

MiSeq System

Custom Primers Guide

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Introduction

Illumina primers are in positions 12, 13, and 14 of the MiSeq reagent cartridge.

Reservoir	Read
12	Read 1 Primer Mix
13	Index Primer Mix
14	Read 2 Primer Mix

You can use custom sequencing primers for a run on the MiSeq. To do so, specify the use of custom primers in the sample sheet, and then load prepared custom primers into the empty reservoirs on the MiSeq reagent cartridge. The empty reservoirs are labeled 18, 19, and 20, and each reservoir is associated with a specific read in a sequencing run.

Reservoir	Read
18	Read 1 custom primer
19	Index Read custom primer
20	Read 2 custom primer

You can use a combination of custom primers and standard Illumina primers provided in the reagent cartridge. For example, you can use the Illumina primer for Read 1, a custom primer for the Index Read, and the Illumina primer for Read 2.



NOTE

When custom primers are used for Read 1 and Read 2, the software directs the sippers to reservoirs 18 and 20; therefore, Illumina primers are not used for the sequencing run. If Illumina primers are not used, the optional Illumina PhiX control cannot be sequenced.

For more information about PhiX control, see *MiSeq System Denature and Dilute Libraries Guide (part # 15039740)*.

Editing the Sample Sheet for Custom Primers

You can set up your sample sheet for custom primers using the Illumina Experiment Manager v1.2, or later. If you edit your sample sheet manually, list the custom primers in the Settings section of the sample sheet.

- 1 Open your sample sheet for editing in Excel or Notepad.
- 2 Locate the Settings section. If your sample sheet does not have a Settings section, create one after the Reads section and before the Data section.

Figure 1 Example of Custom Primers in Settings Section

	A	B	C	D	E	F	G	H
1	[Header]							
2	IEMFileVersion		3					
3	Investigator Name	Dr_YourName						
4	Project Name	Custom_Primers						
5	Experiment Name	Custom_Primers						
6	Date	1/16/2012						
7	Workflow	Resequencing						
8	Assay	TruSeq DNA/RNA						
9	Description	Test						
10	Chemistry	Default						
11	[Reads]							
12		151						
13		151						
14	[Settings]							
15	CustomRead1PrimerMix	C1						
16	CustomIndexPrimerMix	C2						
17	CustomRead2PrimerMix	C3						
18	[Data]							
19	Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Sample_PrcIndex	I7_Index	Descriptio	
20	Sample_1		Custom_Primers	A01	ATCACG	A001		
21	Sample_2		Custom_Primers	A02	TTAGGC	A003		
22	Sample_3		Custom_Primers	A03	GGCTAC	A011		

- 3 In the Settings section, add a line for each custom primer using the following example.

Settings	
CustomRead1PrimerMix	C1
CustomIndexPrimerMix	C2
CustomRead2PrimerMix	C3

Include **C1** for the Read 1 primer, **C2** for the Index Read primer, and **C3** for the Read 2 primer.

If you are using a combination of Illumina primers and custom primers, list only the custom primer you plan to use.

Preparing and Loading Custom Primers

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

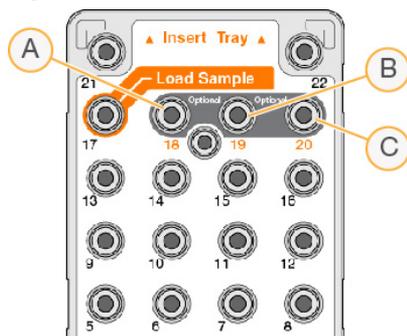
Prepare Custom Primers

- 1 Prepare 600 μ l of custom primer at 0.5 μ M.
- 2 Use HT1 (Hybridization Buffer) to dilute the custom primer, as needed.

Load Custom Primers

- 1 Clean the foil seal covering the reservoir covering the position associated with the primer you are loading.
- 2 Pierce the foil seal with a clean 1 ml pipette.
- 3 Pipette 600 μ l of custom primer in the appropriate position on the reagent cartridge. Avoid touching the foil seal.

Figure 2 Custom Primer Reservoirs



- A Position 18 for Read 1 Custom Primer
- B Position 19 for Index Read Custom Primer
- C Position 20 for Read 2 Custom Primer

- 4 Inspect the bottom of the cartridge to make sure that bubbles are not present in the reservoirs.

Revision History

Document #	Date	Description of Change
Document # 15041638 v01	February 2016	Changed the name of the guide from Using Custom Primers on the MiSeq to MiSeq System Custom Primers Guide. Added locations of the Illumina primer mixes in the MiSeq reagent cartridge.
Part # 15041638 Rev. B	July 2013	Added note stating that PhiX control is not sequenced when custom primers are used for Read 1 and Read 2.
Part # 15041638 Rev. A	March 2013	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

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

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

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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