

NovaSeq

Custom Primers Guide

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NovaSeq 6000 Reagent Kit v1.0

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 in the Advanced Options section.

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. Illumina primers refer to primers already in the cartridge position. See *Primer Positions on the Cluster Cartridge on page 3* for more information.

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

VP10 Primers

The VP10 Custom Read 1 primer (Illumina DNA PCR-Free, Tagmentation) differs from other custom primers in the following ways:

- ▶ It does not require dilution and is ready to use as is. Start at step 1 of Add Custom Primers to the Cluster Cartridge.
- ▶ It can be used for Illumina DNA PCR-Free, Tagmentation libraries, PhiX, and other Illumina compatible libraries.

This does not apply when using the NovaSeq Reagent V1.5 Kit, as it already contains this primer in the Read 1 primer well.

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 kit 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 μ M final concentration. When combining primers to make the custom index read or any custom read primer mixture, the total concentration of the mixture should be 0.3 μ M and an equal volume should be assigned to each single primer in the mixture.

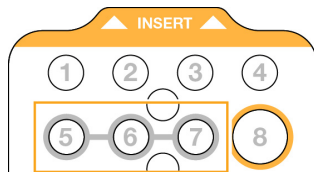
Mode	Custom Primer	Volume (ml)
S4	Read 1	3.5
	Read 2	3.5
	Index 1	5.0
SP/S1/S2	Read 1	2.0
	Read 2	2.0
	Index 1	3.5

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
SP/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 1**—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.

NovaSeq 6000 Reagent Kit v1.5

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 in the Advanced Options section.

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 Index Read 1 and 2 (combined). Due to reverse complement workflow, if using custom index read primers, a mix of index read primers for both Read 1 and Read 2 will be required in the index custom primer position.

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. Illumina primers refer to primers already in the cartridge position. See *Primer Positions on the Cluster Cartridge on page 6* for more information.

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). Due to reverse complement workflow, a custom primer mix for Index Read 1 and 2 can now be added to position 23.

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

Illumina Primer	Illumina Primer Position
Illumina Read 1 primer	24
Illumina Read 2 primer	13
Illumina Index primer mix	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 and 2 primer mix.

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

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

VP10 Primers

The VP10 Custom Read 1 primer (Illumina DNA PCR-Free, Tagmentation) differs from other custom primers in the following ways:

- ▶ It does not require dilution and is ready to use as is. Start at step 1 of Add Custom Primers to the Cluster Cartridge.
- ▶ It can be used for Illumina DNA PCR-Free, Tagmentation libraries, PhiX, and other Illumina compatible libraries.

This does not apply when using the NovaSeq Reagent V1.5 Kit, as it already contains this primer in the Read 1 primer well.

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.

Some Illumina library types including the Illumina DNA PCR-Free library require VP10 read 1 primer. NovaSeq Reagent Kits v1.5 contain VP10 Read 1 Primer and VP14 Index 1 and 2 primer mix and can be used directly without needing custom primers.

HT1 is not provided in the kit 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 μ M final concentration. When combining primers to make the custom index read or any custom read primer mixture, the total concentration of the mixture should be 0.3 μ M and an equal volume should be assigned to each single primer in the mixture.

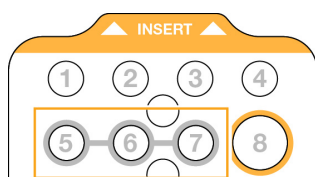
Mode	Custom Primer	Volume (ml)
S4	Read 1	3.5
	Read 2	3.5
	Index 1 and 2	5.0
SP/S1/S2	Read 1	2.0
	Read 2	2.0
	Index 1 and 2	3.5

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 and 2 primer mix

Figure 2 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 and 2 primer mix	5.0
SP/S1/S2	5	Custom Read 1 primer	2.0
	6	Custom Read 2 primer	2.0
	7	Custom Index 1 and 2 primer mix	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 position for Index Read 1 and Index Read 2.
 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 v03	July 2020	Added information in support of the NovaSeq 6000 Reagent Kit v1.5 and VP10 primer requirement for some library types.
Document # 1000000022266 v02	October 2019	Added the SP flow cell to custom primer tables.
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.

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

Product documentation—Available for download from support.illumina.com.



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