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Sequencing primer compatibility of Illumina libraries on Illumina sequencing platforms

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Illumina offers a wide range of library preparation kits and sequencing instruments. Choosing the correct sequencing primer for your library and instrument is critical for a successful run. Table 1 provides a comprehensive sequencing primer compatibility chart.

Sequencing primers HP6 (Read 1), HP7 (Read 2), and HP8 (Index 1) are compatible only with TruSeq adapters, excluding TruSeq Amplicon adapters. Sequencing primers HP10/BP10 (Read 1), HP11/BP11 (Read 2), HP12/BP12 (Index 1), and HP9/BP13 (Index 2) are compatible with TruSeq, Nextera, TruSight, and TruSeq Amplicon adapters.

Table 1. Sequencing primer compatibility based on library type and instrument flow cell type.

Platform	Cluster Kit Cartridge	Flow cell Type	Read1	Index1 (i7)	Index2 (i5)	Read2	Library Type
HiSeq	HiSeq v4 High Output	Single Read (GD-401-4001)	HP10	HP12	HP9	N/A	Nextera DNA, Nextera XT, TruSeq HT, TruSeq Amplicon, TruSeq LT/v2, TruSeq Small RNA
		Paired End (PE-401-4001)	HP10	HP12	Grafted P5 on flow cell	HP11	
	TruSeq V3 High Output*	Single Read (GD-401-3001)	HP6	HP8	HP9 ¹⁾	N/A	TruSeq HT, TruSeq LT/v2, TruSeq Small RNA
		Paired End (PE-401-3001)	HP6	HP8	Grafted P5 on flow cell	HP7	
	HiSeq Rapid V2	Single Read (GD-402-4002)	HP10	HP12	HP9	N/A	Nextera DNA, Nextera XT, TruSeq HT, TruSeq Amplicon, TruSeq LT/v2, TruSeq Small RNA
			HP10	HP12	Grafted P5 on flow cell	HP11	
		Paired End (PE-402-4002)	HP10	HP12	HP9	N/A	Nextera DNA, Nextera XT, TruSeq HT, TruSeq Amplicon, TruSeq LT/v2, TruSeq Small RNA
			HP10	HP12	Grafted P5 on flow cell	HP11	
NextSeq 500	All kits	N/A	BP10	BP12	BP13	BP11	Nextera DNA, Nextera XT, TruSeq HT, TruSeq Amplicon, TruSeq LT/v2, TruSeq Small RNA
		N/A	HP10	HP12	Grafted P5 on flow cell	HP11	
MiSeq	All kits	N/A	HP10	HP12	Grafted P5 on flow cell	HP11	Nextera DNA, Nextera XT, TruSeq HT, TruSeq Amplicon, TruSeq LT/v2, TruSeq Small RNA
		N/A	HP10	HP12	Grafted P5 on flow cell	HP11	
Genome Analyzer Iix	TruSeq v5-Cluster Station*	Single Read (GD-203-5001)	HP6	HP8	HP9 ¹⁾	N/A	TruSeq HT, TruSeq LT/v2, TruSeq Small RNA

	Paired End (PE-203-5001)	HP6	HP8	Grafted P5 on flow cell	HP7
TruSeq v2- cBot*	Single Read (GD-300-2001)	HP6	HP8	HP9 ¹⁾	N/A
	Paired End (PE-300-2001)	HP6	HP8	Grafted P5 on flow cell	HP7

These single-read cluster kits require HP9, which is found in the TruSeq Dual Index Sequencing Primer Box, Single -Read (FC-121-1003).

* The cluster kits **TruSeq v3 High Output**, **TruSeq v5- Cluster Station**, and **TruSeq v2- cBot** contain sequencing primers HP6, HP7, and HP8. Therefore, it is required to purchase a TruSeq Dual Index Sequencing Primer Box.

- Single-read flow cell: use FC-121-1003 (containing **HP10**, **HP12**, and **HP9**)
- Paired-end flow cells: use PE-121-1003 (containing **HP10**, **HP11**, and **HP12**)

To use custom primers on Illumina instruments, some manual setup is required. For instructions on how to set up custom primer on your instrument, see the following guides:

Using Custom Primers on the HiSeq (15061846), Using Custom Primers on the MiSeq (15041638), Using Custom Primers on the NextSeq 500 (15057456) and Using Custom Sequencing Primers on the HiSeq and GAIIX.

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