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Illumina Experiment Manager

Software Guide

Overview 4
Install and Configure the Software 4
Configure Repository Locations 4
Download the Manifest File 5
Manage Custom Genomes 5
Create Custom Library Prep Kit 6
Create a Sample Plate 7
Create an AmpliSeq Library PLUS for Illumina Sample Plate 7
Create a Nextera Sample Plate 8
Create a TruSeq Amplicon Sample Plate 9
Create a TruSight Enrichment Sample Plate 11
Create a TruSight HLA Sample Plate 12
Create a TruSight Tumor 15 Sample Plate 13
Create a Sample Plate 14
Sample Plate File Extensions 15
Create a Sample Sheet 16
NovaSeq Sample Sheets 16
NextSeq or MiniSeq Sample Sheets 18

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MiSeq Sample Sheets 19
HiSeq Sample Sheets 25
Revision History 27
Technical Assistance 34

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Overview

The Illumina® Experiment Manager (IEM) software is used to create and edit well-formed sample sheets for Illumina systems and analysis software.

Create a sample sheet before starting sample or library preparation. IEM detects when an index combination is suboptimal and provides a warning. By creating the sample sheet before sample or library preparation, you can try different index combinations without risking samples.

Creating a sample sheet in IEM consists of two steps:

- **Create a sample plate**—The sample plate stores information about the samples in each well of a plate. The information includes the library prep type, the plate name, and indexes.
- **Create a sample sheet**—The sample sheet is based on the information defined during sample plate creation. A sample plate is not required to generate a sample sheet, but can be useful for organizing samples. The sample sheet transfers run and sample information in the compatible file format (*.csv) to the system software.

Install and Configure the Software

IEM is compatible with all Windows platforms. Use the following instructions to install the latest version of IEM.

1. Uninstall any previous version of IEM.
2. Download IEM from the IEM support page on the Illumina website.
3. Double-click the IEM installer file.
4. Select **Next**.
5. Read the End-User License Agreement and then select the **I accept the terms in the License Agreement** checkbox.
6. **[Optional]** Select **Change…** to choose a different installation path for the software.
7. Select **Next**.
8. Select **Install** to accept all settings and install the software.
9. Select **Finish** to complete the installation.
   An IEM icon appears on the desktop.

Configure Repository Locations

1. From the main screen of IEM, select **Settings**.
2. Review the default repository locations.
3. **[Optional]** Select **Browse** and navigate to each location where IEM saves sample plates, sample sheets, manifests, and genomes. If needed, specify locations on a shared network folder so that the files are accessible from the sequencing system.

   NOTE
   Sample sheets and manifests can also be copied to a USB drive.

4. Select **OK** to save the settings.

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Download the Manifest File

1 Depending on application, download the manifest file from the location indicated in the following table. Save downloaded files in the specified Manifest Repository location.

Application	Manifest File Location
Amplicon DS TruSight® Tumor 26	• TruSight Tumor 26 manifest files for pool A and pool B are on the TruSight Tumor 26 kit support page. • Download Pool A and pool B manifest files for TruSeq® Custom Amplicon dual strand panels designed in DesignStudio® from DesignStudio or the custom products page in MyIllumina.
Enrichment	• TruSight Rapid Capture kits support page. • Download manifest files for custom products designed in DesignStudio from DesignStudio or the custom products page in MyIllumina.
PCR Amplicon	Manifest files are generated in IEM.
Targeted RNA	• TruSeq Targeted RNA Expression kit support page. • Download manifest files for custom products designed in DesignStudio from DesignStudio or the custom products page in MyIllumina.
TruSeq Amplicon	• TruSeq Amplicon - Cancer Panel library prep kit support page. • TruSight Myeloid Sequencing Panel support page. • Download manifest files for custom products designed in DesignStudio from DesignStudio or the custom products page in MyIllumina.
TruSeq Bovine	TruSeq Bovine Parentage kit support page.
TruSight Tumor 15	Manifest files for library MixA and MixB are preloaded with IEM v1.11, or later.

Manage Custom Genomes

Creating a sample sheet in IEM requires a reference genome.

When IEM is installed, the software creates a Genomes directory that mimics the genomes folder structure on the MiSeq® system. The Genomes directory is a copy of the latest genome build, which can be downloaded from MyIllumina.

The Genomes directory consists of a series of folders and the file GenomeSize.xml. This file provides IEM with the genome information to create a sample sheet. A custom reference genome that is not included in the directory must be added.

Add Custom Genome to Genome Repository

1 Make sure that the genome is in a FASTA format (*.fa).

2 Navigate to the genome folder within the Genome directory.
To determine the Genome directory path, see *Configure Repository Locations* on page 4

3 Create a folder labeled with the genome name.
Do not use spaces in the folder name.

4 Add the FASTA file to the new folder.

5 Close and reopen IEM.

6 Convert the FASTA file to XML format:

- Create a MiSeq sample sheet, selecting the **PCR Amplicon** application when prompted. See *Create*

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a MiSeq Sample Sheet on page 20.
- Create a manifest file and select the new genome. See *Create a Manifest File for the PCR Amplicon Workflow* on page 21.
- When prompted to convert the file to XML format, select **Yes**.

7 **[Optional]** Delete the original FASTA file.

Create Custom Library Prep Kit

Creating a custom library prep kit in IEM consists of two steps: adding the custom kit to the list of available sample prep kits, and then adding it to the appropriate application.

CAUTION

To avoid recipe problems during run setup, any custom kit must match the parameters of the selected application. For example, Index Read number and length.

Add Custom Kit to Kits

1. In Program Files, navigate to Illumina\Illumina Experiment Manager\SamplePrepKits.
2. Copy a library prep kit file (*.txt) and name the copy for the custom library prep kit.
   The file name must be unique.
3. Open the new file in a text editor, such as Notepad.
4. In the [Name] section, replace the name of the Illumina kit with the same custom kit name used to name the file.
5. Edit the sequences as needed.
   IEM will not accept duplicated sequences.
6. Save and close the file.
7. If IEM is open, close and reopen it.
   When creating a sample plate, the custom library prep kit is listed as an option.

Add Custom Kit to Applications

1. In Program Files, navigate to Illumina\Illumina Experiment Manager\Applications.
2. Copy a library prep kit file (*.txt) and name the copy for the custom library prep kit.
   The file name must be unique.
3. Using a text editor, such as Notepad, open the application file (*.txt) that represents where you want the custom library prep kit to be displayed.
4. In the [Compatible Sample Prep Kits] section, add the name of the custom library prep kit that was added to the SamplePrepKits folder.
   Make sure to use the name added to the [Name] section, not the name of the library prep kit file (*.txt).
5. Save and close the file.
6. If IEM is open, close and reopen it.
   The custom library prep kit is an option in the Library Prep Kit menu of the selected application.

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Create a Sample Plate

The sample plate stores information about the samples in each well of a plate. The information includes the library prep type, plate name, and sample indexes. A sample plate is not required to generate a sample sheet, but is useful for organizing samples.

The procedure to create a sample plate varies by library prep kit and index adapters. The following applications have unique procedures. For all other applications, see *Create a Sample Plate* on page 14

Application	Instructions
AmpliSeq™ Library PLUS for Illumina®	See *Create an AmpliSeq Library PLUS for Illumina Sample Plate* on page 7.
Nextera™	See *Create a Nextera Sample Plate* on page 8.
TruSeq Amplicon	See *Create a TruSeq Amplicon Sample Plate* on page 9.
TruSight Enrichment	See *Create a TruSight Enrichment Sample Plate* on page 11.
TruSight HLA	See *Create a TruSight HLA Sample Plate* on page 12.
TruSight Tumor 15	See *Create a TruSight Tumor 15 Sample Plate* on page 13.

Create an AmpliSeq Library PLUS for Illumina Sample Plate

1. From the main screen, select **Create Sample Plate**.
2. Select **AmpliSeq Library PLUS for Illumina (96)**, and then select **Next**.
3. In the Unique Plate Name field, enter a unique name for the sample plate.
   For Index Reads, **2** is automatically selected.
4. Select **Next**.
5. Select the **Table** or **Plate** tab, depending on your preferred view.
   - The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
   - The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying drop-down list to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.
6. **[Optional]** Use the fill and copy features to populate all wells quickly.
   - To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
   - To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.
   - Paste content from an Excel table into the Sample ID column.
7. Enter a unique sample ID for each well that contains a sample.
   The sample ID is used to track a sample from preparation through sequencing and analysis. The sample ID is typically a barcode, but any value is acceptable.
8. Select a well in the Index Well field for each well that contains a sample.
9. In the Index1 and Index2 fields, select the index adapter being used for each Index Read.
   - To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
   - To make an edited index layout the default index layout, select **Save As Default Index Layout**.

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- To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.

Shaded wells contain invalid or missing values.

10 **[Optional]** Enter a sample name, project, and description for each well to record more detailed information about the plate.

11 **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.
- To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
- To print an image of the sample plate, select **Print**.

12 Select **Finish**, and then save the sample plate file in the desired location.
For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a Nextera Sample Plate

1 From the main screen, select **Create Sample Plate**.

2 Select from the following options.
- **IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A**
- **Nextera DNA CD Indexes (24 Indexes, tubed)**
- **Nextera DNA CD Indexes (96 Indexes, plated)**
- **Nextera Exome**
- **Nextera Index Kit (24 Indexes, 96 Samples)**
- **Nextera Index Kit (96 Indexes, 384 Samples)**
- **Nextera Mate Pair**
- **Nextera XT Index Kit (24 Indexes, 96 Samples)**
- **Nextera XT Index Kit (96 Indexes, 384 Samples)**
- **Nextera XT v2 A-D**

3 Select **Next**.

4 In the Unique Plate Name field, enter a unique name for the sample plate.

5 For Index Reads, select the indexing scheme.
- **1**—Libraries are single-index.
- **2**—Libraries are dual-index.

The index adapters might limit the selection of index reads.

6 Select **Next**.

7 Select the **Table** or **Plate** tab, depending on your preferred view.
- The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
- The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.

8 **[Optional]** Use the fill and copy features to populate all wells quickly.
- To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
- To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.

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- Paste content from an Excel table into the Sample ID column.

9. Enter a unique sample ID for each well that contains a sample.
   The sample ID is used to track a sample from preparation through sequencing and analysis. The sample ID is typically a barcode, but any value is acceptable.
10. If appropriate, select a well in the Index Well field for each well that contains a sample.
11. In the Index1 and Index2 fields, select the index adapter being used for each Index Read.
    - To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
    - To make an edited index layout the default index layout, select **Save As Default Index Layout**.
    - To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.

    Shaded wells contain invalid or missing values.
12. **[Optional]** Enter a sample name, project, and description for each well to record more detailed information about the plate.
13. **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.
    - To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
    - To print an image of the sample plate, select **Print**.
14. Select **Finish**, and then save the sample plate file in the desired location.
    For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a TruSeq Amplicon Sample Plate

1. From the main screen, select **Create Sample Plate**.
2. Select from the following options.
   - **TruSeq Amplicon Cancer Panel**
   - **TruSeq Custom Amplicon**
   - **TruSeq Custom Amplicon Low Input**
   - **TruSight Myeloid**
3. Select **Next**.
4. In the Unique Plate Name field, enter a unique name for the sample plate.
   For Index Reads, **2** is automatically selected.
5. Select **Next**.
6. Select the **Table** or **Plate** tab, depending on your preferred view.
   - The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
   - The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.
7. **[Optional]** Use the fill and copy features to populate all wells quickly.
   - To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
   - To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.

- Paste content from an Excel table into the Sample ID column.

8 [Standard protocol] For each well that contains a sample, do the following.

   a Enter a unique sample ID.
   The sample ID is used to track a sample from preparation through sequencing and analysis. The sample ID is typically a barcode, but any value is acceptable.

   b In the Index1 and Index2 fields, select the index adapter being used for each Index Read.
   - To autopopulate the indexes for all index reads for 96 wells, select Apply Default Index Layout. If desired, edit the autopopulated indexes.
   - To make an edited index layout the default index layout, select Save As Default Index Layout.
   - To restore the Illumina layout as the default index layout, select Restore Illumina Default Index Layout.

NOTE
Shaded wells contain invalid or missing values.

   c Enter the name of the Illumina manifest file for your assay or control without the .txt extension.

   d [Optional] Enter a sample name, project, and description for each well to record more detailed information about the plate.

9 [Dual strand protocol] For each well that contains a sample, do the following.

   a Enter a unique sample ID and the pool (A or B). Each unique DNA sample must have a unique sample ID for pool A and pool B.
   - Enter pool A sample IDs in cells A01–A06, B01–B06, C01–C06, and so on, to populate the left side of the plate.
   - Enter pool B sample IDs in cells A07–A12, B07–B12, C07–C12, and so on, to create a mirror image on the right side of the plate.

   b Enter the same sample name for matched pool A and pool B samples.

	Sample ID	Sample Name
Sample 1—Pool A	Sample001A	DNASample001
Sample 1—Pool B	Sample001B	DNASample001

   c In the Index1 and Index2 fields, select the index adapter being used for each Index Read. The index pair must be unique for each well.
   - To autopopulate the indexes for all index reads for 96 wells, select Apply Default Index Layout. If desired, edit the autopopulated indexes.
   - To make an edited index layout the default index layout, select Save As Default Index Layout.
   - To restore the Illumina layout as the default index layout, select Restore Illumina Default Index Layout.

NOTE
Shaded wells contain invalid or missing values.

   d Enter the name of the Illumina manifest file for pool A samples and for pool B samples. Do not include the .txt file extension in the name.

   e [Optional] Enter a sample project and description for each well to record more detailed information about the plate.

10 [Optional] Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.

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- **[Dual strand protocol]** Make sure that pool A samples appear in columns 1–6 and pool B samples appear in columns 7–12.
- To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
- To print an image of the sample plate, select **Print**.

11 Select **Finish**, and then save the sample plate file in the desired location.
For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a TruSight Enrichment Sample Plate

1 From the main screen, select **Create Sample Plate**.

2 Select **TruSight Enrichment**, and then select **Next**.

3 In the Unique Plate Name field, enter a unique name for the sample plate.

4 For Index Reads, select the indexing scheme.
- **1**—Libraries are single-index.
- **2**—Libraries are dual-index.

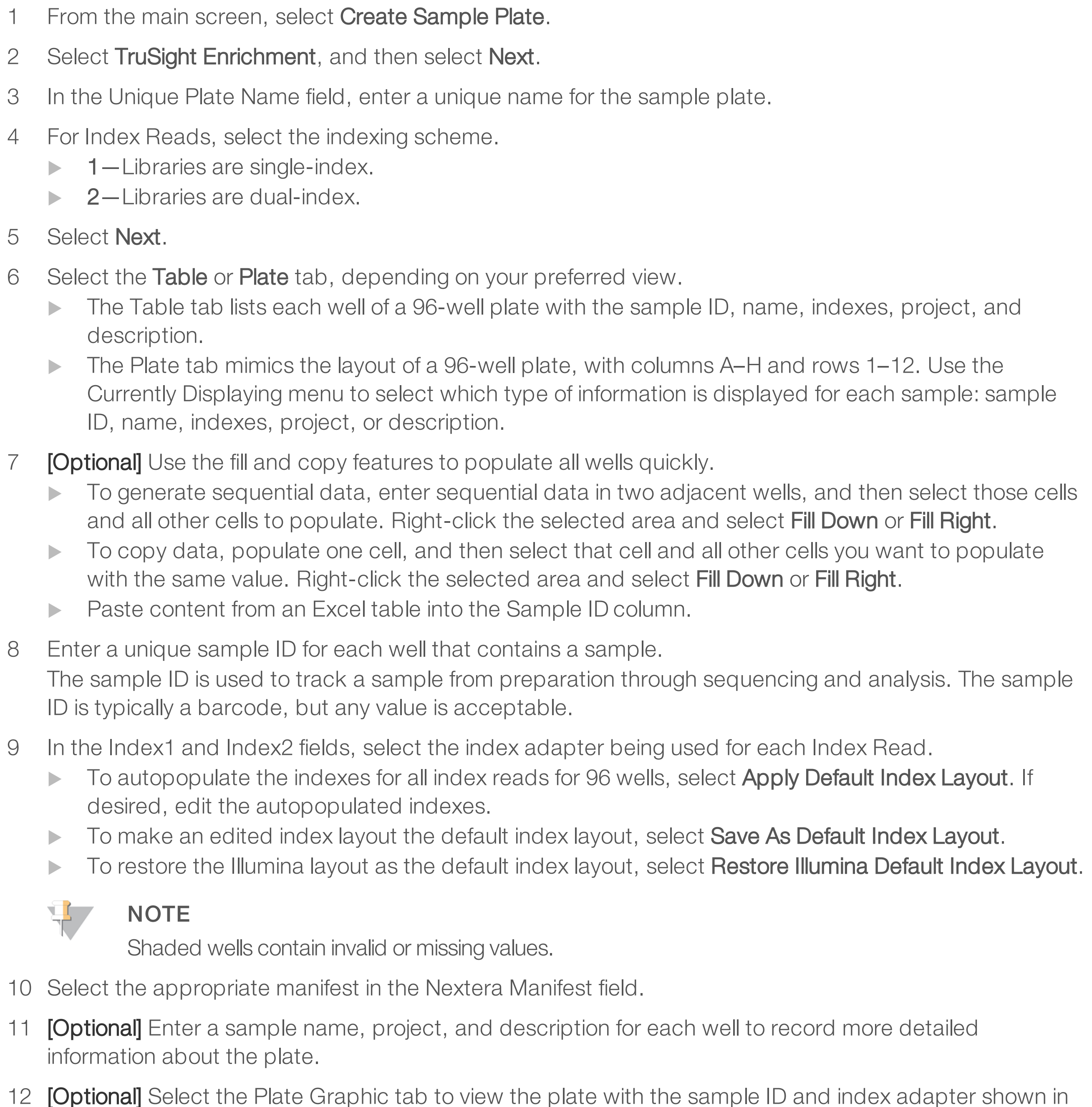
5 Select **Next**.

6 Select the **Table** or **Plate** tab, depending on your preferred view.
- The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
- The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.

7 **[Optional]** Use the fill and copy features to populate all wells quickly.
- To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
- To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.
- Paste content from an Excel table into the Sample ID column.

8 Enter a unique sample ID for each well that contains a sample.
The sample ID is used to track a sample from preparation through sequencing and analysis. The sample ID is typically a barcode, but any value is acceptable.

9 In the Index1 and Index2 fields, select the index adapter being used for each Index Read.
- To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
- To make an edited index layout the default index layout, select **Save As Default Index Layout**.
- To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.



NOTE
Shaded wells contain invalid or missing values.

10 Select the appropriate manifest in the Nextera Manifest field.

11 **[Optional]** Enter a sample name, project, and description for each well to record more detailed information about the plate.

12 **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in

each well.
- To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
- To print an image of the sample plate, select **Print**.

13 Select **Finish**, and then save the sample plate file in the desired location.
For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a TruSight HLA Sample Plate

1 From the main screen, select **Create Sample Plate**.

2 Select from the following options.
- **Nextera XT Index Kit (24 Indexes, 96 Samples)**
- **Nextera XT Index Kit (96 Indexes, 384 Samples)**
- **Nextera XT v2 A-D**

3 Select **Next**.

4 In the Unique Plate Name field, enter a unique name for the sample plate.

5 For Index Reads, select the indexing scheme.
- **1**—Libraries are single-index.
- **2**—Libraries are dual-index.

The index adapters might limit the selection of index reads.

6 Select **Next**.

7 Select the **Table** or **Plate** tab, depending on your preferred view.
- The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
- The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.

8 **[Optional]** Use the fill and copy features to populate all wells quickly.
- To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
- To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.
- Paste content from an Excel table into the Sample ID column.

9 Enter a unique sample ID and identical sample name for each well that contains a sample.
The sample name format is DNASource-HLAGeneTarget-DateOfExperiment with a 20-character limit. Use only alphanumeric characters with a dash between each identifier. For example: Sample1-DQB1-022515.

10 In the Index1 and Index2 fields, select the index adapter being used for each Index Read.
- To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
- To make an edited index layout the default index layout, select **Save As Default Index Layout**.
- To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.

NOTE
Shaded wells contain invalid or missing values.

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11 **[Optional]** Enter a sample project and description for each well to record more detailed information about the plate.

12 **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.
- To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
- To print an image of the sample plate, select **Print**.

13 Select **Finish**, and then save the sample plate file in the desired location.
For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a TruSight Tumor 15 Sample Plate

1 From the main screen, select **Create Sample Plate**.

2 Select **TruSight Tumor 15 genes**, and then select **Next**.

3 In the Unique Plate Name field, enter a unique name for the sample plate.
For Index Reads, **2** is automatically selected.

4 Select **Next**.

5 Select the **Table** or **Plate** tab, depending on your preferred view.
- The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
- The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.

6 **[Optional]** Use the fill and copy features to populate all wells quickly.
- To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
- To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down** or **Fill Right**.
- Paste content from an Excel table into the Sample ID column.

7 For each well that contains a sample, enter a unique sample ID and the pool (A or B). Each unique DNA sample must have a unique sample ID for pool A and pool B.

8 Enter the same sample name for matched pool A and pool B samples.

	Sample ID	Sample Name
Sample 1—Pool A	Sample001A	DNASample001
Sample 1—Pool B	Sample001B	DNASample001

9 In the Index1 and Index2 fields, select the index adapter being used for each Index Read. The index pair must be unique for each well.
- To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
- To make an edited index layout the default index layout, select **Save As Default Index Layout**.
- To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.

Shaded wells contain invalid or missing values.

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10 **[Optional]** Enter a sample project and description for each well to record more detailed information about the plate.

11 **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.
   - To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, PhotoShop, or other graphics-enabled software.
   - To print an image of the sample plate, select **Print**.

12 Select **Finish**, and then save the sample plate file in the desired location.
For the proper file extension for your library prep kit, see *Sample Plate File Extensions* on page 15.

Create a Sample Plate

1 From the main screen, choose whether to create or edit a sample plate.
   - To create a sample plate, select **Create Sample Plate**, and then select the appropriate library kit and select **Next**.
   - To edit a sample plate, select **Edit Sample Plate**, and then navigate to the appropriate sample plate.

2 In the Unique Plate Name field, enter a unique name for the sample plate.

3 For Index Reads, select the indexing scheme.
   - **1**—Libraries are single-index.
   - **2**—Libraries are dual-index.

The index adapters might limit the selection of index reads.

4 Select **Next**.

5 Select the **Table** or **Plate** tab, depending on your preferred view.
   - The Table tab lists each well of a 96-well plate with the sample ID, name, indexes, project, and description.
   - The Plate tab mimics the layout of a 96-well plate, with columns A–H and rows 1–12. Use the Currently Displaying menu to select which type of information is displayed for each sample: sample ID, name, indexes, project, or description.

6 **[Optional]** Use the fill and copy features to populate all wells quickly:
   - To generate sequential data, enter sequential data in two adjacent wells, and then select those cells and all other cells to populate. Right-click the selected area and select **Fill Down** or **Fill Right**.
   - To copy data, populate one cell, and then select that cell and all other cells you want to populate with the same value. Right-click the selected area and select **Fill Down**.
   - Paste content from an Excel table into the Sample ID column.

7 Enter a unique sample ID for each well that contains a sample.
The sample ID is used to track a sample from preparation through sequencing and analysis. The sample ID is typically a barcode, but any value is acceptable.

8 Enter the index adapter being used for each Index Read. Use combinations that result in at least one A or C base (red) and at least one G or T base (green) per cycle.
   - To autopopulate the indexes for all index reads for 96 wells, select **Apply Default Index Layout**. If desired, edit the autopopulated indexes.
   - To make an edited index layout the default index layout, select **Save As Default Index Layout**.
   - To restore the Illumina layout as the default index layout, select **Restore Illumina Default Index Layout**.

Shaded wells contain invalid or missing values.

9 **[Optional]** Enter a sample name, project, and description for each well to record more detailed information about the plate.

10 **[Optional]** Select the Plate Graphic tab to view the plate with the sample ID and index adapter shown in each well.
  - To copy an image of the sample plate, select **Copy to Clipboard**. The image can be pasted into Paint, PowerPoint, Word, Photoshop, or other graphics-enabled software.
  - To print an image of the sample plate, select **Print**.

11 Select **Finish**, and then save the sample plate file in the desired location.
Make sure that the file extension is .plt or the software will not recognize the sample plate.

Sample Plate File Extensions

The sample plate file must contain the following file extensions, based on the index adapter kit used.

System	File Extension
AmpliSeq Library PLUS for Illumina	*.aseq.28.plt
IDT-ILMN TruSeq DNA UD Indexes (24 Indexes)	*.trudud24.28.plt
IDT-ILMN TruSeq DNA UD Indexes (96 Indexes)	*.trudud96.28.plt
IDT-ILMN TruSeq RNA UD Indexes (24 Indexes)	*.trurud24.28.plt
IDT-ILMN TruSeq RNA UD Indexes (96 Indexes)	*.trurud96.28.plt
IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A	*.nexdud.210.plt
Nextera DNA CD Indexes (24 Indexes, tubed)	*.nex24.28.plt
Nextera DNA CD Indexes (96 Indexes, plated)	*.nex96.28.plt
Nextera DNA Single Indexes (24 Indexes, plated)	*.nexsingle.18.plt
Nextera Exome	*.nexex.28.plt
Nextera Index Kit (24 Indexes, 96 Samples)	*.nexfc36.28.plt
Nextera Index Kit (96 Indexes, 384 Samples)	*.nexfc.96.28.plt
Nextera Mate Pair	*.nexmp.16.plt
Nextera Rapid Capture Custom Enrichment	*.nexenr.28.plt
Nextera XT Index Kit (24 Indexes, 96 Samples)	*.nexxt24.28.plt
Nextera XT Index Kit (96 Indexes, 384 Samples)	*.nexfc96.28.plt
Nextera XT v2 Index Kit A	*.nexxtv2a.28.plt
Nextera XT v2 Index Kit B	*.nexxtv2b.28.plt
Nextera XT v2 Index Kit C	*.nexxtv2c.28.plt
Nextera XT v2 Index Kit D	*.nexxtv2d.28.plt
ScriptSeq™ Complete	*.ssc.16.plt
ScriptSeq v2	*.ss2.16.plt
SureCell™ WTA 3'	*.sglc.18.plt
TruSeq Amplicon Cancer Panel	*.ampc.28.plt
TruSeq Bovine	*.bov.28.plt
TruSeq Custom Amplicon	*.amp.28.plt
TruSeq Custom Amplicon Low Input	*.amplow.28.plt

System	File Extension
TruSeq DNA CD Indexes (96 Indexes)	*.trudcd.28.plt
TruSeq DNA Methylation	*.trumeth.16.plt
TruSeq DNA Single Indexes Set A	*.trudsinglea.16.plt
TruSeq DNA Single Indexes Set B	*.trudsingleb.16.plt
TruSeq Exome (8rxn x 12 plex)	*.truht.28.plt
TruSeq Exome (8rxn x 3,6,9 plex)	*.tru.16.plt
TruSeq Methyl Capture EPIC	*.trumce.16.plt
TruSeq Rapid Exome	*.nexenr.28.plt
TruSeq Ribo Profile	*.ribo.16.plt
TruSeq RNA CD Indexes (96 Indexes)	*.trurcd.28.plt
TruSeq RNA Single Indexes Set A	*.trursinglea.16.plt
TruSeq RNA Single Indexes Set B	*.trusingleb.16.plt
TruSeq Small RNA	*.sra.16.plt
TruSeq Synthetic Long-Read DNA	*.trulr.18.plt
TruSeq Targeted RNA Expression	*.ttran.26.plt
TruSight Enrichment	*.truenr.28.plt
TruSight Myeloid	*.myeloid.28.plt
TruSight Tumor 15 genes	*.tst15.26.plt
TruSight Tumor 26 genes	*.tst26.28.plt

Create a Sample Sheet

IEM can create a sample sheet to set up analysis of sequencing data from a run on the following sequencing systems.

- NovaSeq™ 6000
- NextSeq™ 550
- NextSeq 500
- MiSeq
- HiSeq™ (all models except HiSeq X®).

The sample sheet workflows in IEM vary by sequencing system.

System	Sample Sheet Instructions
NovaSeq	See *NovaSeq Sample Sheets* on page 16.
NextSeq or MiniSeq™	See *NextSeq or MiniSeq Sample Sheets* on page 18.
MiSeq	See *MiSeq Sample Sheets* on page 19.
HiSeq	See *HiSeq Sample Sheets* on page 25.

NovaSeq Sample Sheets

Use the following instructions to create a sample sheet for analyzing data from a NovaSeq 6000 sequencing run.

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Create a NovaSeq Sample Sheet

1 From the main screen, choose whether to create or edit a sample sheet.
   - To create a sample sheet, select **Create Sample Sheet**.
   - To edit a sample sheet, select **Edit Sample Sheet**, navigate to the appropriate sample sheet, and proceed to step 4.

2 Select **NovaSeq**, and then select **Next**.

3 Select **Next**.
   NovaSeq FASTQ Only is the only supported application for NovaSeq.

4 In the Reagent Kit Barcode field, enter the reagent kit ID from the label on the reagent kit box.

5 Select the appropriate Library Prep Workflow.

6 Select the appropriate Index Adapters.

7 For Index Reads, select the same indexing scheme that was selected during sample plate creation.
   - **0**—Libraries are not indexed.
   - **1**—Libraries are single-index.
   - **2**—Libraries are dual-index.

8 **[Optional]** Enter the Experiment Name, Investigator Name, Description, and Date.

9 Select the Read Type.
   - **Paired End**—The sequencing run includes Read 1 and Read 2.
   - **Single Read**—The sequencing run includes Read 1 only.

10 In the Cycles Read fields, enter one more than the number of cycles.
   For example, for a 150-cycle read, enter 151.
   For a paired-end run, enter the same number in Cycles Read 1 and Cycles Read 2.

11 **[Optional]** Select the **NovaSeq Xp workflow** checkbox.
   Use the NovaSeq Xp directed flow cell loading option to specify which lane the samples are in (1–4).

12 **[Optional]** Select the **Use Adapter Trimming** checkbox.
   The software trims the adapter sequence, which improves the accuracy and speed of analysis.
   This setting is recommended for Nextera and Nextera XT libraries. The default adapter sequence is the adapter present in all Nextera libraries. If a different adapter sequence is used, edit the sequence that is displayed in the final sample sheet.

13 **[Optional]** Select the **Use Adapter Trimming 2** checkbox.
   Set the AdapterRead2 setting in the sample sheet to trim a different adapter sequence in Read 2.

14 Select **Next** to continue to *Select Samples for a NovaSeq Sample Sheet* on page 17.

Select Samples for a NovaSeq Sample Sheet

1 Select samples by creating a sample plate, or using an existing plate.
   - To create a sample plate, select **New Plate**. See *Create a Sample Plate* on page 7.
   - To use an existing plate, select **Select Plate**, and then navigate to the appropriate sample plate.

2 Choose wells to include in the sequencing run.
   - To include all wells, select **Select All**.
   - To specify which wells to include, select only the applicable wells.

3 If you selected NovaSeq Xp workflow, select the lane you want to insert samples into.

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By default, samples are added to lane 1. Use the radio button to select **1–4**, or **All** to specify the lanes displayed.

4 Select **Add Selected Samples**.
Select the **Maximize** checkbox to hide the sample plate panel and view the sample sheet in full screen.

5 **[Optional]** Select **Add Blank Row** to add rows and manually enter sample information.

6 **[Optional]** To remove rows, select at least one field, and then select **Remove Selected Rows**.

7 For each sample, enter a sample name, reference, project, and description, if applicable.

8 Select **Finish**, and then save the sample sheet file (*.csv) in the desired location.

9 Select **Yes** to review the sample sheet in Excel, or **No** to exit the sample sheet wizard.

Applications and Kits

A NovaSeq sample sheet must specify the following analysis application. The associated analysis workflow is written to the sample sheet.

Application	Analysis Workflow	Application Description
NovaSeq FASTQ Only	GenerateFASTQ	Generates demultiplexed FASTQ files from any type of library.

For compatible library prep kits, see the Illumina Experiment Manager support page on the Illumina website.

NextSeq or MiniSeq Sample Sheets

Use the following instructions to create a sample sheet for analyzing data from a NextSeq FASTQ Only sequencing run. Use the sample sheet when operating in standalone mode with the bcl2fastq2 software package.

Create a NextSeq or MiniSeq Sample Sheet

1 From the main screen, choose whether to create or edit a sample sheet.
- To create a sample sheet, select **Create Sample Sheet**.
- To edit a sample sheet, select **Edit Sample Sheet**, navigate to the appropriate sample sheet, and proceed to step 4.

2 Select **NextSeq/MiniSeq**, and then select **Next**.

3 Select **Next**.
NextSeq FASTQ Only is the only supported application for NextSeq/MiniSeq.

4 In the Reagent Kit Barcode field, enter the reagent kit ID from the label of box 1 or box 2 of the SBS kit. If you do not have a reagent kit ID, enter a unique name instead.

5 Select the appropriate Library Prep Workflow.

6 Select the appropriate Index Adapters.

7 For Index Reads, select the same indexing scheme that was selected during sample plate creation.
- **0**—Libraries are not indexed.
- **1**—Libraries are single-index.
- **2**—Libraries are dual-index.

8 **[Optional]** Enter the Experiment Name, Investigator Name, Description, and Date fields.

9 Select the Read Type.

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- **Paired End**—The sequencing run includes Read 1 and Read 2.
- **Single Read**—The sequencing run includes Read 1 only.

10 In the Cycles Read fields, enter one more than the number of cycles.
For example, for a 150-cycle read, enter 151.
For a paired-end run, enter the same number in Cycles Read 1 and Cycles Read 2.

11 **[Optional]** Select the **Use Adapter Trimming** checkbox.
The software trims the adapter sequence, which improves the accuracy and speed of analysis. This setting is recommended for Nextera and Nextera XT libraries. The default adapter sequence is the adapter present in all Nextera libraries. If a different adapter sequence is used, edit the sequence that is displayed in the final sample sheet.

12 **[Optional]** Select the **Use Adapter Trimming 2** checkbox.
Set the AdapterRead2 setting in the sample sheet to trim a different adapter sequence in Read 2.

13 Select **Next** to continue to *Select Samples for a NextSeq or MiniSeq Sample Sheet* on page 19.

Select Samples for a NextSeq or MiniSeq Sample Sheet

1 Select samples by creating a sample plate, or using an existing plate.
- To create a sample plate, select **New Plate**. See *Create a Sample Plate* on page 7.
- To use an existing plate, select **Select Plate**, and then navigate to the appropriate sample plate.

2 Choose wells to include in the sequencing run.
- To include all wells, select **Select All**.
- To specify which wells to include, select only the applicable wells.

3 Select **Add Selected Samples**.

NOTE
Select the **Maximize** checkbox to hide the sample plate panel and view the sample sheet in full screen.

4 **[Optional]** Select **Add Blank Row** to add rows and manually enter sample information.

5 **[Optional]** To remove rows, select at least one field, and then select **Remove Selected Rows**.

6 For each sample, enter a sample name, reference, project, and description, if applicable.

7 Select **Finish**, and then save the sample sheet file (*.csv) in the desired location.

8 Select **Yes** to review the sample sheet in Excel, or **No** to exit the sample sheet wizard.

Applications and Kits

A NextSeq sample sheet must specify the following analysis application. The associated analysis workflow is written to the sample sheet.

Application	Analysis Workflow	Application Description
NextSeq FASTQ Only	GenerateFASTQ	Generates demultiplexed FASTQ files from any type of library.

For compatible library prep kits, see the Illumina Experiment Manager support page on the Illumina website.

MiSeq Sample Sheets

Use the following instructions to create a sample sheet for analyzing data from a MiSeq sequencing run.

Create a MiSeq Sample Sheet

1. From the main screen, choose whether to create or edit a sample sheet.
   - To create a sample sheet, select **Create Sample Sheet**.
   - To edit a sample sheet, select **Edit Sample Sheet**, navigate to the appropriate sample sheet, and proceed to step 4.
2. Select **MiSeq**, and then select **Next**.
3. Select the appropriate application, and then select **Next**.
4. In the Reagent Kit Barcode field, enter the reagent kit ID from the label of box 1 or box 2 of the SBS kit.
5. Select the appropriate Library Prep Workflow.
6. Select the appropriate Index Adapter.
7. For Index Reads, select the same indexing scheme that was selected during sample plate creation.
   - **0**—Libraries are not indexed.
   - **1**—Libraries are single-index.
   - **2**—Libraries are dual-index.
8. Enter an Experiment Name for the run.
9. **[Optional]** Enter the Investigator Name, Description, and Date.
10. Select the Read Type.
    - **Paired End**—The sequencing run includes Read 1 and Read 2.
    - **Single Read**—The sequencing run includes Read 1 only.
11. In the Cycles Read fields, enter one more than the number of cycles.
    For example, for a 150-cycle read, enter 151.
    For a paired-end run, enter the same number in Cycles Read 1 and Cycles Read 2.
12. See *MiSeq Workflow-Specific Settings* on page 22 for information on workflow-specific settings. Make the appropriate selections.
13. Select **Next** to continue to *Select Samples for a MiSeq Sample Sheet* on page 20.

Select Samples for a MiSeq Sample Sheet

1. Select samples by creating a sample plate, or using an existing plate.
   - To create a sample plate, select **New Plate**. See *Create a Sample Plate* on page 7.
   - To use an existing plate, select **Select Plate**, and then navigate to the appropriate sample plate.
2. Choose wells to include in the sequencing run.
   - To include all wells, select **Select All**.
   - To specify which wells to include, select only the applicable wells.
3. Select **Add Selected Samples**.
   Select the **Maximize** checkbox to hide the sample plate panel and view the sample sheet in full screen.
4. **[Optional]** Select **Add Blank Row** to add rows and manually enter sample information.
5. **[Optional]** To remove rows, select at least one field, and then select **Remove Selected Rows**.
6. For each sample, enter a sample name, reference, project, and description, if applicable.
7. From the Genome Folder menu, select the appropriate genome for your sample.

This step is applicable to the following applications, which use a genomic reference.

Category	Application
Targeted Resequencing	TruSeq Amplicon
	PCR Amplicon
	Enrichment
	Amplicon DS TruSight Tumor 26
Small Genome Sequencing	Resequencing
	Assembly
Other	LibraryQC

8 Proceed as follows, depending on application.
- For the PCR Amplicon application, proceed to *Create a Manifest File for the PCR Amplicon Workflow* on page 21.
- For all other applications, proceed to *Complete the Sample Sheet* on page 21.

Complete the Sample Sheet

1 If you are using the Enrichment application, enter the name of the Illumina manifest file for your assay or control without the .txt file extension.
If the menu does not contain your library prep kit, copy a manifest file in the Manifest Repository folder. For more information, see *Download the Manifest File* on page 5.

2 Select **Finish**, and then save the sample sheet file (*.csv) in the desired location.

3 Select **Yes** to review the sample sheet in Excel, or **No** to exit the sample sheet wizard.

Create a Manifest File for the PCR Amplicon Workflow

The manifest file for the PCR Amplicon workflow contains information about each sample that limits analysis results to user-defined regions of interest. A sample sheet can have multiple manifests. Create a manifest using the reference genome, chromosome coordinates, and PCR primer length.

1 From the Nextera Manifest menu, choose a manifest option:
- To create a manifest, select **New**.
- To choose an existing manifest, select **Edit**.

If you create a manifest, the Create New Amplicon Manifest screen appears.

2 From the Genomes drop-down menu, select a reference genome for each new manifest.
Make sure that the selected genome matches the reference genome used to design the PCR amplicon primers.
The genome must be located in the Genome Repository. Each sample sheet you create must have the same reference genome for all associated manifests.

NOTE
The Genome Repository can be the same location as the MiSeq Reporter Genomes directory, where the reference genome is located.

3 Add a blank row for each region of interest

4 Add a name for each amplicon.

NOTE

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The manifest entries can be copied from an Excel table into IEM. After pasting, make sure that the columns contain the appropriate content.

5 In the Chromosome column, select the appropriate chromosome for each amplicon.
6 In the Amplicon Start and Amplicon End columns, enter the coordinates for each amplicon, including primer lengths.
7 In the Upstream Probe Length and Downstream Probe Length columns, specify the length of each primer for each amplicon.
The upstream and downstream probe lengths use the PCR primers to generate the amplicon. Specifying the length allows variants called in these regions to be flagged during analysis.
8 Name the manifest file to save it to the Manifest Repository.
9 Select **OK**.
10 Before starting the run, copy the manifest into the MCS Manifest Repository on the MiSeq system.

MiSeq Workflow-Specific Settings

When selecting samples for a MiSeq sample sheet, the settings available vary depending on your workflow, determined by the category and application you selected. Use the following settings to specify additional run parameters, depending on your workflow.

Setting	Applications	Description
BWA-Backtrack	Enrichment, Library QC, PCR Amplicon, Small Genome Resequencing	Allows selection of v0.6.1 of the BWA aligner. This setting only applies to MiSeq Reporter v2.6 and later workflows, which use a newer version of the BWA aligner (BWA-MEM, v0.7.9a).
Custom Primer for Read 1	All except TruSeq Amplicon	Use a custom primer for Read 1.
Custom Primer for Index	All except TruSeq Amplicon	Use a custom primer for Index 1 and Index 2.
Custom Primer for Read 2	All except TruSeq Amplicon	Use a custom primer for Read 2.
Use Somatic Variant Caller	TruSeq Amplicon, PCR Amplicon, Enrichment, Resequencing	Select this checkbox if you are using the somatic variant caller. Somatic variant caller is an Illumina variant calling algorithm for TruSeq Amplicon – Cancer Panel and TruSight Myeloid Sequencing Panel. It detects low frequency mutations (even below 5%) in a mixed cell population. For more information, see the *Somatic Variant Caller Technical Note (Pub. No. 970-2012-014)* on the Illumina website.
Indel Realignment GATK	Resequencing, Enrichment	Locally realign reads around indels to minimize mismatches.
Flag PCR Duplicates	Resequencing, PCR Amplicon, Enrichment, Library QC	Flag apparent PCR duplicates in the BAM files and omit them from variant calling. PCR duplicates are two clusters from a paired-end run that have the same alignment positions for each read.
Variant Quality Filter	TruSeq Amplicon, PCR Amplicon, Resequencing, Enrichment	A cutoff parameter with a default setting of 30. For more information, see the *MiSeq Sample Sheet Quick Reference Guide (document # 15028392)* on the Illumina website.

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Setting	Applications	Description
Use Adapter Trimming	All except TruSeq Amplicon	The software masks the adapter sequence, which improves the accuracy and speed of analysis. This setting is recommended when sequencing libraries prepared with the Nextera, Nextera XT, Nextera Rapid Capture Enrichment, or TruSight Enrichment kits. The default adapter sequence is the adapter present in all Nextera libraries. If a different adapter sequence is used, edit the sequence that is displayed in the final sample sheet. For TruSight HLA, do not change the default.
Use Adapter Trimming Read 2	Plasmids, Assembly, RNA-Seq, Library QC, FASTQ Only, ChIP-Seq	Set the AdapterRead2 setting in the sample sheet to trim a different adapter sequence in Read 2.
Run Picard HsMetrics	Enrichment	Perform Picard hybrid selection (HS) analysis of the BAM file.
Reverse Complement	Resequencing, Library QC, FASTQ Only, Assembly	Convert Nextera Mate Pair libraries from a mate pair to a paired-end orientation as required by BWA and Velvet.
K-mer size	Assembly	Set the k-mer size used for assembly. The range is 2–255. Larger k-mer sizes require more memory and can impact the stability and performance of the analysis. K-mer optimization is suggested for optimal assemblies.
Genome	Small RNA	Provide the relative or absolute path to the following reference sequence folders. The typical settings for human runs are shown in parentheses: • Contaminants (HumanRNAContaminants) • RNA (HumanRNA) • miRNA (HumanRNAMature)
Export to gVCF	PCR Amplicon, TruSeq Amplicon, Enrichment	Enable output of gVCF files to the run folder.
Genus-Level Classification	Metagenomics	Enable genus-level classification, which overrides the species-level taxonomic classification default.

Applications and Kits

A MiSeq sample sheet must specify one of the following analysis applications. The analysis workflow for each application is written to the sample sheet.

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Category	Application	Workflow	Application Description
Targeted Resequencing	TruSeq Bovine	Custom Amplicon	Sequencing TruSeq Bovine Parentage Sequencing Panel libraries. A manifest file from the TruSeq Bovine Parentage Sequencing Panel support page is required for alignment.
	TruSight Tumor 15	TruSight Tumor 15	Performs alignment and somatic variant calling for formalin-fixed paraffin-embedded (FFPE) samples. Two libraries per sample are required to perform analysis. The manifest files for these libraries are preinstalled in IEM and MiSeq Reporter.
	TruSeq Amplicon	Custom Amplicon	Sequencing libraries prepared using a TruSeq Amplicon kit or the TruSight Myeloid Sequencing Panel. A manifest file from DesignStudio or the TruSight Myeloid support page is required for alignment. Reads are aligned against the manifest files specified in the sample sheet.
	PCR Amplicon	PCR Amplicon	Sequencing PCR amplicon libraries prepared with the Nextera XT Library Prep kit. The amplicons are generated from primers targeting particular genome positions (up to ~384 loci from up to 96 samples). Reads are aligned against the specified reference genome. The user-generated manifest file specified in the sample sheet provides the targeted regions. Variant calling is performed only within the targeted regions, and coverage statistics are reported for them.
	Metagenomics 16S rRNA	Metagenomics	Sequencing of genetic material from uncultured samples. No genomic reference is required for a Metagenomics workflow. Reads are classified using a database of 16S rRNA data included with the MiSeq Reporter software.
	Enrichment	Enrichment	Sequencing DNA obtained from hybrid capture-based enrichment. Reads are aligned against the specified reference genome. The Illumina manifest file specified in the sample sheet provides the targeted regions. Variant calling is performed only within the targeted regions, and coverage statistics are reported for them.
	Clone Checking	GenerateFASTQ	Verification of clone production by sequencing typically done with custom primers. A pseudogene (in FASTA format) representing the clone of interest is required for mapping and alignment.
	Amplicon DS TruSight Tumor 26	Amplicon DS	Detects somatic mutations in FFPE samples. This workflow independently processes variants from the forward and reverse strands of the library, and then algorithmically reconciles the calls.
Small Genome Sequencing	Resequencing	Resequencing	Sequencing a small genome, such as *E. coli*. Reads are aligned against the reference and variants are reported.
	Plasmids	GenerateFASTQ	Sequencing entire plasmid DNA. A reference plasmid genome is required for mapping and alignment. Output files are in BAM and VCF format.
	Assembly	Assembly	Assembly of small (< 20 Mb) genomes from reads without the use of a genomic reference. If a genomic reference is included, a dot plot is generated for the reference.

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Category	Application	Workflow	Application Description
RNA Sequencing	Targeted RNA	Targeted RNA	Sequencing TruSeq Targeted RNA Expression libraries. A target reference and a manifest file from DesignStudio are required. Reads are aligned against the manifest files specified in the sample sheet.
	Small RNA	Small RNA	Sequencing cDNA after reverse transcription of small RNA. Annotation backed by mirBase and Rfam. FASTQ files and stats files are available for subsequent downstream analysis.
	RNA-Seq	GenerateFASTQ	Sequencing cDNA following reverse transcription and library preparation. FASTQ files are generated and can be used with third-party software for subsequent analysis.
Other	TruSight HLA	GenerateFASTQ	Sequencing Long Range PCR HLA gene amplicons prepared using the Nextera XT Library Prep kit.
	Library QC	LibraryQC	Fast resequencing of a reference genome to check the DNA library and generate statistics for each sample.
	FASTQ Only	Generate FASTQ	Generating demultiplexed FASTQ files from any type of library.
	ChIP-Seq	Generate FASTQ	Sequencing TruSeq ChIP libraries. FASTQ files are generated and can be used with third-party software for subsequent analysis.

For compatible library prep kits, see the Illumina Experiment Manager support page on the Illumina website.

HiSeq Sample Sheets

Use the following instructions to create a sample sheet for analyzing data from a sequencing run on any of the following HiSeq systems.

- HiSeq 1000
- HiSeq 1500
- HiSeq 2000
- HiSeq 2500
- HiSeq 3000
- HiSeq 4000

For HiSeq protocols that support individually addressable lanes, loaded libraries are specified for each lane.

Create a HiSeq Sample Sheet

1. From the main screen, choose whether to create or edit a sample sheet.
   - To create a sample sheet, select **Create Sample Sheet**.
   - To edit a sample sheet, select **Edit Sample Sheet**, navigate to the appropriate sample sheet, and proceed to step 5.
2. Select **HiSeq**, and then select **Next**.
3. Select the appropriate HiSeq system, and then select **Next**.
4. Select **Next**.
   HiSeq FASTQ is the only supported application for HiSeq.
5. In the Reagent Kit Barcode field, enter the reagent kit ID from the label of box 1 or box 2 of the SBS kit.

If you do not have a reagent kit ID, enter a unique name instead.

6 Select the appropriate Library Prep Workflow.

7 Select the appropriate Index Adapters.

8 For Index Reads, select the same indexing scheme that was selected during sample plate creation.
   - 0—Libraries are not indexed.
   - 1—Libraries are single-index.
   - 2—Libraries are dual-index.

9 [Optional] Enter the Experiment Name, Investigator Name, Description, and Date.

10 Select the Read Type.
   - Paired End—The sequencing run includes Read 1 and Read 2.
   - Single Read—The sequencing run includes Read 1 only.

11 In the Cycles Read fields, enter one more than the number of cycles.
   For example, for a 150-cycle read, enter 151.
   For a paired-end run, enter the same number in Cycles Read 1 and Cycles Read 2.

12 See *HiSeq Workflow-Specific Settings* on page 26 for information on workflow-specific settings. Make the appropriate selections.

13 Select **Next** to continue to *Select Samples for a HiSeq Sample Sheet* on page 26.

Select Samples for a HiSeq Sample Sheet

1 Select samples by creating a sample plate, or using an existing plate.
   - To create a sample plate, select **New Plate**. See *Create a Sample Plate* on page 7.
   - To use an existing plate, select **Select Plate**, and then navigate to the appropriate sample plate.

2 Choose wells to include in the sequencing run.
   - To include all wells, select **Select All**.
   - To specify which wells to include, select only the applicable wells.

3 Select the lane you want to insert samples into. By default, samples are added to lane 1. Use the radio dials to select **1 - 8**, or **All** to specify the lanes displayed.

4 Select **Add Selected Samples**.
   Select the **Maximize** checkbox to hide the sample plate panel and view the sample sheet in full screen.

5 [Optional] Select **Add Blank Row** to add rows and manually enter sample information.

6 [Optional] To remove rows, select at least one field, and then select **Remove Selected Rows**.

7 For each sample, enter a sample name, reference, project, and description, if applicable.

8 Select **Finish**, and then save the sample sheet file (*.csv) in the desired location.

9 Select **Yes** to review the sample sheet in Excel, or **No** to exit the sample sheet wizard.

HiSeq Workflow-Specific Settings

When selecting samples for a HiSeq sample sheet, the settings available vary depending on your workflow, determined by the HiSeq system you selected. Use the following settings to specify additional run parameters available, depending on your workflow.

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Setting	Applications	Description
Custom Primer for Read 1	HiSeq FASTQ Only	Use a custom primer for Read 1.
Custom Primer for Index	HiSeq FASTQ Only	Use a custom primer for Index 1 and Index 2.
Custom Primer for Read 2	HiSeq FASTQ Only	Use a custom primer for Read 2.
Use Adapter Trimming	HiSeq FASTQ Only	The software masks the adapter sequence, which improves the accuracy and speed of analysis. This setting is recommended for Nextera and Nextera XT libraries. The default adapter sequence is the adapter present in all Nextera libraries. If a different adapter sequence is used, edit the sequence that is displayed in the final sample sheet.
Use Adapter Trimming Read 2	HiSeq FASTQ Only	Set the AdapterRead2 setting in the sample sheet to trim a different adapter sequence in Read 2.
Reverse Complement	HiSeq FASTQ Only	Convert Nextera Mate Pair libraries from a mate pair to a paired-end orientation as required by BWA and Velvet.

Applications and Kits

A HiSeq sample sheet must specify one of the following analysis applications. The associated analysis workflow is written to the sample sheet.

Application	Analysis Workflow	Application Description
HiSeq FASTQ Only	GenerateFASTQ	Generates demultiplexed FASTQ files from any type of library.

For compatible library prep kits, see the Illumina Experiment Manager support page on the Illumina website.

Revision History

Document	Date	Description of Change
Document # 15031335 v08	December 2018	• Updated software descriptions to IEM v1.16. • Added IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A as an option in sample plate creation. • Added IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A to sample plate file extensions section. • Experiment Name is now required when creating a MiSeq sample sheet.
Document # 15031335 v07	January 2018	• Updated software descriptions to IEM v1.15. • Added section to cover sample sheet creation for AmpliSeq Library PLUS for Illumina. • Revised step-by-step instructions to improve clarity.

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Document	Date	Description of Change
Document # 15031335 v06	September 2017	• Renamed guide to *Illumina Experiment Manager Software Guide*. • Renamed, reorganized, and divided some procedures to improve continuity. • Revised step-by-step instructions to be more succinct. • Separate selection of a Library Kit workflow and Index Adapters in the procedure to create a sample sheet. • Removed information on the NeoPrep system, CASAVA software, and HiSeq Enrichment. • Added directed flow cell loading option for NovaSeq instrument sample sheet creations (NovaSeq Xp workflow) • Separated out procedures to create a sample sheet and select samples for that sheet. • Incorporated workflow-specific information from QRCs into the user guide. • Added sections to cover sample sheet creation for TruSeq Amplicon, TruSight HLA, TruSight Enrichment, TruSight Tumor 15, and Nextera.
Document # 15031335 v05	February 2017	• Added NovaSeq as an option for instruments supported and removed HiScanSQ and Genome Analyzer as supported instruments in IEM. • Added a *NovaSeq-Compatible Sample Sheets* section.
Document # 15031335 v04	October 2016	• Added TruSeq Bovine in Targeted Resequencing description. • Added note that CASAVA software has been retired but is still supported in this release of IEM.
Document # 15031335 v03	January 2016	• Added TruSight Myeloid in TruSeq Amplicon application description. • Specified in the Targeted RNA application description that it does not support TruSight RNA Pan-Cancer libraries. • Added TruSight RNA Pan-Cancer in RNA-Seq application description. • Removed supported library prep kits for each application. Specified that supported library prep kits are listed in the IEM support page. • Moved Revision History to the back of the guide.
Document # 15031335 v02	October 2015	Updated software descriptions to IEM v1.11. Added: • Added TruSight Tumor 15 as a library prep kit option for MiSeq
Document # 15031335 v01	September 2015	Updated software descriptions to IEM v1.10. Added: • Changed references of sample prep to library prep to reflect changes to software user interface • Updated name of TruSight Tumor library prep kit to TruSight Tumor 26 • The Amplicon DS application within the MiSeq sample sheet wizard screen was renamed to Amplicon DS TruSight Tumor 26 • Function to edit HiSeq sample sheets in IEM has been disabled • Enabled dual-index, single read sample sheet configurations on the HiSeq 3000/4000
Part # 15031335 J	March 2015	Updated software descriptions to IEM v1.9. Added: • NeoPrep Library Prep System sample sheet • TruSight HLA application for MiSeq • HiSeq 3000 and 4000 sequencing system Removed unnecessary checkboxes for NextSeq custom primers Nextera XT v2 sample sheets can be created for 384 samples in a single sheet
Part # 15031335 H	June 2014	Updated software descriptions to IEM v1.8. Added TruSeq Synthetic Long-Read DNA as a library prep kit option to HiSeq FASTQ Only application run settings

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Part # 15031335 G	April 2014	Updated software descriptions to IEM v1.7. The following changes were made to the software: • Added NextSeq instrument option for use with the NextSeq FASTQ application • Added Nextera XT v2 Set A, B, C, and D as library prep kit options • Removed the wording, "Recommended for cancer samples" that appears next to the Use Somatic Variant Caller checkbox • Added the option to save and edit CASAVA sample sheets for the HiSeq to use with CASAVA or bcl2fastq v1.8.4 software. This functionality was removed in v1.5 and v1.6 • Underscores can now be used in the text you enter in sample sheets • Added an export to gVCF option in the workflow-specific settings for PCR Amplicon, TruSeq Amplicon, and Enrichment • Added a genus-level classification option to Metagenomics workflow-specific settings • Added the TruSight Enrichment library prep kit to HiSeq Enrichment application run settings • Cycle counts are no longer reset to the default of 2 x 150 when you edit an existing sample sheet. • IEM now supports samples with different reference genomes within the same sample sheet • IEM no longer checks the base pair diversity in the indexes specified for MiSeq Targeted Resequencing in the TruSeq Amplicon workflow. MiSeq RTA v1.17.28 and higher processes low-plexity index reads for the TruSeq Amplicon workflow, which makes base pair diversity checks in IEM unnecessary. • Index 517 is now an option for the Nextera, Nextera XT, and Nextera Rapid Capture Enrichment library prep kit. The following changes were made to the user guide: • Added sample sheet run settings parameters for use with CASAVA. • Added supported Library Prep Kit information for CASAVA. • Added a description of the Export to gVCF option in workflow-specific settings for the MiSeq • Added a description of the Genus-level Classification option to workflow-specific settings for the MiSeq • Added TruSight Enrichment to the list of library prep kits supported for the HiSeq Enrichment application • Added information on downloading target and probe manifest files for Nextera Rapid Capture Custom Enrichment

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Document	Date	Description of Change
Part # 15031335 F	May 2013	Updated software descriptions to IEM v1.6. The following changes were made to the software: • Added the Amplicon DS workflow option to the MiSeq Targeted Resequencing category • When creating a sample sheet, IEM automatically checks that all samples with the same manifest also have the same reference genome. Otherwise, the software displays an error message if not • Improved the error messages provided when attempting to edit an invalid sample sheet • The Genome Folder and Manifest columns for Applications that require the fields to be populated were moved to the left of the Sample Project and Description columns for increased usability • IEM now generates HiSeq sample sheets that are in the correct format for use with the HiSeq Analysis Software analysis package. These sample sheets are fully compatible with HCS 2.0 • Corrected the indexes available for use with the Nextera Mate Pair kit • For some analysis options under the MiSeq and HiSeq Enrichment categories, additional kits were incorrectly listed. These kits could not actually be analyzed with the currently available downstream MiSeq and HiSeq analysis software and these kit names were removed • For the MiSeq TruSeq Custom Amplicon analysis, the workflow value listed in the resulting sample sheet is now set to Custom Amplicon. Previously it was Amplicon The following changes were made to the user guide: • Added the Amplicon DS workflow description to the *MiSeq Applications and Sample Prep Kits* section • Added the Amplicon DS workflow as an option in the *Targeted Resequencing* section

Document	Date	Description of Change
Part # 15031335 E	March 2013	Updated software descriptions to IEM v1.5. The following changes were made to the software: • Creating sample sheets for use with CASAVA or AVC is no longer supported • Manifests are no longer created as part of Create Sample Plate • Illumina must provide manifests or you can create them using the Create Sample Sheet function, depending on the workflow • Removed Project Name from the Sample Sheet Workflow Parameters • Added Nextera Mate Pair, Nextera Rapid Capture, Targeted RNA Expression, and TruSight Enrichment to library prep kit types • Removed TruSeq Amplicon - Cancer Panel from the TruSeq Amplicon application for the MiSeq sample sheet • Added Nextera Rapid Capture and TruSight Enrichment to the Enrichment and FASTQ Only applications for the MiSeq sample sheet • Added Nextera Mate Pair to the Resequencing, Assembly, Library QC, and FASTQ Only applications for the MiSeq sample sheet • Added TruSeq Amplicon to the FASTQ Only application for the MiSeq sample sheet • Added a Targeted RNA application to the RNA Sequencing Category for the MiSeq sample sheet and added Targeted RNA Expression to the application • Added Indel Realignment GATK, Run Picard Hs Metrics, Reverse Compliment, and K-mer size workflow-specific settings • FASTA reference files are no longer required for the PCR Amplicon and Enrichment applications for MiSeq sample sheets • Added the HiSeq 2500 label to the Create Sample Sheet function • Renamed the Workflow Selection screen to Application Selection • Added applications to the Create Sample Sheet function for HiSeq, HiScanSQ, and Genome Analyzer™ • Removed Sample Sheet Name, flow cell ID, Operator, and Recipe fields from the HiSeq, HiScanSQ, and Genome Analyzer sample sheet run settings parameters. • Added Reagent Cartridge Barcode, Experiment Name, Investigator Name, Description, Date, Read Type, and Cycles Read as well as workflow-specific settings to the HiSeq, HiScanSQ, and Genome Analyzer sample sheet run settings parameters. • TruSeq Small RNA Library Preparation, TruSeq Custom Amplicon, and TruSeq Amplicon - Cancer Panel Library Preparation sample sheets can now be created for HiSeq, HiScanSQ, and Genome Analyzer.
Part # 15031335 E (continued)	March 2013	The following changes were made to the user guide: • Added instructions for downloading manifests in Getting Started • Moved the MiSeq Applications and Library Prep Kits section into the Creating a Sample Sheet section • Added a HiSeq, HiScanSQ, and Genome Analyzer Applications and Library Prep Kits section into the Creating a Sample Sheet section

Document	Date	Description of Change
Part # 15031335 D	January 2013	Updated software descriptions to IEM v1.4. The following changes were made to the software: • Added Variant Filter Quality cutoff parameter to Resequencing, PCR Amplicon, Custom Amplicon, and Enrichment applications • Added Flag PCR Duplicates option to Resequencing, Library QC, PCR Amplicon, and Enrichment workflows • Added Use Somatic Variant Caller option to Resequencing, PCR Amplicon, and Enrichment workflows The following changes were made to the user guide: • Added Resequencing, PCR Amplicon, and Enrichment workflows to the Use Somatic Variant Caller description as options. • Added Flag PCR Duplicates description in the *Creating a MiSeq-Compatible Sample Sheet* section. Added the following new sections: • Creating a Custom Library Prep Kit Type • Creating a Custom Library Prep Kit
Part # 15031335 C	September 2012	Updated software descriptions to IEM v1.3. The following changes were made to the software: • Added the Nextera Enrichment to kit options • Modified TruSeq DNA v2 and TruSeq RNA v2 sample prep kit assay and workflow options to be called TruSeq LT • Enrichment application now uses GenerateFASTQ workflow • Displays the index sequence in the sample sheet sample table (read only) and outputs it to the sample sheet • Changed order of sample sheet columns • When creating more than one manifest per sample sheet, all samples no longer need to be aligned to the same reference genome. The following user interface changes were made: • Reset default installer option to be Just Me rather than All Users • Changed Application to Category and Workflow to Application in the MiSeq Workflow Selection screen • Changed name of TruSeq DNA v2 and TruSeq RNA v2 kit options to TruSeq LT • Prompt to select Assay changed to select Sample Prep Kit Type • Changed Custom Amplicon to TruSeq Amplicon in the workflow select panel • Changed Custom Enrichment to Enrichment in the workflow select panel • Enrichment now maps to the PCR Amplicon workflow • Renamed Amplicon Manifest column to Nextera Manifest in the plate editor • Print function added to Plate Samples screen • Select All, Add Blank Row, and Remove Selected Rows functions added to Sample Selection screen • Genome Folder now selected from a drop-down menu when creating a sample sheet The following changes were made to the user guide: • Removed kit-specific sections and created a quick reference card for each user interface workflow. The cards provide specific options and settings. • The guide now provides detailed general information on how to use the IEM application and definitions of the sample sheet applications.

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Document	Date	Description of Change
Part # 15031335 B	June 2012	Updated software descriptions to IEM v1.2 Added the following sample and library preparation kits to assay and workflow options: • Nextera XT Library Preparation Kits • TruSeq Amplicon - Cancer Panel Preparation Kits Added the following new procedures and sections: • Genome Repository Preferences • Workflows and Sample Prep Kits table listing all the workflows, the associated sample prep kits, and the analysis workflow written to the sample sheet. Updated the following information: • Support for the new MiSeq Reporter FASTQ Only workflow. • Option to specify the use of custom primers and associated ports.
Part # 15031335 A	December 2011	Initial release

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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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