

MiniSeq System

Custom Primers Guide



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Revision History

Document	Date	Description of Change
Document # 1000000002700 v02	November 2020	Added primer position information for MiniSeq Rapid Reagent Kits.
Document # 1000000002700 v01	September 2016	Updated custom primer positions to 15 for Read 1, 14 for Read 2, and 13 for Index 1 and Index 2.
Document # 1000000002700 v00	February 2016	Initial release.

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Introduction

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

1. Specify that custom primers are part of the run.
 - For runs connected to Local Run Manager, use of custom primers are specified on the Create Runs screen.
 - For runs connected to BaseSpace or BaseSpace Onsite, use of custom primers are specified on the Planned Runs screen of the Prep tab.
 - For runs using the standalone configuration, use of custom primers are specified on the MiniSeq Control Software Run Setup screen.
2. Prepare and add 2 ml of each custom primer to the custom primer positions on the MiniSeq reagent cartridge. See [Prepare Custom Primers on page 3](#).

All other steps follow the run setup workflow as described in the *MiniSeq System Guide (document # 1000000002695)*.

Custom Primers and PhiX

When custom primers are used for Read 1 or Read 2, the software directs the instrument to pull from reservoirs 14 and 15. 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.

i | 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 specified, the software pulls the primer from the appropriate reservoir.

Example: If a custom primer is used for Read 2, but not for Read 1, the software pulls the Read 1 primer from Illumina Read 1 primer position (#24). The Read 2 primer is pulled from the custom Read 2 primer position (#14).

Custom Primer	Custom Primer Position
Custom Read 1 primer	15
Custom Read 2 primer	14
Custom Index 1 primer Custom Index 2 primer	13

Illumina Primer	Illumina Primer Position
Illumina Read 1 primer	24
Illumina Read 2 primer	25
Illumina Index 1 primer and Index 2 primer mix	28

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

 Custom Index 2 and custom Read 2 primers cannot be used with MiniSeq Rapid reagent kits.

Compatible Primer Combinations

Due to the primer design and dual-indexing workflow on the MiniSeq standard reagent kit, combinations of custom primers and Illumina primers are limited.

MiniSeq Standard Reagent Kits:

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

- 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 *Indexed Sequencing Overview Guide (document # 15057455)*.

MiniSeq Rapid Reagent Kits:

The MiniSeq Rapid recipe allows for dual-indexed single-end runs. The Index 2 read is primed off the grafted P5 primer on the flow cell, rather than using an Illumina Index 2 sequencing primer. It is not possible to use custom Index 2 or custom Read 2 primers with MiniSeq Rapid reagents.

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

Prepare and Add Custom Primers

Make sure that the MiniSeq 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.

Position #	Custom Primer
15	Custom Read 1 primer
14	Custom Read 2 primer
13	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
15	1 ml	Custom Read 1 primer
14	1 ml	Custom Read 2 primer
13	1 ml	Custom Index 1 primer
13	0.5 ml of each primer	Custom Index 1 primer <i>and</i> Custom Index 2 primer
13	1 ml	Custom Index 2 primer

i | Custom Index 2 and custom Read 2 primers cannot be used with MiniSeq Rapid reagent kits.

Set Up a Run for Custom Primers

The use of custom primers is specified during run setup.

- If the instrument is configured for Local Run Manager, specify custom primers on the Create Run page.
- If the instrument is configured for BaseSpace or BaseSpace Onsite, specify custom primers on the BaseSpace Prep tab. You can later edit these settings on the MiniSeq Control Software Run Setup screen.
- If the instrument is configured for standalone mode, specify custom primers on the MiniSeq Control Software Run Setup screen.

Specify Custom Primers Using BaseSpace Prep Tab

1. On the Plan Run screen of the BaseSpace Prep tab, select the checkbox for each custom primer to be used with the run.
 - **R1**—Custom primer for Read 1.
 - **R2**—Custom primer for Read 2.
 - **Index**—Custom primer for Index 1 Read.

To specify custom primers for Index 1 or Index 2 separately, edit the custom primers settings on the MiniSeq Control Software Run Setup screen.

Specify Custom Primers Using Local Run Manager

1. On the Create Run page of Local Run Manager, select the checkbox for each custom primer to be used with the run.
 - **Read 1**—Custom primer for Read 1.
 - **Index 1**—Custom primer for Index 1 Read.
 - **Index 2**—Custom primer for Index 2 Read.
 - **Read 2**—Custom primer for Read 2.

Specify Custom Primers in Standalone Configuration

1. On the MiniSeq Control Software 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**.

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.

