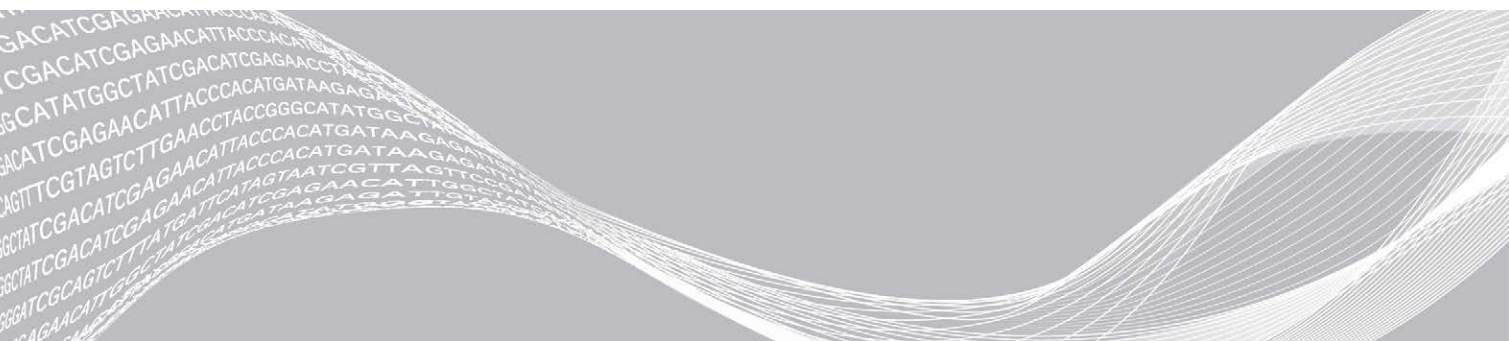


NextSeq System

Custom Primers Guide

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Introduction

Using custom sequencing primers for a run on the NextSeq system requires two additional steps during run setup.

- 1 Prepare and add 2 ml of each custom primer to the custom primer positions on the NextSeq reagent cartridge. See *Prepare Custom Primers on page 4*.
- 2 Specify that custom primers are part of the run during run setup.

All other steps follow the run setup workflow as described in the *NextSeq 500 System Guide (document # 15046563)* or *NextSeq 550 System Guide (document # 15069765)*.



NOTE

NCS v1.4 or later and NextSeq 500/550 Kit v2 or later is required for use of custom primers for an Index 2 Read.

Custom Primers and PhiX

When custom primers are used for Read 1 or Read 2, the software directs the instrument to pull from reservoirs 7 and 8. 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.

Primer Positions on the Reagent Cartridge

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

Example: If you use a custom primer for Read 2, but not for Read 1, the software pulls the Read 1 primer from Illumina primer position (#20) and pulls the Read 2 primer from the custom primer position (#8).

Custom Primer	Custom Primer Position #
Custom Read 1 primer	7
Custom Read 2 primer	8
Custom Index 1 primer Custom Index 2 primer*	9

* Using a custom Index 2 primer is possible with NCS v1.4, or later, and the NextSeq 500/550 Kit v2 or later.

Illumina Primer	Illumina Primer Position # (v2 or later Reagent Cartridge)
BP10—Read 1 primer	20
BP11—Read 2 primer	21
BP14—Index 1 primer and Index 2 primer mix	22

Illumina Primer	Illumina Primer Position # (v1 Reagent Cartridge)
BP10—Read 1 primer	20
BP11—Read 2 primer	21
BP12—Index 1 primer	22
BP13—Index 2 primer	18

Primer positions used for the run are recorded in the file `Primers.xml`, which is written to the Reagents folder.

Compatible Primer Combinations

Due to the primer design and dual-indexing workflow on the NextSeq, 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 custom Read 2 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)*.

Using the v2 or Later Reagent Cartridge

Combination	Read 1 Primer	Index 1 Primer	Index 2 Primer	Read 2 Primer
All Illumina	BP10—Read 1	BP14—Index 1	BP14—Index 2	BP11—Read 2
Custom Read 1	Custom Read 1	BP14—Index 1	Custom Index 2	BP11—Read 2
Custom Read 2	BP10—Read 1	Custom Index 1	BP14—Index 2	Custom Read 2
All custom	Custom Read 1	Custom Index 1	Custom Index 2	Custom Read 2

Using the v1 Reagent Cartridge

Combination	Read 1 Primer	Index 1 Primer	Index 2 Primer	Read 2 Primer
All Illumina	BP10—Read 1	BP12—Index 1	BP13—Index 2	BP11—Read 2
Custom Read 1	Custom Read 1*	BP12—Index 1	Not compatible	BP11—Read 2
Custom Read 2	BP10—Read 1	Custom Index 1	BP13—Index 2	Custom Read 2
All custom	Custom Read 1*	Custom Index 1	Not compatible	Custom Read 2

* Using the v1 reagent cartridge, a custom Read 1 primer is possible with single-indexed runs only.

Prepare and Add Custom Primers

Make sure that the NextSeq reagent cartridge is thawed and inspected before proceeding.

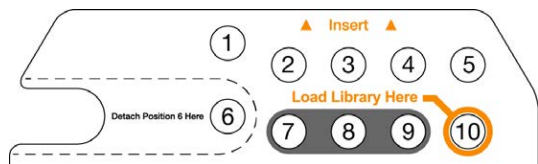
Prepare Custom Primers

- 1 If frozen, thaw each custom primer to be used.
- 2 Use HT1 to dilute each custom primer to yield 2 ml at 0.3 μ M final concentration.

Add Custom Primers to Reagent Cartridge

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

Figure 1 Custom Primer Positions



Position #	Custom Primer
7	Custom Read 1 primer
8	Custom Read 2 primer
9	Custom Index 1 primer Custom Index 2 primer

- 2 With a clean pipette tip, pierce the foil seal covering each position associated with the custom primer.
- 3 Add the following volumes of custom primer in the appropriate position on the reagent cartridge. Avoid touching the foil seal as you dispense the primer.

Position #	Volume	Custom Primer
7	2 ml	Custom Read 1 primer
8	2 ml	Custom Read 2 primer
9	2 ml	Custom Index 1 primer
9	1 ml of each primer	Custom Index 1 primer <i>and</i> Custom Index 2 primer
9	2 ml	Custom Index 2 primer

Set Up a Run for Custom Primers

The use of custom primers is specified during run setup.

- ▶ For Local Run Manager run mode, specify custom primers on the Create Run page of the Local Run Manager software.
- ▶ For manual run mode, specify custom primers on the NCS Run Setup screen.

Specify Custom Primers in Local Run Manager Mode Configuration

- 1 On the Create Run screen of the Local Run Manager software, specify custom primer information.
- 2 When you finish specifying run parameters, select **Next**.

Specify Custom Primers in Manual Run Mode Configuration

- 1 On the NCS Run Setup screen, select the checkbox for each custom primer to be used with the run. During the run, the software directs the sippers to pull primers from the appropriate reservoir.
- 2 When you finish specifying run parameters, select **Next**.

Revision History

Document	Date	Description of Change
Document # 15057456 v02	June 2019	Revised directions for compatibility with NCS 4.0. <ul style="list-style-type: none"> • Added instructions for specifying primers in Local Run Manager mode. • Removed instructions for specifying primers in the BaseSpace Prep Tab. • Updated standalone mode to manual mode. Updated references to v2 reagent cartridges to v2 or later.
Document # 15057456 v01	October 2015	Emphasized that the software directs the sippers to pull primers from the appropriate reservoirs on the reagent cartridge depending on which reads use custom primers opposed to Illumina primers. Listed that 2 ml custom Index 2 primer is loaded in position #9.
15057456 D	May 2015	Added reference to the <i>NextSeq 550 System Guide (part # 15069765)</i> . Updated kit names to include 550, as in the NextSeq 500/550 Kit v2.
15057456 C	February 2015	Added Illumina primers provided in the v2 reagent cartridge. Added information about using a custom Index 2 primer, which requires the NextSeq 500 Kit v2.
15057456 B	September 2014	Updated URL for Safety Data Sheets (SDS) to support.illumina.com/sds.html . Updated NextSeq product markings from ™ to ®.
15057456 A	August 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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