 primer	2.0
	6	Custom Read 2 primer	2.0
	7	Custom Index 1 primer	3.5

Set Up a Run for Custom Primers

The use of custom primers is specified during run setup.

- 1 Select the **Custom Primers** checkbox, and then select the appropriate checkboxes:
 - ▶ **Read 1**—Use custom primer for Read 1.
 - ▶ **Read 2**—Use custom primer for Read 2.
 - ▶ **Custom Index**—Use custom primer for Index 1.

During the run, the software directs the sippers to pull primers from the appropriate reservoir.

- 2 When run parameters are complete, select **Review** to proceed with run setup.

Revision History

Document	Date	Description of Change
Document # 1000000022266 v01	August 2018	Added custom primer volumes, concentrations, and reagent cartridge positions for S1 and S4 flow cells. Updated custom primer volumes and concentrations for S2 flow cells.
Document # 1000000022266 v00	March 2017	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
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Finland	+358 800918363	+358 974790110
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Ireland	+353 1800936608	+353 016950506
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Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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



Illumina

5200 Illumina Way

San Diego, California 92122 U.S.A.

+1.800.809.ILMN (4566)

+1.858.202.4566 (outside North America)

techsupport@illumina.com

www.illumina.com

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