

NextSeq 1000 and 2000

Custom Primers Guide



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Revision History

Document #	Date	Description of Change
1000000139569 v01	November 2020	Added NextSeq 1000/2000 P2 (v2) Reagents kit information. Updated custom primer addition instructions.
1000000139569 v00	October 2020	Initial release.

Introduction

Using custom primers for a run on an Illumina® NextSeq™ 1000 or 2000 Sequencing System requires two additional steps during run setup:

- Prepare and add the appropriate volume of each custom primer to the custom primer position of the reagent cartridge.
- Select the custom primers in the control software during run setup.

All other steps follow the run setup workflow described in the *NextSeq 2000 Sequencing System Guide* (document # 1000000109376). To use custom primers with the NextSeq 1000/2000 P2 (v2) Reagent kits you must use a custom recipe. Download the custom recipe from the NextSeq 1000/2000 support site page. The NextSeq 1000/2000 P2 (v3) Reagents kit and NextSeq 2000 P3 Reagents kit do not require a custom recipe to use custom primers.

NextSeq 1000/2000 allows the use of two custom read primers or custom index primer combinations per custom well. Read 1 custom primers are not compatible with Index 1 custom primers and Read 2 custom primers are not compatible with Index 2 custom primers. There are two available custom primer wells.

Depending on your library preparation kit, you might be required to use Illumina primer mixes. See [VP10 and VP14 Custom Primers on page 4](#) for more information.

Illumina cannot guarantee the performance or compatibility of custom primers. You are responsible for validating custom primers for sequencing on the NextSeq 1000 and 2000 Sequencing Systems.

Custom Primers and PhiX

When custom primers are selected in the control software during run setup, the software directs the system to pull reagents from the Custom 1 and Custom 2 wells. Illumina primers are not used for the read or index during the sequencing run. Illumina primers refer to primers already in the reagent cartridge wells

If Illumina primers are not used for Read 1 or Read 2, the optional PhiX control is not sequenced.

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

VP10 and VP14 Custom Primers

If your library prep kit requires VP10 Custom Read 1 Primer or VP14 Custom Index 2 Primer, proceed to [Add Custom Primers to the Reagent Cartridge on page 5](#). VP10 and VP14 custom primers do not require preparation.

To confirm if your library prep kit requires VP10 or VP14 custom primers, see the Compatible Products page for your library kit on the [Illumina support site](#).

Prepare and Add Custom Primers

Custom primers are prepared using HT1, and then added to the custom wells in the NextSeq 1000/2000 reagent cartridge. Make sure that the reagent cartridge is thawed and inspected before proceeding.

If using custom or third-party libraries alongside PhiX or Illumina libraries, prepare your custom read primers by spiking into HP21 and custom index primers by spiking into BP14.

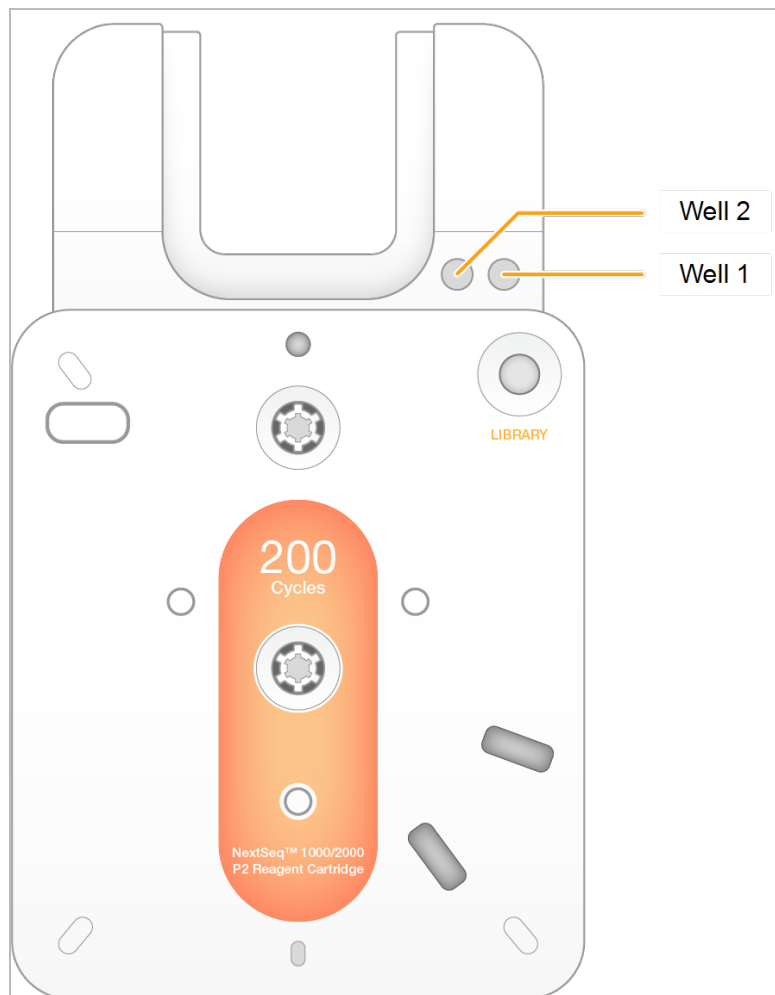
HT1, HP21, and BP14 are included in the NextSeq 1000/2000 primer kits. See [Kit Configurations on page 7](#) for more information.

Prepare Custom Primers

1. If frozen, thaw each custom primer to be used.
2. If using Illumina libraries, prepare custom read primers and/or custom index primers as follows.
 - Use HT1 to dilute each custom read primer mix to yield 600 μ l at 0.3 μ M final concentration.
 - Use HT1 to dilute each custom index primer mix to yield 600 μ l at 0.6 μ M final concentration.
3. If using custom or third-party libraries alongside PhiX or Illumina libraries, prepare custom read primers or custom index primers as follows.
 - Add each custom read primer mix to 600 μ l HP21 for a 0.3 μ M final concentration.
 - Add each custom index primer mix to 600 μ l BP14 for a 0.6 μ M final concentration.

Add Custom Primers to the Reagent Cartridge

1. Using a clean pipette tip, pierce the foil seal covering the custom 1 well or custom 2 well.



2. Add 550 μ l custom primer mix to custom 1 well or custom 2 well on the reagent cartridge. If using both wells, add 550 μ l custom primer mix to the second well. Dispense the liquid slowly to avoid spillage and cross-contamination.
3. Initiate the sequencing run within 1 hour. Storing the cartridge inside the system can cause the custom primer mix to evaporate.

Set Up a Run for Custom Primers

For more information on setting up your run, see *NextSeq 2000 Sequencing System Guide (document # 1000000109376)*.

1. Select one of the following options for each read and index in the NextSeq 1000/2000 Control Software run setup.
 - **No**—Use Illumina primer. No is the default selection.
 - **Custom 1**—Use the primer added to the Custom 1 well.
 - **Custom 2**—Use the primer added to the Custom 2 well.

- When run parameters are complete, select **Prep**.

Kit Configurations

The following are the available kit configurations for NextSeq 1000/2000 custom primers. Store kit contents at -25°C to -15°C.

Kit Name	Catalog #
NextSeq 1000/2000 Read & Index Primers	20046115
NextSeq 1000/2000 Index Primer Kit	20046116
NextSeq 1000/2000 Read Primer Kit	20046117

NextSeq 1000/2000 Read & Index Primers

Quantity	Acronym	Reagent Name	Cap Color
1	BP14	BP14 index primer mix	Yellow
1	HP21	HP21 read primer mix	Blue
2	HT1	Hybridization Buffer 1	Clear

NextSeq 1000/2000 Index Primer Kit

Quantity	Acronym	Reagent Name	Cap Color
10	BP14	BP14 index primer mix	Yellow
10	HT1	Hybridization Buffer 1	Clear

NextSeq 1000/2000 Read Primer Kit

Quantity	Acronym	Reagent Name	Cap Color
10	HP21	HP21 read primer mix	Blue
10	HT1	Hybridization Buffer 1	Clear

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Technical Support Telephone Numbers

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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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