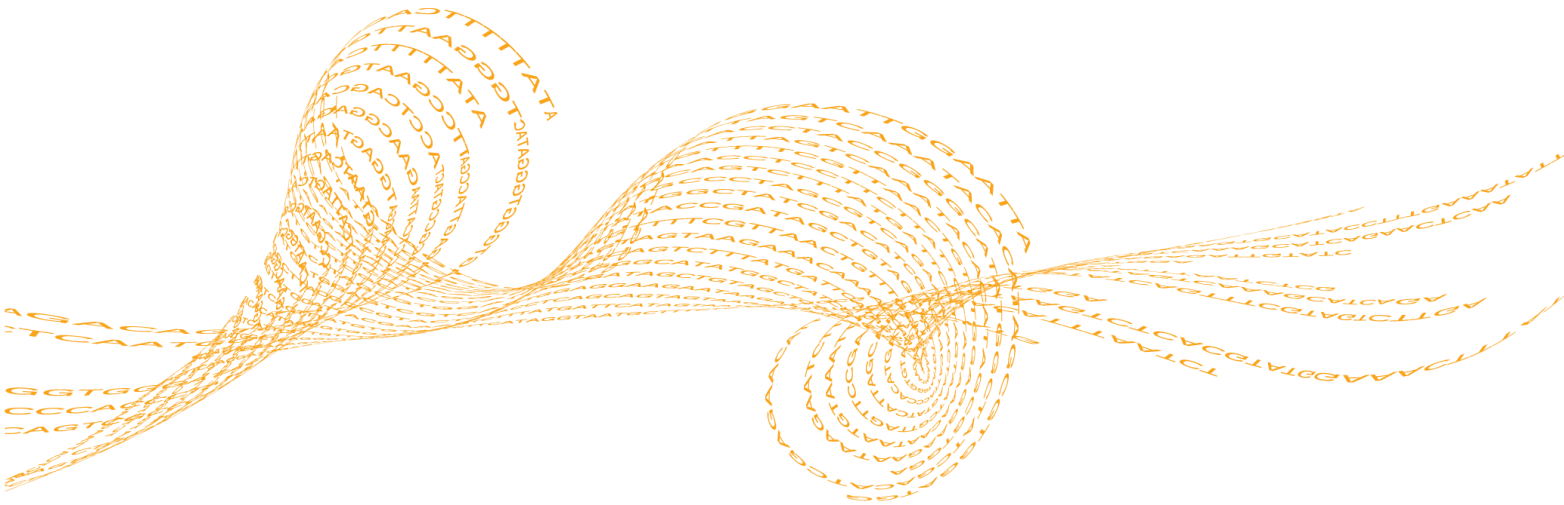
MiSeq® Sample Sheet
Quick Reference Guide

FOR RESEARCH USE ONLY



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Part # 15028392 Rev. J
August 2013

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Revision History

Part #	Revision	Date	Description of Change
15028392	J	August 2013	Added the following information: • Sample sheet settings BaitManifestFileName, OutputGenomeVCF, PicardHSmetrics, and StitchReads; added description of read stitching • Sample ID requirements for the Targeted RNA workflow • Adapter sequences for TruSeq libraries; moved adapter sequences to Adapter Settings section
15028392	H	May 2013	Added sample sheet requirements for the Amplicon - DS workflow. Changed the Custom Amplicon workflow to TruSeq Amplicon workflow.
15028392	G	April 2013	Updated the Adapter sample sheet setting to include the adapter sequence for Nextera Mate Pair libraries. Added the ReverseComplement sample sheet setting for Nextera Mate Pair libraries using the Resequencing or Assembly workflows.
15028392	F	March 2013	Updated the following sample sheet settings: • CustomAmpliconAlignerMaxIndelSize—Changed default to 25 • FilterPCRDuplicates—Changed to FlagPCRDuplicates • VariantCaller—Added Resequencing workflow • EnrichmentMaxRegionStatisticsCount and ExcludeRegionsManifestA—Added for the Enrichment workflow • Added VariantMinimumQualCutoff • Removed VariantFilterQualityCutoff; VariantMinimumGQCutoff is preferred • Added requirements for Targeted RNA workflow • Removed Index 2 as an option for Assembly and Small RNA workflows • Noted that custom indices can be used for the Custom Amplicon workflow with MCS v2.2, or later Removed custom primer instructions. See *Using Custom Primers on the MiSeq (part # 15041638)*.

Part #	Revision	Date	Description of Change
15028392	E	November 2012	Added the following information: • Description of Enrichment workflow, manifest, and data section requirements • Data section requirements for PCR Amplicon workflow • Descriptions of sample sheet settings AdapterRead2 and QualityScoreTrim • Note about supported option for listing genome references for multiple species in the same sample sheet when using MiSeq Reporter v2.1 Updated the following information: • Organized sample sheet settings into settings for sequencing, analysis, and variant calling • Updated Small RNA workflow to list the genome folder as required in the sample sheet • Updated sample sheet settings for variant calling to add VariantMinimumGQCutoff, and to update StandBiasFilter and MinimumCoverageDepth for the Enrichment workflow
15028392	D	July 2012	Added the following information: • Added the PCR Amplicon analysis workflow for Nextera XT libraries and information about the manifest file • Noted that adapter trimming is recommended for longer read lengths up to 250 cycles • Added descriptions of sample sheet settings for PercentTilesToScan and StrandBiasFilter • Changed Setup Options screen to Run Options screen per MCS v1.2
15028392	C	April 2012	Updated the following information: • Updated name of Amplicon workflow to Custom Amplicon • Updated name of DenovoAssembly workflow to Assembly • Added GenerateFASTQ workflow • Listed genome folder as required for amplicon sequencing in Sample Sheet Parameters
15028392	B	December 2011	Updated the steps in Setting Up the Sample Sheet. Listed manifest files as required for TruSeq Custom Amplicon libraries.
15028392	A	September 2011	Initial release

Introduction

The sample sheet is a comma-separated values (*.csv) file that stores information required to set up, perform, and analyze a sequencing run.

Illumina recommends that you create your sample sheet before preparing your sample libraries. You can create your sample sheet using the Illumina Experiment Manager or create it manually using a text editor, such as Excel or Notepad.

Before starting the run, make sure that the sample sheet is accessible to the instrument. You can either copy the sample sheet to a network location or copy the sample sheet from a USB flash drive using the Manage Files feature in MiSeq Control Software (MCS).

When the run begins, the software copies the sample sheet from the designated sample sheet folder to the root of the MiSeqOutput folder. At the end of the run, the sample sheet is used for secondary analysis by the MiSeq Reporter software.

Illumina Experiment Manager

The Illumina Experiment Manager is a wizard-based application that guides you through the steps to create your sample sheet.

Using the Illumina Experiment Manager not only reduces syntax errors, but also provides prompts for information that applies to your sample type and analysis workflow. It provides a feature for recording parameters for the sample plate, such as sample ID, project name, dual indices, and barcode information. Then, the sample plate information can be imported to the sample sheet using the Illumina Experiment Manager.

The Illumina Experiment Manager can be run on any Windows platform. To download the software, go to the Illumina Experiment Manager support page on the Illumina website (support.illumina.com/sequencing/sequencing_software/experiment_manager/downloads.ilmn).

For more information, see the *Illumina Experiment Manager User Guide (part # 15031335)*.

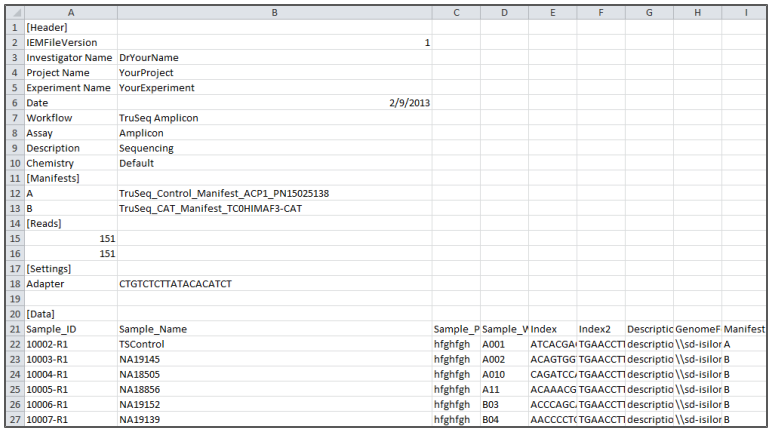
Sample Sheet Workflow

1. Create your sample sheet using one of the following methods:
   - **Illumina Experiment Manager**—See the *Illumina Experiment Manager User Guide (part # 15031335)*.
   - **Excel or Notepad**—See *Sample Sheet Parameters* on page 6.
2. Name your sample sheet with the reagent cartridge barcode number associated with the sequencing run, and use a *.csv extension. For more information, see *Naming the Sample Sheet* on page 20.
3. Copy the sample sheet to the sample sheet folder specified in Run Options in MCS or other location accessible to the instrument computer.

Sample Sheet Parameters

The sample sheet is organized in sections titled Header, Reads, Manifests, Data, and Settings. Section headings are case-sensitive and shown in brackets [ ] in the following example:

Figure 1 Sample Sheet Example in Excel



	A	B	C	D	E	F	G	H	I
1	[Header]								
2	IEMFileVersion	1							
3	Investigator Name	DrYourName							
4	Project Name	YourProject							
5	Experiment Name	YourExperiment							
6	Date	2/9/2013							
7	Workflow	TruSeq Amplicon							
8	Assay	Amplicon							
9	Description	Sequencing							
10	Chemistry	Default							
11	[Manifests]								
12	A	TruSeq_Control_Manifest_ACP1_PN15025138							
13	B	TruSeq_CAT_Manifest_TC0HIMAF3-CAT							
14	[Reads]								
15	151								
16	151								
17	[Settings]								
18	Adapter	CTGTCTCTTATACACATCT							
19									
20	[Data]								
21	Sample_ID	Sample_Name	Sample_P	Sample_W	Index	Index2	Descriptic	GenomeF	Manifest
22	10002-R1	TSControl	hfghfgh	A001	ATCACGA	TGAACCT	descriptio	\\sd-isilor	A
23	10003-R1	NA19145	hfghfgh	A002	ACAGTGG	TGAACCT	descriptio	\\sd-isilor	B
24	10004-R1	NA18505	hfghfgh	A010	CAGATCA	TGAACCT	descriptio	\\sd-isilor	B
25	10005-R1	NA18856	hfghfgh	A11	ACAAACG	TGAACCT	descriptio	\\sd-isilor	B
26	10006-R1	NA19152	hfghfgh	B03	ACCCAGC	TGAACCT	descriptio	\\sd-isilor	B
27	10007-R1	NA19139	hfghfgh	B04	AACCCCT	TGAACCT	descriptio	\\sd-isilor	B

Header Section

Parameter	Description
Investigator Name	Your name.
Project Name	Project name of your preference.
Experiment Name	Experiment name of your preference.
Date	Date of your experiment.
Workflow	**Required** The workflow field must list the analysis workflow name recognized by MiSeq Reporter.
Assay	The name of the assay used to prepare your samples.
Chemistry	The recipe fragments used to build the run-specific recipe. If this field is blank, the system uses the default recipe fragments. **Optional**—For non-indexed or single-indexed TruSeq RNA or TruSeq DNA libraries, leave this field blank. **Required**—For any workflows that use dual indexing, such as Nextera and TruSeq Custom Amplicon, the chemistry field is required. Enter **amplicon** in this field.

Reads Section

Parameter	Description
Number of cycles in Read 1	**Required**
Number of cycles in Read 2	**Required** for paired-end runs.

NOTE
The index sequence defined in the Data section specifies the number of cycles for the index read.

Data Section

The following table summarizes the Data section requirements for each analysis workflow. Column order is not important.

Workflow	Required Data Columns	Optional Data Columns
Amplicon - DS	Sample_ID, Sample_Name, Manifest, GenomeFolder, Index	Index 2*
Assembly	Sample_ID	Sample_Name, Index, GenomeFolder
Enrichment	Sample_ID, Manifest, GenomeFolder	Sample_Name, Index, Index 2
Generate FASTQ	Sample_ID	Sample_Name, Index, Index 2
LibraryQC	Sample_ID, GenomeFolder	Sample_Name, Index, Index 2
Metagenomics	Sample_ID	Sample_Name, Index, Index 2
PCR Amplicon	Sample_ID, Manifest, GenomeFolder	Sample_Name, Index, Index 2
Resequencing	Sample_ID, GenomeFolder	Sample_Name, Index, Index 2
Small RNA	Sample_ID, GenomeFolder, Contaminants, miRNA, RNA	Sample_Name, Index
Targeted RNA	Sample_ID**, Sample_Name, Manifest, GenomeFolder, Index, Index 2	--
TruSeq Amplicon (*formerly Custom Amplicon*)	Sample_ID, Manifest, GenomeFolder, Index	Sample_Name, Index 2*

* For the TruSeq Amplicon and Amplicon - DS workflows—Using MCS v2.2, or later, you can optionally specify a single-index run.

** For the Targeted RNA workflow, the sample ID and sample name must be specified in the sample sheet in such a way to specify replicates. For more information, see *Data Columns for the Targeted RNA Workflow* on page 10.

Column Heading	Description
SampleID	**Required** Every sample must have a ***unique sample ID***. At least one sample must be listed. List one sample per line.
Sample_Name	**Optional** The sample name is used in reporting and file naming.
Index	**Required** for multi-sample assays with single or dual indexing. Nucleotide sequence—Valid characters are A, C, G, T, and N, where N matches any base. Enter the index sequence of the i7 index.
Index2	**Required** for multi-sample assays with dual indexing. Nucleotide sequence—Valid characters are A, C, G, T, and N, where N matches any base. Enter the index sequence of the i5 index.

NOTE
For the appropriate index sequences, see the user guide for your sample preparation kit.

Genome Folder Path

The GenomeFolder contains the reference genome in FASTA file format. For optimal results, store reference genomes on the local drive or use BaseSpace.

Enter the full path (UNC path) to the GenomeFolder in the sample sheet. Do not enter the path using a mapped drive.

NOTE
Introduced in MiSeq Reporter v2.1, you can specify genome references for multiple species in the same sample sheet for all workflows ***except*** the Small RNA workflow.

Data Section Requirements by Workflow

Not all columns used in the Data section of the sample sheet apply to every analysis workflow. For example, the Manifests column is required for some workflows, and not required for other workflows.

Data Columns for the Amplicon - DS Workflow

Column Heading	Description
Sample Name	**Required** Group sample names in pairs. List exactly two samples with identical sample names, one sample for the forward direction and one sample for the reverse direction.

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step. It is the same reference genome specified in the manifest file. Specifying the genome folder provides coordinates and chromosome mapping. For example, the genome folder for human is hg19 (Homo_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA).
Manifest	**Required** Specify the manifest key for this sample, which is located in the first column of the Manifests section. Two manifests must be specified, one manifest containing the reverse complement of the probes listed in the other manifest.

Data Columns for the Assembly Workflow

Column Heading	Description
GenomeFolder	**Optional** If provided, MiSeq Reporter compares the *de novo* assembly against the reference genome, and generates a dot-plot that graphically summarizes the results. • If the specified folder does not exist, Illumina Experiment Manager combines the GenomePath configuration setting with the genome string. • If the path does not exist, MiSeq Reporter stops processing.

Data Columns for the Enrichment Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step.
Manifest	**Required** Specify the manifest key for this sample, which is located in the first column of the Manifests section.

Data Columns for the Library QC Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step.

Data Columns for the PCR Amplicon Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step.
Manifest	**Required** Specify the manifest key for this sample, which is located in the first column of the Manifests section.

Data Columns for the Resequencing Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step.

Data Columns for the Small RNA Workflow

Column Heading	Description
GenomeFolder	**Optional** If provided, reads are aligned against the full reference genome.
Contaminants	**Required** Specify the path to the folder containing the FASTA files of contaminants.
miRNA	**Required** Specify the path to the folder containing FASTA files of mature miRNAs.
RNA	**Required** Specify the path to the folder containing FASTA files of small RNAs.

Data Columns for the Targeted RNA Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step.
Manifest	**Required** Specify the manifest key for this sample, which is located in the first column of the Manifests section of the sample sheet.

Data Columns for the TruSeq Amplicon Workflow

Column Heading	Description
GenomeFolder	**Required** Specify the path to the reference genome folder, which contains the FASTA files to be used in the alignment step. The reference genome must be the same genome used to generate the manifest file. The genome folder is used to provide variant annotations and set the chromosome sizes in the BAM file output.
Manifest	**Required** Specify the manifest key for this sample, which is located in the first column of the Manifests section of the sample sheet.

Sample Sheet Settings

The Settings section of the sample sheet is optional for all workflows. Settings control various sequencing and analysis parameters. Each line in the Settings section contains a setting name in the first column and a value in the second column.

Sample Sheet Settings for Sequencing

Parameter	Description
CustomRead1PrimerMix CustomIndexPrimerMix CustomRead2PrimerMix	Create one line for each custom primer used. Indicate C1 for the Read 1 primer, C2 for the Index primer, or C3 for the Read 2 primer. Custom primers are supported for Read 1, Index 1 Read, and Read 2 only. For more information, see *Using Custom Primers on the MiSeq (part # 15041638)*.
PercentTilesToScan	If set to the default value of 1, 100% of the tiles are scanned. Valid values are 0 through 1. • If set to 0, the software rounds *up* to one tile. • For all other settings, the software rounds *down*. For example, a value of 0.99 results in one less tile than the maximum number of tiles possible on the flow cell. For more information about dual-surface scanning, see the *MiSeq System User Guide (part # 15027617)*.

Sample Sheet Settings for Analysis

Parameter	Description
Adapter	**For all workflows** Specify the 5' portion of the adapter sequence to prevent reporting sequence beyond the sample DNA. Illumina recommends adapter trimming for Nextera libraries and Nextera Mate Pair libraries. To specify two or more adapter sequences, separate the sequences by a plus (+) sign. For example: CTGTCTCTTATACACATCT+AGATGTGTATAAGAGACAG For more information, see *Adapter Settings* on page 15 and *Adapter Sequences* on page 16.
AdapterRead2	**For all workflows** Specify the 5' portion of the Read 2 adapter sequence to prevent reporting sequence beyond the sample DNA. Use this setting to specify a different adapter other than the one specified in the **Adapter** setting. For more information, see *Read 1 and Read 2 Adapters* on page 16.
Aligner	**For the Resequencing workflow and Library QC workflow** As of MiSeq Reporter v2.2, Eland has been deprecated, but not removed. For backward compatibility, use the Aligner setting to specify Eland. When using the default aligner for any workflow, you do not need to specify the alignment method in the sample sheet.
BaitManifestFileName	**For the Enrichment workflow** Specify the full path to the bait file. This setting is used only if the PicardHSmetrics setting is used and set to true.
CustomAmpliconAlignerMax IndelSize	**For the TruSeq Amplicon workflow** By default, the maximum detectable indel size is 25. A larger value increases sensitivity to larger indels, but requires more time to complete alignment.
EnrichmentMaxRegion StatisticsCount	**For the Enrichment workflow** Default is 40000. Sets the maximum number of rows shown in the Targets table and recorded EnrichmentStatistics.xml.

Parameter	Description
ExcludeRegionsManifestA	**For the Enrichment workflow** This setting excludes one or more region groups (separated by plus signs) from consideration. For example, if this setting specifies ABC+DEF, any region that has either ABC or DEF specified in the **Group** column of the manifest is ignored when parsing the manifest. No variant calling is performed for this region or reported in enrichment statistics. If the sample sheet contains more than one manifest, use multiple lines, such as ExcludeRegionsManifestB, ExcludeRegionsManifestC.
FlagPCRDuplicates	**For the Enrichment workflow, Library QC workflow, PCR Amplicon workflow, and Resequencing workflow** Settings are 0 or 1. Default is 1, filtering. If set to 1, PCR duplicates are flagged in the BAM files and not used for variant calling. PCR duplicates are defined as two clusters from a paired-end run where both clusters have the exact same alignment positions for each read. *(Formerly FilterPCRDuplicates. FilterPCRDuplicates is acceptable for backward compatibility.)*
Kmer	**For the Assembly workflow** This setting overrides the k-mer size used by Velvet. Default is 31; odd-numbered values up to 255 are supported.
OutputGenomeVCF	**For the Enrichment workflow, PCR Amplicon workflow, and TruSeq Amplicon workflow** Settings are 0 or 1. If set to true (1), this setting turns on genome VCF (gVCF) output for single sample variant calling. This setting requires MiSeq Reporter v2.3.
QualityScoreTrim	**For all workflows** If set to a value > 0, then the 3' ends of non-indexed reads with low quality scores are trimmed. Trimming is automatically applied by default at a value of 15 when using BWA for alignment.
PicardHSmetrics	**For the Enrichment workflow** Settings are 0 or 1. Default is 0. If set to true (1), this setting generates Picard HS metrics for the given bait and manifest file. If the bait file is not explicitly identified, the manifest file is used as the bait file. Use the BaitManifestFileName setting to specify the bait file.

Parameter	Description
ReverseComplement	**For the Assembly workflow, Library QC workflow, and Resequencing workflow** Settings are 0 or 1. Default is 1, reads are reverse-complemented. If set to true (1), all reads are reverse-complemented as they are written to FASTQ files. This setting is necessary in certain cases, such as processing of mate pair data using BWA, which expects paired-end data. Per-cycle metrics might be disrupted by this setting. Set this setting to 1 when using the Resequencing workflow or Assembly workflow with Nextera Mate Pair libraries. Set this setting to 1 when using the Workflow workflow with Nextera Mate Pair libraries.
StitchReads	**For the Amplicon - DS workflow, Generate FASTQ workflow, and TruSeq Amplicon workflow** Settings are 0 or 1. Default is 0, paired-end reads are not stitched. If set to true (1), paired end reads that overlap are stitched to form a single read. To be stitched, a minimum of 10 bases must overlap between Read 1 and Read 2. Paired-end reads that cannot be stitched are converted to two single reads. This setting requires MiSeq Reporter v2.3. For more information, see *Read Stitching* on page 17.
TaxonomyFile	**For the Metagenomics workflow** This setting overrides the taxonomy database; default is taxonomy.dat. As of MiSeq Reporter v2.3, species-level classification is enabled, by default. For faster, but less granular genus-level classification, specify gg_13_5_genus_32bp.dat.
VariantCaller	**For the Enrichment workflow, PCR Amplicon workflow, Resequencing workflow, and TruSeq Amplicon workflow** Specify one of the following variant caller settings: • GATK (default) • Somatic (recommended for tumor samples) • Starling (legacy variant caller) • None (no variant calling) When using the default variant caller for the workflow, it is not necessary to specify the variant calling method in the sample sheet.

Adapter Settings

Some clusters can sequence beyond the sample DNA and read bases from a sequencing adapter, particularly with longer read lengths up to 250 cycles.

The Adapter sample sheet setting prevents reporting sequence beyond the sample DNA by trimming the specified sequence in FASTQ files. Trimming the adapter sequence avoids reporting of spurious mismatches with the reference sequence, and improves performance in accuracy and speed of alignment.

For workflows using BWA, the Adapter setting trims reads from the start of the adapter sequence. When using ELAND (deprecated in MiSeq Reporter v2.2), reads are N-masked or replaced with Ns (no-call) from the start of the adapter sequence.

Read 1 and Read 2 Adapters

If you specify an adapter sequence for the Adapter setting in the sample sheet, the same adapter sequence is trimmed for Read 1 and Read 2. To trim a different adapter sequence in Read 2, use the AdapterRead2 setting in the sample sheet.

To trim two or more adapters, separate the sequences by a plus (+) sign.

How Adapter Trimming Works

MiSeq Reporter considers each potential adapter start position (n) within the sequence, starting at the first base (n=0). The process continues to count matches and mismatches between sequence (n) and adapter (0), sequence (n + 1), and adapter (1), and so on. This loop terminates if the following occurs:

```
MismatchCount > 1 and MismatchCount > MatchCount
```

Otherwise, the count continues until the end of the sequence or end of the adapter is reached, whichever comes first. The sequence is trimmed starting at position n, if:

```
MatchCount / (MatchCount + MismatchCount) > Cutoff
```

By default, the cutoff is 0.9 or < 10% mismatch rate. This default setting can be modified using the configurable setting **AdapterTrimmingStringency**.

Masking Short Reads

MiSeq Reporter includes the NMaskShortAdapterReads configuration setting, which prevents reads that have been almost entirely trimmed or masked from confounding downstream analysis. This setting is applied under the following conditions:

- If the adapter is encountered within the first 32 bases of the read, then the adapter sequence is N-masked.
- If the adapter is identified in the first 32 bases ***and*** the read includes ten or more bases from the start of the adapter, the entire read is N-masked. The configuration setting NMaskShortAdapterReads controls the ten-base limit.

Adapter Sequences

Library Type	Adapter Sequence
Nextera libraries	Illumina recommends adapter trimming for Nextera libraries. **CTGTCTCTTATACACATCT**
Nextera Mate Pair libraries	Illumina recommends multiple adapter trimming for Nextera Mate Pair libraries. Read 1: **CTGTCTCTTATACACATCT** Read 2: **AGATGTGTATAAGAGACAG**

Library Type	Adapter Sequence
Small RNA libraries	By default, adapter trimming is performed in the Small RNA workflow using the standard adapter sequence: **TGGAATTCTCGGGTGCCAAGGC** Use the Adapter setting to specify an adapter sequence other than the standard sequence.
TruSeq libraries	Read 1: **AGATCGGAAGAGCACACGTCTGAACTCCAGTCA** Read 2: **AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**

Read Stitching

MiSeq Reporter v2.3, or later, is required to use the optional StitchReads setting. Read stitching is compatible only with the Amplicon - DS workflow, GenerateFASTQ workflow, and TruSeq Amplicon workflow. Read stitching is not possible with any other Illumina alignment method or analysis workflows, but might be allowable input with some third-party analysis tools.

When set to true (1), paired-end reads that overlap are stitched to form a single read in the FASTQ file. At each overlap position, the consensus stitched read has the base call and quality score of the read with higher Q-score.

When the StitchReads setting is applied, the stitched read and each individual read are aligned and the alignment information is used in variant calling. A BAM file is written for the stitched read, Read 1, and Read 2. In some cases, this setting can improve accuracy of variant calling.

For each paired read, a minimum of 10 bases must overlap between Read 1 and Read 2 to be a candidate for read stitching. The minimum threshold of 10 bases minimizes the number of reads that are stitched incorrectly due to a chance match. Candidates for read stitching are scored as follows:

- For each possible overlap of 10 base pairs or more, a score of 1 – MismatchRate is calculated.
- Perfectly matched overlaps have a MismatchRate of 0, resulting in a score of 1.
- Random sequences have an expected score of 0.25.
- If the best overlap has a score of ≥ 0.9 ***and*** the score is ≥ 0.1 higher than any other candidate, then the reads are stitched together at this overlap.

Paired-end reads that cannot be stitched are converted to two single reads in the FASTQ file.

Sample Sheet Settings for Variant Calling

Setting Name	Description
FilterOutSingleStrandVariants	**For the PCR Amplicon workflow and Resequencing workflow** This setting filters variants if they are found in one read-direction only. **Default value:** • 1 (on)—Somatic variant caller (0 for TruSeq Amplicon workflow) This setting does not apply to the TruSeq Amplicon workflow.
IndelRepeatFilterCutoff	This setting filters indels if the reference has a 1-base or 2-base motif over eight times (by default) next to the variant. **Default value:** • 8—GATK • 8—Somatic variant caller • 8—Starling
MinimumCoverageDepth	**For the Enrichment workflow** The variant caller filters variants if the coverage depth at that location is less than the specified threshold. Decreasing this value increases variant calling sensitivity, but raises the risk of false positives. **Default value:** • 20—GATK (Enrichment workflow only; 0 for any other workflow)
MinQScore	This setting specifies the minimum base call Q-score to use as input to variant calling. **Default value and variant caller:** • 20—Somatic variant caller • 0—Starling
StrandBiasFilter	**For the Enrichment workflow** This setting filters variants with a significant bias in read-direction. Variants filtered in this way have **SB** in the filter column of the VCF file, instead of **PASS**. **Default value:** • -10—GATK (Enrichment workflow only; no filter for any other workflow) • 0.5—Somatic variant caller
VariantFrequencyEmitCutoff	This variant caller does not report variants with a frequency less than the specified threshold. **Default value:** • 0.01—Somatic Variant Caller

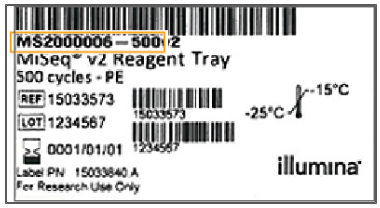
Setting Name	Description
VariantFrequencyFilterCutoff	This setting filters variants with a frequency less than the specified threshold. **Default value:** • 0.01—Somatic variant caller • 0.20—GATK • 0.20—Starling
VariantMinimumGQCutoff	This setting filters variants if the genotype quality (GQ) is less than the threshold. GQ is a measure of the quality of the genotype call and has a maximum value of 99. (*Formerly, VariantFilterQualityCutoff, which is acceptable for backward compatibility.*) **Default value:** • 30—GATK • 30—Somatic variant caller • 20—Starling
VariantMinimumQualCutoff	This setting filters variants if the quality (QUAL) is less than the threshold. QUAL indicates the confidence that the variant is genuine. **Default value:** • 30—GATK • 30—Somatic variant caller • 20—Starling

Naming the Sample Sheet

During the run setup steps, MCS looks for a sample sheet with a name matching the barcode number of the reagent cartridge. Therefore, Illumina recommends that you name your sample sheet with the barcode number of the reagent cartridge followed by *.csv extension. The barcode number is on the reagent cartridge label directly below the barcode.

In the following example, the sample sheet name is MS2000006-500.csv. You do not need to include the kit version in the sample sheet name.

Figure 2 Reagent Cartridge Label



If the barcode number is not known, use a preferred name for the sample sheet followed by *.csv. When the software cannot locate the sample sheet during the run setup steps, browse to the appropriate sample sheet.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Illumina 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	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support, select a product, then click **Documentation & Literature**.

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