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BaseSpace Onsite v1.1 LT System Guide

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Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

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Revision History

Document	Date	Description of Change

Document # 1000000002666 v01	January 2016	• Updated for BaseSpace Onsite v1.1:
		  • Added compatibility with the MiniSeq System
		  • Added new apps—Amplicon DS v1.2, TruSight Tumor 15 v1.0
		  • Added MiniSeq Connection section
		  • Added Prepare a MiniSeq Run section
		• Changed to chapter format and created the following chapters: Overview, Workflow, Admin Tasks, Replacement Procedures, Troubleshooting
		• Removed BAM Files, gVCF Files, and VCF Files sections.
		• Replaced FASTQ Files sections with boilerplate content.
		• Moved Fix Sample Sheet and Common Sample Sheet Fixes section to Troubleshooting chapter.

Document # 1000000002666 v00	October 2015	Initial release.

Table of Contents

Overview

Chapter 1

Introduction

The BaseSpace Onsite LT System is a genomics analysis platform that is a directly integrated end-to-end solution for the following systems:

- **MiSeq System**—With MiSeq Control Software v2.6 or later
- **MiniSeq System**

BaseSpace Onsite has the following features:

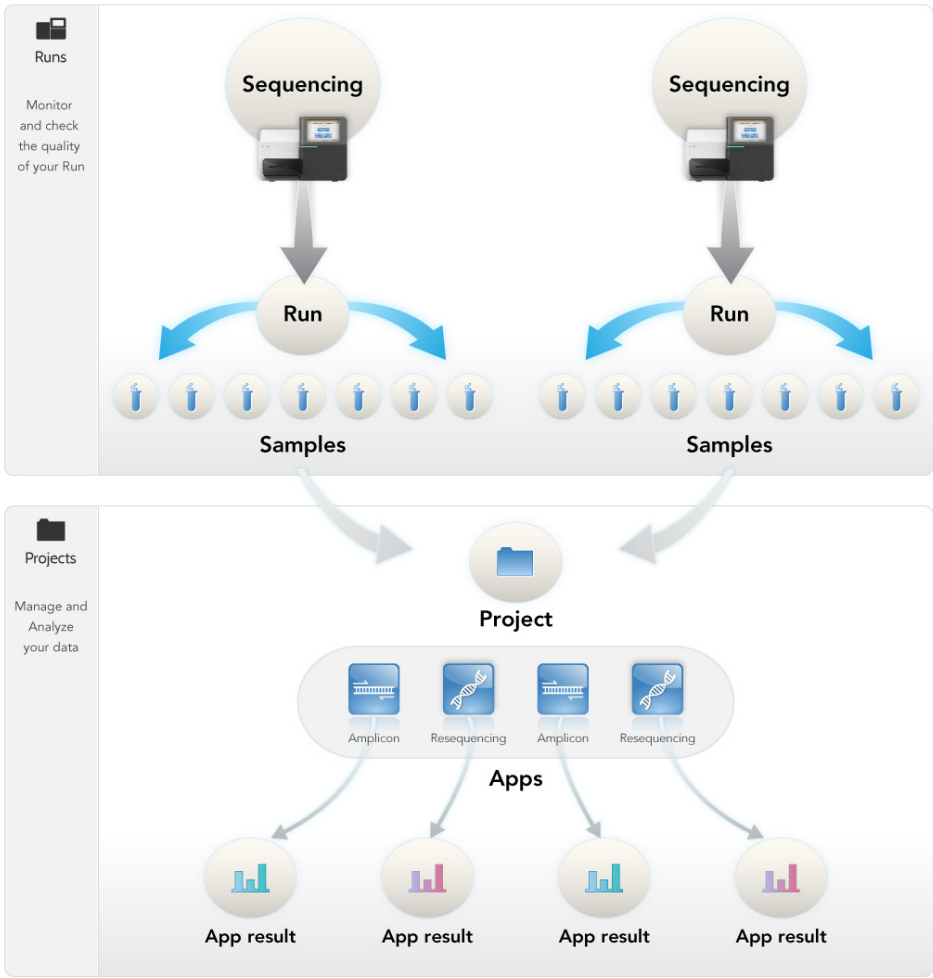
- BaseSpace Onsite runs locally; no need to connect to the cloud.
- You can prepare a MiniSeq run on the BaseSpace Onsite Prep Tab, and then start that run from your instrument.
- The instrument seamlessly transfers base call (BCL) files and associated files to BaseSpace Onsite for automatic analysis and storage.
- You can share data with others and easily scale storage and computing needs.

Workflow Model

Samples go through the following workflow model:

- A sequencing instrument produces a run, which contains log files, instrument health data, run metrics, a sample sheet, and base call information (BCL files).
- The base call information is demultiplexed in BaseSpace Onsite to create samples for analysis.
- BaseSpace Onsite apps analyze the samples.
- The result files from an app session are stored in an analysis. App results generally contain BAM and VCF files for each samples, but can also contain other file types. App results can be also be used as inputs for other apps.
- Samples and analyses are stored in projects.

Figure 1 BaseSpace Onsite Data Model



BaseSpace Onsite Apps

BaseSpace Onsite supports the following apps.

App	Description
Illumina Core Apps	The following Illumina Core Apps are available: • Isaac Whole-Genome Sequencing v4 • Isaac Enrichment v1.0 and v2.1 • BWA Whole-Genome Sequencing v1 • BWA Enrichment v1.0 and v2.1 • TopHat Alignment v1 (multilaunch feature not available) • Cufflinks Assembly and Differential Expression v1 • TruSeq Amplicon v1.1 • Amplicon DS v1.1 and v1.2 • 16S Metagenomics v1.0.1 • MethylSeq v1.0 • Small RNA v1.0 • TruSeq Targed RNA v1.0 • TruSight Tumor 15 v1.0 For documentation, see the Illumina support page for BaseSpace.
VariantStudio	Use VariantStudio to view annotation information for variants and transcripts and to filter to the variants of interest. For more information, see *Run the VariantStudio App* on page 32 and the *BaseSpace VariantStudio Software User Guide (document # 15047059)*.
Integrative Genomics Viewer	The Integrative Genomics Viewer (IGV) of the Broad Institute is a fully featured genome browser that allows you to visualize your sequence data in more detail. For more information, see *Launch the IGV App* on page 31.
BaseSpace Labs Apps	The following apps are developed using an accelerated development process. Illumina provides limited support for BaseSpace Labs Apps and are provided without a warranty. • FastQC v1.0 • FASTQ Toolkit v1.0 • Kraken Metagenomics v1.0 • Variant Calling Assessment Tool (VCAT) v.2.0
Third-Party Apps	Currently, BaseSpace Onsite provides SPAdes Genome Assembler v3.0 and v3.5. For more information, see the SPAdes App page on the Apps tab.

App	Description
BaseMount	BaseMount is a tool that allows command line read access to your BaseSpace Onsite data. The BaseMount app is a standalone installable application that you can install on your own computer. Then you can connect BaseMount to the data on your BaseSpace Onsite server or servers. For more information, see the following links: • BaseMount Web Page • BaseMount Help • BaseSpace Blog **Note**—BaseSpace is the default API endpoint for BaseMount. Make sure to launch BaseMount using the correct server configuration option for BaseSpace Onsite: `basemount --config {config_file_prefix} --api-server=http://{BSO_windows_IP}:8080 {mount-point_folder_path}` • The `--config` parameter is optional. Use this parameter to mount against multiple user accounts. • The `BSO_windows_IP` is the IP address that you enter in your browser to access your BaseSpace Onsite account. The API url is at port 8080. • The `mount-point_folder_path` is the path to a user-created directory on the system where BaseMount is running.

Annotation Options

Depending on the application used, the following annotation options are available.

Option	Description
Illumina Annotation Service RefSeq or Ensembl	This option utilizes the Illumina Annotation Service (hosted at annotation.basespace.illumina.com) to provide annotation of variants (SNPs, insertions, and deletions) in the sample VCF files. This service requires an external network connection and also requires you to whitelist the IP address of your BaseSpace Onsite system.
Basic Annotation with local genome files from the UCSC hg19 genome	This option utilizes a basic set of annotations, including SNPs and indels, using the genome files hosted locally on the BaseSpace Onsite system. This option does ***not*** require an external network connection.
On-node Analysis RefSeq or Ensembl	This option is a localized version of the Illumina Annotation Service provided with the BaseSpace Onsite v2.1 software update. It provides annotations for known variants as provided by Illumina Annotation Service, and can also predict novel variants. This option does ***not*** require an external network connection.
None	No annotation is performed with this option. The reports generated for analyses using this option shows either a 0 or N/A for the various annotations.

The following apps provide either the Illumina Annotation Service or None options:

- Amplicon DS v1.0 and v1.2
- BWA Enrichment v2.1
- Isaac Enrichment v2.1
- TruSeq Amplicon v1.1

The following apps provide the Illumina Annotation Service, Basic, or None options:

- BWA Enrichment v1.0
- BWA Whole Genome Sequencing v1.0
- Isaac Enrichment v1.0
- Isaac Whole Genome Sequencing v2

The following apps provide the On-node Analysis option:

- Isaac Whole Genome Sequencing v4.0
- TruSight Tumor 15 v1.0—Performs annotation internally, but does not have annotation options in the app input form

All other applications on BaseSpace Onsite do not provide annotation options. To perform annotation on the results of these analyses, you can use the VariantStudio application, which also performs annotation using the Illumina Annotation Service.

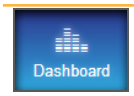
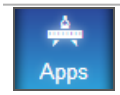
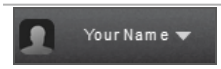
Supported Browsers and Operating Systems

BaseSpace Onsite v1.1 runs on Windows 7 or later and is compatible with the following browsers:

- Internet Explorer 10 or later
- Google Chrome 43 or later
- Mozilla FireFox 38 or later
- Safari 8 or higher

BaseSpace Onsite User Interface

Toolbar

Icon	Name	Description
	Dashboard Tab	See *Dashboard Tab* on page 9.
	Prep Tab	See *Prep Tab* on page 10.
	Runs Tab	See *Runs Tab* on page 10.
	Projects Tab	See *Projects Tab* on page 11.
	Apps Tab	See *Apps Tab* on page 14.
	Support Page	Provides access to the BaseSpace Onsite Knowledge Base, User Guide, and Illumina Technical Support.
	Search	Alows you to find runs, projects, samples, files, or apps. For more information, see *Search* on page 41.
	Account	Provides access to: • MyAccount—See *MyAccount* on page 8 • Trash—See *Delete Runs, Projects, Analyses, or Samples* on page 39 • Terms—Leads to the User Agreement • Admin Panel—Only available if you have admin privileges; see *Admin Panel* on page 9 • Blog—Leads to the blog, which includes latest news, developments, and updates • Sign out

MyAccount

Page	Description
Settings	Edit your notifications settings, profile, and profile picture.
Transfer History	Review transferred projects and runs. For more information, see *Transfer Owner* on page 38.
Library Prep Kits	Shows all available library prep kits and allows you to create custom kits. To enter a custom kit, click **New**. You can define nonstandard index combinations and your own default layout for the indexes on a plate.
Storage	Shows the total amount of storage used (owned by you and shared) and the amount of storage used in Runs, Projects, and Trash.

Admin Panel

The Admin Panel allows you to manage analysis, notifications, storage, users, system health, planned runs, software updates, and alarms. See *Admin Tasks* on page 1 for a description.

NOTE
You need administrator privileges to see and work in the admin panel.

Dashboard Tab

Pane	Description
Storage	This pane shows the total amount of storage used (used by you and shared). Click the **Details** button to open the Storage page under My Account.
Developers	Click the **Details** button on the Developers pane to go to the BaseSpace developer portal. Access to the BaseSpace developer portal requires an external internet connection outside of your local network.
Newsfeed	This pane shows the most recent posts from the BaseSpace blog. Click an article name to open the article, or click the **Read more** link at the bottom of the pane to open the BaseSpace blog. The Newsfeed pane requires an external internet connection outside of your local network to show content. Access to the BaseSpace blog also requires an external internet connection.
Notifications	This pane shows notifications and alerts about runs, collaborators, analyses, and projects. The most recent notifications are listed first. **Note:** If you did not configure SMTP during installation, all notifications show up on the dashboard. No notifications are sent through email.
Latest Runs	This pane shows the 3 most recent runs with their dates and status, and is updated automatically. Click **All Runs** to open the Runs tab. Clicking a run opens the Runs tab with the run loaded. For more information, see *Runs Tab* on page 10.
Latest Analyses	This pane shows the 3 most recent analysis with their dates and status, and is updated automatically. Click **All Analyses** to open the Analyses page, which lists all analysis results, along with the application used, project name, date updated, size of the result, and status. Clicking an analysis on the dashboard pane opens the specific analysis report. For more information, see *Analyses Page* on page 13.

NOTE
When you bookmark the BaseSpace Onsite location in your browser, make sure to bookmark the Dashboard page after logging on. Do not bookmark the Login page.

Prep Tab

The Prep tab enables you to set up a run on the MiniSeq system. Other sequencing instruments use a sample sheet to provide sample information to BaseSpace Onsite.

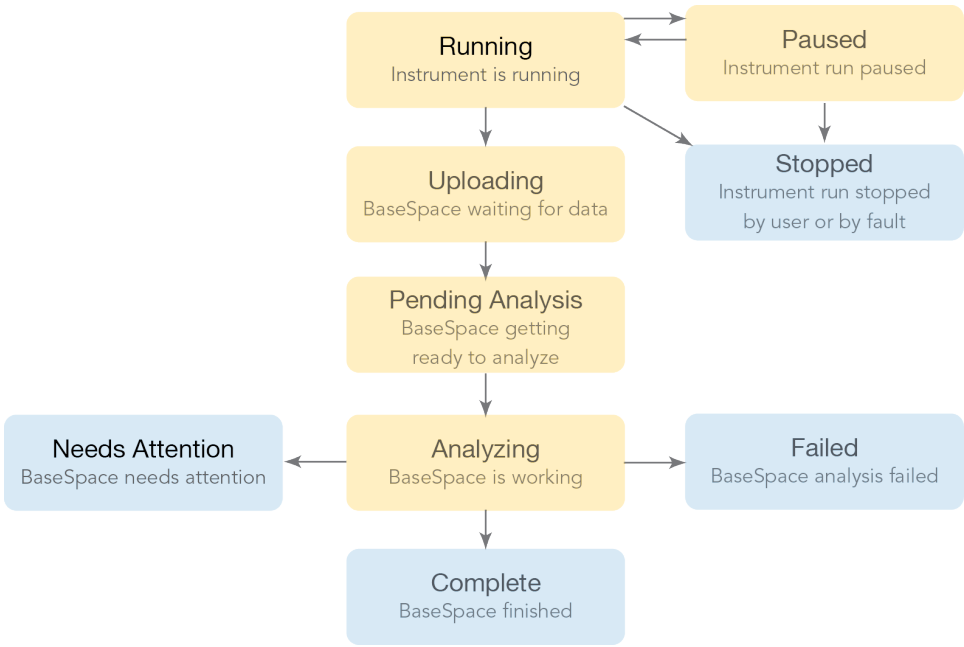
Fore more information, see *Prepare a MiniSeq Run* on page 18.

NOTE
BaseSpace Onsite LT only supports the MiniSeq System on the Prep tab. Do not attempt to set up NeoPrep or NextSeq runs.

Runs Tab

The Runs button leads to the runs list, which allows you to sort your runs based on experiment name, state, workflow, created date, machine, and owner.

The following run states are possible. The blue boxes indicate final states.



If you want to look at a run in detail, click the name to view metrics in more detail. For more information, see *Run Overview Page* on page 11.

To manage the projects, use the buttons above the projects list.

Button	Description
Share	Manage sharing a run with a particular collaborator. See *Share a Project or Run Using the Email Option* on page 36 on page 1.
Get Link	Forward the sharing link to any number of collaborators. See *Share a Project or Run using the Get Link option* on page 36.
Download Run	Download files from this run. See *Download Run File Package* on page 33.

Button	Description
Transfer Owner	Hand control of data over to a collaborator or customer. This button is visible if a run is selected. See *Transfer Owner* on page 38.
Move to Trash	Delete a project. This button is visible if a run is selected. See *Delete Project* on page 1.
View Trash	View the deleted items in the trash, so you can restore them or empty the trash. See *Delete Runs, Projects, Analyses, or Samples* on page 39.

Run Overview Page

The following panes are shown on the Run Overview page.

Pane	Description
Run Details	Provides a summary of the run with links to view files and download and share options. For more information, see *Share Data* on page 36, *View Files and Results* on page 24, or *Download Files* on page 33.
Samples	Provides a list of all the app results in the run, the associated projects, and the number of samples in that analysis.
Charts	Shows an intensity by cycle chart. Clicking the header takes you to the Charts page, which contains 5 charts with run metrics.
Run Summary	Shows tables with basic data quality metrics. Clicking the header takes you to the Run Summary page.
Indexing QC	Lists count information for indexes used in the run. Clicking the header takes you to the Indexing QC page.
Side Navigation ribbon	Provides navigation of the Run Details pane.

Run Samples List

The samples list allows you to sort the samples in your run based on sample ID, app, date created, and project. For more information, see the following sections:

- *Sample Overview Page* on page 13.
- *Analyses Page* on page 13.
- *Project Overview Page* on page 12.

In addition, the Side Navigation ribbon provides easy navigation in the Run Details area.

Projects Tab

The Projects button opens a list of your projects. You can sort the list by name, last update, or owner. Clicking a project provides access to the app results and samples within that project.

To manage the projects, use the buttons above the projects list.

Button	Description
New Project	Generate a new project. See *Set Up a New Project* on page 37.
Edit Project	Edit the name and description of the project. See *Edit Project Details* on page 37.
Share Project	Share a project with a particular collaborator. See *Share a Project or Run Using the Email Option* on page 36.
Get Link	Forward the sharing link to any number of collaborators. See *Share a Project or Run using the Get Link option* on page 36.
Transfer Owner	Hand control of data over to a collaborator or customer. See *Transfer Owner* on page 38.
Move to Trash	Delete a project. This button is visible if a project is selected. See *Delete Project* on page 1.
View Trash	View the deleted items in the trash, so you can restore them or empty the trash. See *Delete Runs, Projects, Analyses, or Samples* on page 39.

Project Overview Page

The following panes are shown on the Project Overview page. You can also access these panes using the left navigation bar.

Pane	Description
About	Provides summary information about the project, including the project owner, shared status, date created, and collaborators.
Analyses	Provides a list of all the App Sessions in the project. This tab can be sorted based on analysis name, last modified date created, status, or application used to generate the analysis. Clicking the analysis links to the app results for that sample. For more information, see *Analyses Page* on page 13.
Samples	Provides a list of all the samples in the project. Clicking a sample links to the page for that sample. For more information, see *Sample Overview Page* on page 13. Selecting the samples allows you to launch it in an app, copy to a different project, or combine with another result.

Project Toolbar

The project toolbar allows you to perform the following actions.

Button	Description
Launch App	Run apps on your sample. Clicking the app name leads to a page with more information about launching that app, including access permissions. See *Analyze Samples Further* on page 1.
Download Project	Download all files in a project. See *Download Project or Analysis Package* on page 1.

Button	Description
Import	Upload files to a project. See *Upload Files to Projects* on page 38.
Share Project	Manage sharing a project with a particular collaborator. See *Share a Project or Run Using the Email Option* on page 36.
Get Link	Forward the sharing link to any number of collaborators. See *Share a Project or Run using the Get Link option* on page 36.
Edit Project	Edit the name and description of the project. See *Edit Project Details* on page 37.
Transfer Owner	Hand control of data over to a collaborator or customer. See *Transfer Owner* on page 38.
Move to Trash	Hand control of data over to a collaborator or customer. See *Transfer Owner* on page 38.
View trash	View all deleted runs, projects, analyses, and samples. See *Delete Runs, Projects, Analyses, or Samples* on page 39

Options that are not available for the particular analysis or sample are grayed out.

If you have selected samples in the **Samples** pane, you can perform additional actions.

Button	Description
Copy to...	Copy samples from this project to another. See *Copy Samples* on page 37.
Combine	Combine samples. See *Combine Samples* on page 37.

The app session states are defined as follows.

State	Description
Running	The app is processing or uploading data.
Complete	Processing and file upload has finished and the data are now available to use.
Aborted	This AppResult or Sample has been aborted and cannot be resumed.
Needs Attention	Cannot continue without user intervention.

Analyses Page

The Analyses page provides access to the results for that app session. There is a general information pane to the left, and several graphs, depending on the app run.

Sample Overview Page

The following panes are shown on the Sample Overview page.

Pane	Description
Sample Details	Provides a summary of the run with a links to launch a custom BaseSpace Onsite app on your sample. Clicking the app name leads to a page with more information about that app, including access permissions.

Pane	Description
Files	Provides a list of files associated with that sample. You can either look at all FASTQ files, or look at files specific for an app session.

For more information, see *View Files and Results* on page 24. You can also download selected files; see *Download Multiple FASTQ Files* on page 33.

Apps Tab

The Apps page provides an overview of the custom BaseSpace Onsite apps that you can run.

- Clicking the app name leads to a page with more information about that app, including the version, a link to the developer, and their app support contact details.
- Clicking the Launch button allows you to set up the app session. Specify parameters, such as the project, sample, or output folder used by the app, depending on the app, and accept access permissions.
- You can search for apps using Search.

Connect BaseSpace Onsite

This section includes procedures for connecting BaseSpace Onsite to your sequencing instrument.

The MiSeq System and the MiniSeq System feature an option to send instrument health to BaseSpace and sequencing data to BaseSpace Onsite in real time to streamline instrument quality control and analysis. Real-time monitoring of runs enables fast troubleshooting.

For more information, see the system guide for your instrument.

NOTE

When connecting your instrument to BaseSpace Onsite, you are asked to enter the full path to your BaseSpace Onsite server. Your BaseSpace Onsite IP address can be found in the broker configuration file of the sequencing system that you want to connect BaseSpace Onsite to. The BaseSpace Onsite IP address is included as part of the API URL value (http://{BaseSpace_Onsite_IP}:8080).

Connect the MiSeq System

NOTE

Unlike BaseSpace, BaseSpace Onsite does not have a MiSeq Reporter app. BaseSpace Onsite directs all workflow types uploaded from MiSeq through the GenerateFASTQ app. To perform additional secondary analysis, manually launch the relevant app or apps on the resulting FASTQ samples.

1. Make sure that you have a stable network connection of at least 10 Mbps upload speed from the MiSeq.
2. During run configuration, enter the full path to your BaseSpace Onsite server.
3. Select or clear **When using BaseSpace or BaseSpace Onsite, replicate analysis locally on MiSeq**.

   The Replicate Analysis Locally setting specifies analysis processing locations when using BaseSpace Onsite. The setting provides the option to perform analysis both locally on the instrument and in BaseSpace Onsite. Consider the following items when selecting or deselecting this option:

   - If you select this option, MiSeq Reporter launches automatically after the run and performs analysis locally.
   - If you do not select this option, MiSeq Reporter does not launch automatically after the run and analysis is performed in BaseSpace Onsite only.
   - If performing the VeriSeq PGS workflow with BlueFuse Multi, select this option.
4. Click **Save and Return**.
5. When starting a sequencing run, select **Use BaseSpace Onsite for storage and analysis** on the BaseSpace Options screen.

NOTE

To use BaseSpace Onsite, load a sample sheet at the start of your run.

BaseSpace Onsite automatically disconnects from the MiSeq system at the end of the run or when all RTA analysis files have finished uploading. If the network connection is interrupted, analysis files continue uploading after the connection is restored from the point when the interruption occurred.

Connect the MiniSeq System

1. Make sure that you have a stable connection of at least 10 Mbps upload speed from the MiniSeq.
2. From the home screen, click **Manage Instrument**.
3. Click **System Configuration**.
4. Click **Analysis Configuration**.
5. Select the **BaseSpace Onsite** tab.
6. In the **Server Name** field, enter the full path to your BaseSpace Onsite server.
7. [Optional] Select **Browse** and navigate to a secondary network **Output Folder** location to save a copy of data in addition to the BaseSpace Onsite server.
8. [Optional] Select **Save the credentials as the default** and enter your user name and password.
9. [Optional] Select **Bypass Analysis Method login screen** to bypass the BaseSpace Onsite login screen.
10. [Optional] Select **Send instrument health information to Illumina to help Illumina improve its products**.
    This option sends instrument health information to BaseSpace and requires an internet connection.
11. Click **Save**.
12. Log in to BaseSpace Onsite when setting up the run on the MiniSeq system.

Workflow

BaseSpace Onsite Prep Tab

Prepare a MiniSeq Run

You can prepare MiniSeq runs through the BaseSpace Onsite Prep tab, which organizes samples, libraries, pools, and run in a single environment. This option allows MiniSeq sequencing data to stream seamlessly to BaseSpace Onsite.

Do not use the Prep Tab to prepare sequencing runs for other sequencing instruments. If you do not want to use BaseSpace Onsite to set up a run, you can also start a run on the MiniSeq system.

1 Log in to BaseSpace Onsite. If it is your first time logging in, accept the user agreement.

2 Click the **Prep** icon.

3 Set up a MiniSeq run on the Prep Tab in 4 consecutive steps.

   a Biological Samples
   b Libraries
   c Pools
   d Planned Runs

Biological Samples

You can create new samples, import samples, or use existing samples.

Create New Biological Samples

1 Under Manual Prep, click the **Biological Samples** icon.

2 Click **+ Create**.

3 Fill out the required fields Sample ID, Name, and Nucleic Acid type.

NOTE
The sample ID and sample name can only contain alphanumeric characters, dashes, or underscores. The sample ID must be unique and short. The sample name can be more descriptive to provide a human-readable identifier.

4 [Optional] Fill out the Organism (species) field.

5 [Optional] Fill out the Project fields. You can also generate a new project. A project is optional here but is required later because the output data gets stored to the project.

6 If you only want to select the newly created sample, click **Next: Prep Libraries**.

7 If you want to select multiple samples, click **Save & Continue Later**. This selection takes you back to the Biological Samples list, with the recently created sample at the top of the list.

Import Biological Samples

1 Under Manual Prep, click the **Biological Samples** icon.

2 Click **Import**.

3 If you have not generated an import file, click the template link and fill out the samples. Note the following items when filling out the template:
   - The sample ID and sample name can only contain alphanumeric characters, dashes, or underscores. The sample ID must be unique and short. The sample name can be more descriptive to provide a human-readable identifier.
   - The Organism (species) field is optional.
   - The Project field is optional, but a project is required later because the output are stored to the project.
   - Fill out the Nucleic Acid column with DNA or RNA.

Figure 2 Import Sample Template

	A	B	C	D	E	F
1	[Header]					
2	FileVersic	1				
3						
4	[Data]					
5	SampleID	Name	Species	Project	NucleicAcid	
6	TestSamp	SampleA	PhiX	Project_A	DNA	
7	RealSamp	SampleB	PhiX	Project_B	RNA	

4 Click the **Choose File** button.

5 Browse to the import file, and then click **Open**.

6 Click **Import**.

7 If you only want to select the newly created samples, click **Next: Prep Libraries**.

8 If you want to select multiple samples, click **Save & Continue Later**. This selection takes you back to the Biological Samples list, with the recently created sample at the top of the list.

Use Existing Biological Samples

The Biological Samples list shows all available samples that you have created on your account.

1 To select samples, use the following methods:
   - Select checkboxes.
   - Click anywhere on a sample row while holding **Ctrl** to add to a selection.
   - Click anywhere on a sample row while holding **Shift** to select all samples in between.
   - Click the checkbox next to SampleID to select all samples on the current page.

   The box next to the Biological Samples header tracks the total number of samples, and how many are selected. Click **X** to clear the current selection.

2 Click the **Prep Libraries** button in the top navigation bar.

Prep Libraries

On the Prep Libraries page, assign indexes to biological samples based on the indexes available in the chosen library prep. Every used well or tube contains a separate library. The best practice is to first set up the libraries in BaseSpace Onsite. Then export a file of your library settings, and use that to pipette the biological samples into the proper wells or tubes.

To import libraries and associate them to new biological samples at the same time, see *Import Samples and Libraries* on page 21.

NOTE
If you do not want to use indexed sequencing, you still assign your biological sample to an index. When you set up your sequencing run, you can specify that you do not want to sequence the index.

1 Select the library prep type.
BaseSpace Onsite automatically assigns indexes to wells or tubes.
To create a custom library prep kit, see *Set Up Custom Library Prep Kit* on page 20.

2 Enter the plate ID.
The ID must be unique.

3 Click the **Auto Prep** button to fill the plate or tubes automatically with all samples listed.
Alternatively, manually drag the samples to the wells or tubes.

   a Select 1 or more samples. Hold the Shift key to select multiple samples. To select multiple samples on Firefox or Internet Explorer, click the well twice.
   b Drag the selected samples to a position.

4 Click **Download CSV** to save a file of your library settings.

5 If you want to select the new plate or tubes, click **Pool Libraries**.

6 If you want to select multiple library preps or plates, perform the following steps.

   a Click **Save & Continue Later**. This selection takes you to the Libraries list, with the recently created set-up at the top of the list.
   b Select the checkboxes in the Libraries list.
   c Click the **Pool Libraries** button in the top navigation bar.

NOTE
If any samples are not assigned to a project, you cannot continue. Select the sample, click the **Set Project** button, and assign it to a project. You can also generate a new project.

Set Up Custom Library Prep Kit

1 When prepping a library, select **+ Custom Library Prep Kit** in the Library Prep Kit dropdown menu.
The Custom Library Prep Kit Definition page opens.

2 Enter a name for the custom prep that meets the following requirements:
- Unique for your account
- Contains only alphanumeric, hyphen, underscores, and spaces
- Contains ≤ 50 characters

3 Select at least 1 of the supported read types.

4 Select at least 1 of the indexing strategies. You cannot only select **None**.

5 Fill out the default number of cycles.

6 Click **template** to download the index definition file template.

7 Fill out the **Settings** section:
- For single read only—No adapter (blank), or 1 adapter sequence for Read 1.
- For paired-end—No adapter (blank), or 2 adapter sequences, 1 for Read 1 and 1 for Read 2.
- Make sure that each adapter sequence meets the following criteria:
  - Sequence of A, T, C, or G character
  - Length from 1 to 20 characters

8 Fill out the **Index1Sequences** and **Index2Sequences** sections:
- For Single Index, with or without None:
  - 1 to 100 Index 1 names
  - For each Index 1 name an associated Index 1 sequence
- For Dual Index, with or without None and Single Index:
  - 1 to 100 Index 1 names
  - For each Index 1 name an associated Index 1 sequence
  - 1 to 100 Index 2 names
  - For each Index 2 name an associated Index 2 sequence
- Each index name meets the following criteria:
  - Unique within the file
  - Length from 1 to 8 characters alphanumeric, hyphen, or underscore characters
- Each index sequence meets the following criteria:
  - Sequence of A, T, C, or G characters
  - Length from 1 to 20 characters
  - All index sequence lengths (Read 1 and Read 2) are equal
  - Index 1 sequences are unique within the file set of Index 1 sequences
  - Index 2 sequences are unique within the file set of Index 2 sequences

9 If the supported indexing strategy specifies Single Index, you can set up **Default Layout By Well**:
- Each well unique from A01 to H12
- For each well, an associated index name exists in the specified Index1Sequences section

10 If the supported indexing strategy specifies Single Index or Dual Index, you can set up **Default Layout By Column**:
- Each column number unique from 1 to 12
- For each column, an associated index name exists in the specified Index1Sequences section

11 If the supported indexing strategy specifies Dual Index, you can set up **Default Layout By Row**:
- Each row letter unique from A to H
- For each row, an associated index name exists in the specified Index2Sequences section

12 Click the **Choose.csv File** button to select and upload your custom index file.

13 Click **Create New Kit**.
Your custom library prep is added to the library kit dropdown.

Import Samples and Libraries

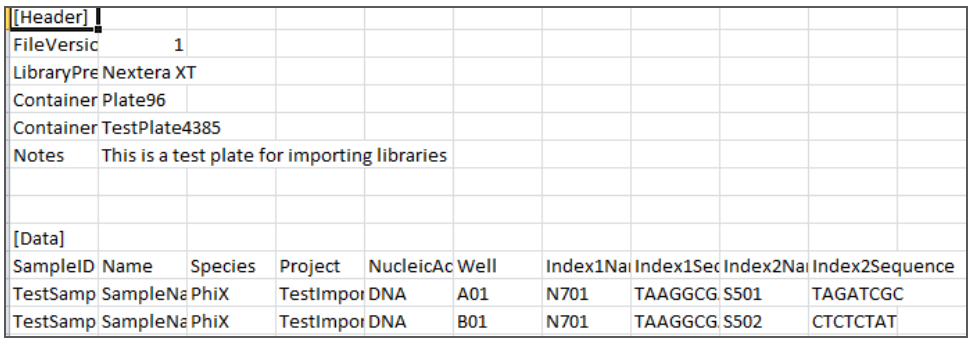
1 Click the **Prep** icon.

2 Click **Libraries**.

3 Click the **Import** button.

4 If you have not generated an import file, click the template link and fill out the samples. Note the following items when filling out the template:
- The sample ID and sample name can only contain alphanumeric characters, dashes, or underscores. The sample ID must be unique and short. The sample name can be more descriptive to provide a human-readable identifier.
- The Species field is optional.
- The Project field is optional, but a project is required later because the output data are stored to the project.
- Fill out the Nucleic Acid column with DNA or RNA.

Figure 3 Import Sample Template



[Header]									
FileVersic	1								
LibraryPre	Nextera XT								
Container	Plate96								
Container	TestPlate4385								
Notes	This is a test plate for importing libraries								
[Data]									
SampleID	Name	Species	Project	NucleicAc	Well	Index1Na	Index1Sec	Index2Na	Index2Sequence
TestSamp	SampleNa	PhiX	TestImpor	DNA	A01	N701	TAAGGCG	S501	TAGATCGC
TestSamp	SampleNa	PhiX	TestImpor	DNA	B01	N701	TAAGGCG	S502	CTCTCTAT

5 Click the **Choose File** button.

6 Browse to the import file, and then click **Open**.

7 Click **Import**.

8 If you only want to select the newly created libraries, click the **Pool Libraries** button.

9 If you want to select other libraries, click **Save & Continue Later**. This selection takes you back to the Libraries list, with the recently created sample at the top of the list.

Pool Libraries

The Pool Libraries page allows you to pool samples and sequence them in the same run, using the same analysis parameters.

1 Fill out the first pool ID. The pool ID must be unique.

2 If needed, you can create additional pools on the right by clicking **+ Add Pool** and filling out the pool IDs.
- Colors of the wells correspond to the colors of the pools.
- You can hover over the wells to see the library IDs.

3 Drag and drop individual samples from their well on the plate to a pool.
You can select multiple samples by holding **Shift**. To select multiple samples on Firefox or Internet Explorer, click the well twice.

4 If you want to pool libraries from multiple plates, specify the plate in the drop-down menu.

5 If you want to merge pools, perform the following steps.

a Click **Save & Continue Later**. This selection takes you to the Pools list, with the

recently created plate at the top of the list.

b Select the checkboxes in the Pools list.

c Click **Merge Pools** in the top navigation bar.

6 Click **Plan Run**.

Plan Run

On the Plan Run page, you can set up the parameters for the sequencing run on your MiniSeq instrument.

1 Select **MiniSeq** from the instrument drop-down.

2 Enter a name for your planned run.

3 [Optional] Enter a reagent barcode to link a reagent kit to the run.

4 [Optional] Select to use a custom primer—**R1**, **R2**, or **Index**.

5 Select **Single Read** or **Paired End**.

6 Enter the number of cycles per read.

7 Verify the Review Indexes section for the indexing strategy.
The indexing strategy is set to the previously chosen index/library prep type. To override the default indexing scheme, select the Index type—**Single Index**, **Dual Index**, or **No Index**. Make sure that you enter the number of index cycles accordingly.
If you have selected multiple libraries, you cannot specify No Index.
BaseSpace Onsite automatically checks if the indexes chosen all start with 2 Gs; if so, you are warned to change your index strategy.

8 Verify the pool that is included in the planned run.

9 When your settings are complete, choose 1 of these options to continue:

10 Click **Sequence**, which opens the Planned Runs list and sets the state of the recently planned run to *Ready to Sequence*.
Alternatively, click **Save & Continue Later**, which opens the Planned Runs list and sets the state of the recently planned run to *Planning*.

NOTE
A planned run must be in the *Ready to Sequence* state in order for it to show up in the Planned Runs list in the instrument control software.

11 To change a planned run to the *Ready to Sequence* state, select the planned run from the list. Click the **Sequence** arrow link in the top navigation bar on the Planned Runs list page.

Your run now shows up in the Planned Runs list in the MiniSeq Control Software. Complete the run from your sequencing instrument. A sample sheet is not required. BaseSpace Onsite automatically generates FASTQ files when the sequencing run is complete.

NOTE
You can connect as many instruments as you have BaseSpace Onsite nodes installed, up to a maximum of 6.

View Files and Results

View Files from a Run

BaseSpace Onsite gives you an option to view your run files or download them individually.

1 Click the **Runs** icon.

2 Click a run.

3 From the Run Overview Page, select the **Files** icon from the left navigation menu.

4 Select a file.

See *BaseSpace Onsite Files* on page 33 for a description of the available files.

View Indexing QC Page

The Indexing QC page lists count information for indexes used in the run. The Indexing QC is only available if the run is an index run.

By viewing the indexing QC results, you can see unexpected results for a sample with a particular index and use the information for troubleshooting purposes. You can also use this feature to confirm that all indexed samples were represented properly.

1 Click the **Runs** icon.

2 Click a run.

3 From the Run Overview page, click the **Indexing QC** or the Indexing QC icon icon from the left navigation menu.

You can select the displayed lane through the drop-down list.

The first table provides an overall summary of the indexing performance for that lane, including the following information.

Total Reads	The total number of reads for this lane.
PF Reads	The total number of passing filter reads for this lane.
% Reads Identified (PF)	The total fraction of passing filter reads assigned to an index.
CV	The coefficient of variation for the number of counts across all indexes.
Min	The lowest representation for any index.
Max	The highest representation for any index.

Further information is provided regarding the frequency of individual indexes in both table and graph form. The table includes the following columns.

Index Number	A unique number assigned to each index by BaseSpace Onsite for display purposes.
Sample ID	The sample ID assigned to an index in the sample sheet.

Project	The project assigned to an index in the sample sheet.
Index 1 (I7)	The sequence for the first Index Read.
Index 2 (I5)	The sequence for the second Index Read.
% Reads Identified (PF)	The number of reads (only includes Passing Filter reads) mapped to this index.

This information is also displayed in graphical form. In the graphical display, indexes are ordered according to the unique Index Number assigned by BaseSpace Onsite.

View Run Charts

The Charts page shows charts with run metrics.

1 Click the **Runs** icon.

2 Select a run.

3 From the Run Overview page, click **Charts** or the Charts icon from the left navigation menu.

Flow Cell Chart

The Flow Cell chart shows color-coded graphical quality metrics per tile for the entire flow cell.

Use the Flow Cell chart to judge local differences per cycle, per lane, or per read in sequencing metrics on a flow cell. It is also an easy way to see the %Q30 metric, which is an excellent single metric to judge a run. Do not use the Flow Cell chart to look at downstream analysis metrics.

The Flow Cell chart has the following features:

- You can select the displayed metric, surface, cycle, and base through the drop-down lists.
- The color bar to the right of the chart indicates the values that the colors represent.
- The chart is displayed with tailored scaling by default.
- Tiles that have not been measured or are not monitored are gray.

You can monitor the following quality metrics using the Flow Cell chart.

Intensity	This chart shows the intensity by color and cycle of the 90% percentile of the data for each tile.
FWHM	The average full width of clusters at half maximum (in pixels). Used to display focus quality.
% Base	The percentage of clusters for which the selected base (A, C, T, or G) has been called.
%Q > 20, %Q > 30	The percentage of bases with a quality score of > 20 or > 30, respectively. These charts are generated after the 25th cycle, and the values represent the current scored cycle.

Median Q-Score	The median Q-Score for each tile over all bases for the current cycle. These charts are generated after the 25th cycle. This plot is best used to examine the Q-scores of your run as it progresses. Bear in mind that the %Q30 plot can give an over simplified view due to its reliance on a single threshold.
Density	The density of clusters for each tile (in thousands per mm^2).
Density PF	The density of clusters passing filter for each tile (in thousands per mm^2).
Clusters	The number of clusters for each tile (in millions).
Clusters PF	The number of clusters passing filter for each tile (in millions).
Error Rate	The calculated error rate, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is not available.
% Phasing, % Prephasing.	The estimated percentage of molecules in a cluster for which sequencing falls behind (phasing) or jumps ahead (prephasing) the current cycle within a read.
% Aligned	The percentage of reads from clusters in each tile that aligned to the PhiX genome.
Perfect Reads	The percentage of reads that align perfectly, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is all gray.
Corrected Intensity	The intensity corrected for cross talk between the color channels by the matrix estimation and phasing and prephasing.
Called Intensity	The intensity for the called base.
Signal to Noise	The signal to noise ratio is calculated as mean called intensity divided by standard deviation of noncalled intensities.

Note the variable scales used on these different parameters.

Data By Cycle Plot

The Data by Cycle plot shows the progression of quality metrics during a run as a line graph.

Use the Data By Cycle plot to judge the progression of quality metrics during a run on a cycle by cycle basis. Do not use the Data By Cycle plot to look at downstream analysis metrics, or aggregate analysis for a whole lane regardless of cycle.

The Data by Cycle plots have the following features:

- You can select the displayed metric and base through the drop-down lists.
- The symbol in the top right-hand corner toggles the plot between pane view and full screen view.

You can monitor the following quality metrics with this plot.

Intensity	This chart shows the intensity by color and cycle of the 90% percentile of the data for each tile.
FWHM	The average full width of clusters at half maximum (in pixels). Used to display focus quality.
% Base	The percentage of clusters for which the selected base (A, C, T, or G) has been called.
%Q > 20, %Q > 30	The percentage of bases with a quality score of > 20 or > 30, respectively. These charts are generated after the 25th cycle, and the values represent the current scored cycle.
Median Q-Score	The median Q-Score for each tile over all bases for the current cycle. These charts are generated after the 25th cycle. This plot is best used to examine the Q-scores of your run as it progresses. Bear in mind that the %Q30 plot can give an over simplified view due to its reliance on a single threshold.
Error Rate	The calculated error rate, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is not available.
Perfect Reads	The percentage of reads that align perfectly, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is all gray.
Corrected Intensity	The intensity corrected for cross talk between the color channels by the matrix estimation and phasing and prephasing.
Called Intensity	The intensity for the called base.
Signal to Noise	The signal to noise ratio is calculated as mean called intensity divided by standard deviation of noncalled intensities.

To expand a chart, click the expand button  .

Q-score Distribution

The Q-score Distribution plot shows a bar graph that allows you to view the number of bases by quality score. The quality score is cumulative for current cycle and previous cycles, and only bases from reads that pass the quality filter are included.

Use it to judge the Q-score distribution for a run, which is an excellent indicator for run performance. Do not use the Q-score Distribution plot to look at metrics other than quality scores.

The Q-score Distribution pane shows plots that allow you to view the number of reads by quality score. The quality score is cumulative for current cycle and previous cycles, and only reads that pass the quality filter are included.

These plots have the following features:

- You can select the displayed read, and cycle through the drop-down lists.
- The symbol in the top right-hand corner toggles the plot between pane view and full screen view.

The Q-score is based on the Phred scale. The following list shows Q-scores and the corresponding chance that the base call is wrong:

- Q10—10% chance of wrong base call
- Q20—1% chance of wrong base call

- Q30—0.1% chance of wrong base call
- Q40—0.01% chance of wrong base call

You can slide the threshold (set at ≥ Q30 by default) to examine the proportion of bases at or above any particular Q-score. When using Q-score binning, this plot reflects the subset of Q-scores used.

Data by Lane Plot

The Data by Lane plots allow you to view quality metrics per lane.

Use the Data By Lane plot to judge the difference in quality metrics between lanes. Do not use the Data By Lane plot to look at alignment or variant calling analysis metrics.

The Data by Lane plots have the following features:

- You can select the displayed metric through the drop-down lists.
- The symbol in the top right-hand corner toggles the plot between pane view and full screen view.

The plots share several characteristics:

- The plots show the distribution of mean values for a given parameter across all tiles in a given lane.
- The red line indicates the median tile value for the parameter displayed.
- Blue boxes are for raw clusters, green boxes for clusters passing filter.
- The box outlines the interquartile range (the middle 50% of the data) for the tiles analyzed for the data point.
- The error bars delineate the minimum and maximum without outliers.
- The outliers are the values that are more than 1.5 times the interquartile range below the 25th percentile, or more than 1.5 times the interquartile range above the 75th percentile. Outliers are indicated as dots.

You can monitor the following quality metrics with this plot:

- The density of clusters for each tile (in thousands per mm^2).
- The number of clusters for each tile (in millions).
- The estimated percentage of molecules in a cluster for which sequencing falls behind (phasing) or jumps ahead (prephasing) the current cycle within a read.
- The percentage of reads from clusters in each tile that aligned to the PhiX genome.

To expand a chart, click the expand button .

Q-score Heat Map

The Q-score heat map shows plots that allow you to view the Q-score by cycle.

View the Q-score heat map for a quick overview of the Q-scores over the cycles. Do not use the Q-score heat map to look at metrics other than quality scores.

These plots have the following features:

- The color bars to the right of each chart indicate the values that the colors represent. The charts are displayed with tailored scaling; the scale is always 0% to 100% of maximum value.
- The symbol in the top right-hand corner toggles the plot between pane view and full screen view.

To expand a chart, click the expand button .

View Run Samples List

The Run Samples List contains a list of all the samples in the run.

Use this option when you want to see a list of all the samples in the run or to navigate to details regarding a specific sample.

1 Click the **Runs** icon.

2 Click a run.

3 From the Runs Overview page, click **Samples** or click the Samples icon from the left navigation menu.

View Run Summary

The Run Summary page has the overall statistics about the run.

Use this option when you want to view information about the run such as percent alignment, cycles, and densities.

1 Click the **Runs** icon.

2 Select a run.

3 From the Run Overview page, click **Run Summary** or click the Run Summary icon from the left navigation menu.

The following metrics are displayed in the top table, split out by read and total.

Level	The sequencing read level.
Yield Total	The number of bases sequenced, which is updated as the run progresses.
Projected Total Yield	The projected number of bases expected to be sequenced at the end of the run.
Aligned	The percentage of the sample that aligned to the PhiX genome, which is determined for each level or read independently.
Error Rate	The calculated error rate of the reads that aligned to PhiX.
Intensity Cycle 1	The average of the A channel intensity measured at the first cycle averaged over filtered clusters.
%Q ≥ 30	The percentage of bases with a quality score of 30 or higher, respectively. This chart is generated after the 25th cycle, and the values represent the current cycle.

The following metrics are available in the Read tables, split out by lane.

Tiles	The number of tiles per lane.
Density	The density of clusters (in thousands per mm^2) detected by image analysis, +/- 1 standard deviation.
Clusters PF	The percentage of clusters passing filtering, +/- 1 standard deviation.

Phas./Prephas.	The value used by RTA for the percentage of molecules in a cluster for which sequencing falls behind (phasing) or jumps ahead (prephasing) the current cycle within a read. For MiSeq, RTA generates phasing and prephasing estimates empirically for every cycle. The value displayed here is therefore not used in the actual phasing/prephasing calculations, but is an aggregate value determined from the first 25 cycles. For most applications, the value reported is very close to the value that is applied. For low diversity samples or samples with unbalanced base composition, the reported value can diverge from the values being applied because the value changes from cycle to cycle.
Reads	The number of clusters (in millions).
Reads PF	The number of clusters (in millions) passing filtering.
%Q ≥ 30	The percentage of bases with a quality score of 30 or higher, respectively. This chart is generated after the 25th cycle, and the values represent the current cycle.
Yield	The number of bases sequenced which passed filter.
Cycles Err Rated	The number of cycles that have been error-rated using PhiX, starting at cycle 1.
Aligned	The percentage that aligned to the PhiX genome.
Error Rate	The calculated error rate, as determined by the PhiX alignment. Subsequent columns display the error rate for cycles 1–35, 1–75, and 1–100.
Intensity Cycle 1	The average of the A channel intensity measured at the first cycle averaged over filtered clusters.

View the Project Sample List

1 Click the **Projects** icon.

2 Select a project.

3 Click **Samples** from the left navigation menu.

View the Analyses List

The Analyses List contains a list of app sessions in a project. Use this option when you want to navigate to details regarding a specific app session.

1 Click the **Projects** icon.

2 Select a project.

3 Click an analysis to see the results.

Launch Apps

Launch an app using the following methods:

- Navigate to a project, sample or analysis, click **Launch Apps**, and select an app.
- Click the Apps icon, select an app, and then click **Launch**. The app guides you through the start-up process.

If you are running BWA Enrichment v2.1 or Isaac Enrichment v2.1, read *Enrichment v2.1 Apps Adapter Trimming* on page 32.

BaseSpace Onsite has limited storage capacity and checks the free space available before starting an app. If there is not enough available space, BaseSpace Onsite displays an error message. For more information, see *Storage Check* on page 45.

Launch the IGV App

The Integrative Genomics Viewer (IGV) of the Broad Institute is a fully featured genome browser that allows you to visualize your sequence data in great detail. Illumina has modified IGV to display alignment and variant data from BaseSpace Onsite (BAM and VCF files).

IGV enables you to perform variant analysis after launching Resequencing or Amplicon workflows in BaseSpace Onsite. IGV is run on a project, which is the highest level directory and contains one or more AppResults. IGV retains all its native functions, including loading data from your local computer.

NOTE
An active internet connection is required to load reference genome data from the Broad Institute.

NOTE
Make sure that the Java Runtime Environment is installed on the computer. Download Java at java.com/en/.

1. Click the **Projects** icon.
2. Select a project.
3. Click **Launch Apps** and select the IGV application.
4. Click **Accept**.
5. Depending on your browser, open or save the *.jnlp file.
   - Internet Explorer—Click **Open**.
   - Google Chrome—Click **Keep** and then click the file to open.
   - Firefox—Select **Open with Java(TM) Web Start Launcher (default)**.

   The IGV App opens on your desktop with the requested project loaded.

BaseSpace Onsite Data in IGV

The BaseSpace Onsite file browser shows data in BaseSpace Onsite that is available for viewing in IGV. The directory structure is shown according to how data are organized in BaseSpace Onsite.

A project is the highest level directory and it contains 1 or more AppResults. If an AppResult is the result of analyzing a single sample, then the sample name is appended to the AppResult name.

The file browser shows alignment (BAM) and variant (VCF) files, and, if produced, BED, GTF, and BEDGRAPH files. Double-click a file to load it as an IGV track. Load VCF files

before BAM files because read tracks can take up an entire IGV screen, which requires scrolling to see variants.

Run the VariantStudio App

The Illumina VariantStudio data analysis software application enables researchers to identify and classify disease-relevant variants quickly, and then communicate significant findings in concise and actionable reports.

This application provides an intuitive framework for nonexpert users and offers the following features:

- Flexible filtering options
- Streamlined variant classification
- Rapid and rich annotations
- Customizable reporting

Use VariantStudio to explore and isolate key variants and to categorize variants and determine biological impact.

1. Click the **Apps** icon.
2. Select the VariantStudio app, and then click **Launch**.
3. Select a project.
   You can only select projects you own.
4. Click **Continue**.
5. If you are using the app for the first time, install VariantStudio.
   a. Click **Install VariantStudio**.
   b. Run the setup.exe file. Your web browser can ask you to save the file first. After the download has completed, double-click the setup file.
   c. If you are prompted with a security warning, click **Install**.
6. Click **Launch VariantStudio**.
   The VariantStudio app opens on your desktop with the requested project loaded. For more information about running VariantStudio, see the *VariantStudio User Guide (document # 15040890)*.

Enrichment v2.1 Apps Adapter Trimming

BWA Enrichment v2.1 and Isaac Enrichment v2.1 Apps do not support adapter trimming in this BaseSpace Onsite release.

- **MiniSeq**—You do not need to use adapter trimming for **MiniSeq** data in any of the secondary analysis apps. Adapter trimming is performed automatically in FASTQ generation based on the library prep kit type selected during run setup in the Prep Tab workflow.
- **MiSeq**—You can upload pretrimmed MiSeq FASTQ files through BaseSpace Onsite Import or upload a MiSeq run with a sample sheet containing adapter sequences for trimming.

Download Files

Download Individual Files

1. Click the **Runs** icon or **Projects** icon.
2. Navigate to a file.
3. Click **Download**.

Download Multiple FASTQ Files

Use this option to download FASTQ files per sample.

1. Click the **Runs** icon or **Projects** icon.
2. Select a run or project.
3. Click a sample in the Samples pane.
4. In the Files pane, select the checkboxes for the FASTQ files.
5. Click **Download Selected**.
   The BaseSpace Onsite Downloader guides you through the download process.

Download Run File Package

There are 4 types of data packages:

- Variant Data, containing VCF files with variant calls
- Aligned Data, containing BAM files with aligned reads
- Unaligned Data, containing FASTQ files with unaligned reads
- SAV Data, containing files describing the set-up of the run and InterOp files

The packages available depend on your workflow; packages that are grayed out are not available for download.

For more information about file types, see *BaseSpace Onsite Files* on page 33.

1. Click the **Runs** icon.
2. Click a run.
3. Click **Download Run**.
4. If not yet installed, click **Install the Downloader**.
   BaseSpace Onsite Downloader is a desktop client that allows for fast, reliable downloads transferred securely over SSL.
5. Select the files to be downloaded.
6. Click **Download your files**.
7. In the Confirm Download dialog, browse to a download folder, and then click **Start Download**.
   BaseSpace Onsite Downloader tracks the progress of the download.

BaseSpace Onsite Files

The apps available on BaseSpace Onsite use and produce various file types. For file format descriptions, see the app guide for the app you are using.

FASTQ File Format

FASTQ file is a text-based file format that contains base calls and quality values per read. Each record contains 4 lines:

- The identifier
- The sequence
- A plus sign (+)
- The quality scores in an ASCII encoded format

The identifier is formatted as **@Instrument:RunID:FlowCellID:Lane:Tile:X:Y ReadNum:FilterFlag:0:SampleNumber** as shown in the following example:

```
@SIM:1:FCX:1:15:6329:1045 1:N:0:2
TCGCACTCAACGCCCTGCATATGACAAGACAGAATC
+
<>;##=><9=AAAAAAAAAA9#:<#<;<<<????#=
```

FASTQ File Names

FASTQ files are named with the sample name and the sample number. The sample number is a numeric assignment based on the order that the sample is listed for the run. For example:

Data\Intensities\BaseCalls\samplename_S1_L001_R1_001.fastq.gz

- **samplename**—The sample name listed for the sample. If a sample name is not provided, the file name includes the sample ID.
- **S1**—The sample number based on the order that samples are listed for the run starting with 1. In this example, S1 indicates that this sample is the first sample listed for the run.

  NOTE
  Reads that cannot be assigned to any sample are written to a FASTQ file for sample number 0, and excluded from downstream analysis.

- **L001**—The lane number.
- **R1**—The read. In this example, R1 means Read 1. For a paired-end run, a file from Read 2 includes R2 in the file name.
- **001**—The last segment is always 001.

FASTQ files are compressed in the GNU zip format, as indicated by *.gz in the file name. FASTQ files can be uncompressed using tools such as gzip (command-line) or 7-zip (GUI).

Quality Scores

A quality score, or Q-score, is a prediction of the probability of an incorrect base call. A higher Q-score implies that a base call is more reliable.

Based on the Phred scale, the Q-score serves as a compact way to communicate small error probabilities. Given a base call, X, the probability that X is not true, P(~X), results in a quality score, Q(X), according to the relationship:

$$Q(X) = -10 \log_{10}(P(\sim X))$$

where P(~X) is the estimated error probability.

The following table shows the relationship between the quality score and error probability.

Quality Score Q(X)	Error Probability P(~X)
Q40	0.0001 (1 in 10,000)

Quality Score Q(X)	Error Probability P(~X)
Q30	0.001 (1 in 1,000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

For more information on the Phred quality score, see en.wikipedia.org/wiki/Phred_quality_score.

During the sequencing run, base call quality scores are calculated after cycle 25 and results are recorded in base call (*.bcl) files, which contain the base call and quality score per cycle.

Health Runs

You can choose whether to send anonymous system health information to Illumina. Health runs help Illumina diagnose issues and improve our products. The information consists of InterOp files and log files, and is not tied to any user account. This option is on by default.

Share Data

You can either share data at a run or project level using an email invitation or through a hyperlink.

Sharing is for read-only access. If you want a collaborator to have write access, see *Transfer Owner* on page 38.

NOTE
Runs and projects have separate permissions. If you share a run, the project associated with that run is not shared automatically. Therefore, samples and app results are not accessible to collaborators of the run.

Share a Project or Run using the Get Link option

The Get Link option allows you to share a project or a run with any collaborator who is in your company and has an account on the BaseSpace Onsite system.

Use the activate or deactivate to turn on or turn off the hyperlink. Anyone who previously accepted the link still has access to the run even when the link is deactivated.

1. Click the **Projects** or **Runs** icon.
2. Select a project or run.
3. Click **Get Link**.
4. Click **Activate** or **Deactivate**.
5. Copy the URL to share with collaborators.

NOTE
Runs and projects have separate permissions. If you share a run, the project associated with that run is not shared automatically. Therefore, samples and app results are not accessible to collaborators of the run.

Share a Project or Run Using the Email Option

Use the Share Project option to share a project using an email link. The specified collaborators receive an email with a link to the Project. Only the specified collaborators can view the corresponding data.

1. Click the **Projects** or **Runs** icon.
2. Select a project or run.
3. Click **Share**.
4. In the Share Settings dialog box, enter the collaborators email address, and then click **Invite**.

   NOTE
   The invitation email address must match your BaseSpace Onsite login email address. Otherwise, your collaborator is not able to view the project.
5. Click **Save Settings**.

Manage Projects and Samples

View the Project Sample List

1. Click the **Projects** icon.
2. Select a project.
3. Click **Samples** from the left navigation menu.

Edit Project Details

1. Click the **Projects** icon.
2. Select a project.
3. Click **Edit Project**.
4. Change project details in the Edit Project dialog box.
5. Click **Save**.

Set Up a New Project

1. Click the **Projects** icon.
2. Click **New Project** in the top left corner.
3. Enter a new name and description.
4. Click **Create**.

Copy Samples

1. Click the **Projects** icon.
2. Select a project.
3. Click **Samples** from the left navigation menu.
4. Select the samples to copy.
5. Click **Copy**.
6. Select the new project.
7. Click **Copy**.

Combine Samples

You can combine data from multiple sequencing runs on the same sample. You can only combine samples that have the same read lengths.

1. Click the **Projects** icon.
2. Select a project.
3. Click **Samples** from the left navigation menu.
4. Select the samples to combine.
5. Click **Combine**.

6 Click **Combine** in the pop-up screen.

Upload Files to Projects

Some apps need additional files generated outside of BaseSpace Onsite. Some apps also provide downstream analysis for results generated outside of BaseSpace Onsite.

You can only upload manifest files, VCF files, and sample FASTQ files.

1 Click the **Projects** icon.

2 Select a project.
You can only upload files to projects you own.

3 Click **Import**.

4 Select the file to upload using the following methods:
- Drag and drop the file in the **Drag and Drop** box.
- Click **Select File**, browse to the file, and then click **Open**.

5 Enter the analysis name in the **Name of analysis** box.

6 [Optional] Associate the file with the samples used as inputs.
The analysis and files are listed in the sample overview page.

7 Click **Complete Import**.

FASTQ File Upload Requirements

The FASTQ file is a text format file used to represent sequences. Each record has 4 lines. The 4 lines of data are an identifier (read descriptor), the sequence, +, and the quality scores. For a detailed description of the FASTQ format, see *FASTQ Files* on page 1.

Make sure that the FASTQ file adheres to the following upload requirements:
- The uploader only supports gzipped FASTQ files generated on Illumina instruments
- The name of the FASTQ files conforms to the naming conventions described in *FASTQ File Names* on page 34.

The uploader rejects any upload session containing files that do not meet the requirements. If a file is rejected, a troubleshooting message is provided.

Transfer Owner

You can use the Transfer Owner option to hand control of data over to a collaborator or customer. You can only transfer data to collaborators who have an account on BaseSpace Onsite

NOTE
If items from a project are in the trash, you cannot transfer ownership of the project.

1 Select a project or run.
- To transfer a project, perform the following steps.

  a Click the **Projects** icon.
  b Select a project.
  c Click the **Transfer Owner** button.

- To transfer a run, perform the following steps.

  a Click the **Runs** icon.
  b Select a run.
  c Click the **More** button, and then select **Transfer Ownership**.

2 Enter the email of the new owner and an optional message.

3 Click **Continue**.

Fix Indexes in the Prep Tab

You can correct errors in your indexes through the Prep tab and regenerate the FASTQ files. You can use this feature to change indexes and regenerate FASTQ files for samples have already been sequenced.

You cannot use this feature if the wrong library prep kit was selected in the Prep tab.

1 Go to the run with the wrong indexes.

2 Click the Run Settings button in the navigation task pane.

3 At the bottom of the page, select the pool.

4 Click the Plate ID.

5 Click **Edit**.

6 Correct the index in the dropdown menu.

7 Go to the affected run.

8 In the **More** dropdown menu, select **Generate FASTQ Files**.
BaseSpace Onsite now starts regenerating the FASTQ files with the corrected indexes. The new FASTQ files get added to the sample list and you can identify the new files by date.

NOTE
If you do not want to identify the samples by date, you can also rename the sample_ID, or assign the new FASTQ files to a new project in the Prep tab.

Delete Runs, Projects, Analyses, or Samples

1 Click the **Runs** or **Projects** icon.
Analyses and samples are deleted from the project page.

2 Select a run or project.

3 Click **Move to Trash**.

4 If deleting a run, confirm that you want to delete associated analyses or samples.

5 Click **Confirm**.
If you are the owner of an item, it is present in the trash.

Note
If you received the run, project, analysis, or sample as a share, it does not appear in the trash. To restore an item, click the previously sent share link or contact the owner.

Items cannot be deleted if they are in a nonterminal state (eg, running, uploading, or analyzing).

Do not perform this action if you want to archive an item.

Empty Trash

1 Click **View Trash**.

2 Click **Empty Trash**.

3 Click **Confirm**.
The admin can now purge the items. For more information, see *Purge Deleted Items* on page 45.

Restore Trash

1 Click **View Trash**.

2 Select the items you want to restore.

3 Click **Restore Trash**.
The item is restored to its original location. Restored items keep all their original attributes except for the share recipients.

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Search

The Search function allows you to find runs, projects, samples, files, and apps.

1. Click the search icon.
2. Type in the run, project, or sample name in the search field and hit enter.
3. Select a run, project, sample, file, or app in the search results. You can also filter the search results by these categories using the drop-down list at the right of the results page.

Admin Tasks

Chapter 3

Manage BaseSpace Onsite

If you have admin privileges, the Account drop-down list provides access to the Admin Panel. The Admin Panel allows you to manage analysis, notifications, storage, users, system health, planned runs, software updates, and alarms.

Manage Analysis

On the Analysis page, you see the analyses that are currently running, and the analyses that are queued. You can sort the analyses by the column headers.

You can perform the following actions:

- Select an active analysis and click **Stop** to stop the analysis.
- Select a queued analysis and click **Remove** to remove the analysis from the queue.
- Click **Settings** to set the administrator notifications you get through email.

Manage Storage

The following tabs are shown on the Storage page.

Tab	Description
Active Storage	Provides an overview of the total amount of active storage used and free, and the storage by user. Active samples, runs, and analyses are stored on the BaseSpace Onsite system and can be used for analysis. At the bottom, you see the list of active samples, runs, and analyses stored. You can sort this list by clicking the column headers. Any changes that add or remove data usually take 3–5 minutes to be reflected in the pie chart. Active samples, runs and analyses are stored on the BaseSpace Onsite system and can be used for analysis. **Note:** If there are many merged samples and copied samples on the system, the usage per-user values are overestimated in the Storage Use By Owners pie chart. The Total Active Storage is not affected.
Archive Storage	Provides an overview of the archived analyses. Archived samples, runs, and analyses are stored on the archive system and cannot be used for analysis on BaseSpace Onsite without restoring them. At the bottom, you see the list of archived samples, runs, and analyses. You can sort this list by clicking the column headers. Setting up an archive location is highly recommended.
Deleted Items	Provides an overview of the items that have been deleted. At the bottom, you see the list of deleted samples, runs, and analyses. You can sort this list by clicking the column headers. You can restore these items to active storage, or purge them from BaseSpace Onsite, which deletes them permanently.

Archive or Backup Analysis

1. On the Storage page, go to the **Archive Storage** tab.
2. Select the analysis.
3. Click **Archive**.
4. A dialog box appears, asking you if you want to keep the data in active storage.

- If you keep the data in active storage, you can keep working with the analysis, while making a backup in the archive.
- If you do not keep the analysis in active storage, the data are archived, and you cannot work with the analysis in BaseSpace Onsite. You can always move it back to active storage as described in *Restore Analysis From Archive* on page 45.

The maximum speed for archiving is 25 Mb/s, so it does not interfere with other BaseSpace Onsite tasks.

Restore Analysis From Archive

1. On the Storage page, go to the **Archive Storage** tab.
2. Select the analysis.
3. Click **Unarchive**.
   If there is sufficient free space, BaseSpace Onsite restores the analysis. If there is not enough space, BaseSpace Onsite displays an error message.

NOTE
You cannot restore an archived item that has been deleted and purged from BaseSpace Onsite.

Set Up Archive Location

1. On the Storage page, go to the **Archive Storage** tab.
2. Click **Mount**.
3. Fill out the form.
   You can set up 1 archive location per BaseSpace Onsite system.

Restore Deleted Item

1. On the Storage page, go to the **Deleted Items** tab.
2. Select the analysis.
3. Click **Unarchive**.
   If there is sufficient free space, BaseSpace Onsite restores the item. If there is not enough space, BaseSpace Onsite displays an error message.

Purge Deleted Items

1. On the Storage page, go to the **Deleted Items** tab.
2. Click **Purge**.
   All items in the trash are removed permanently.

Storage Check

BaseSpace Onsite has limited storage capacity, and checks the free space available before uploading a run or starting an app. The necessary space is then reserved until the process completes.

If there is not enough space, you see an error message and the run or app does not start. If the space check fails before starting FASTQ generation, the run gets into ***Needs Attention*** state. To manage available space, see *Archive or Backup Analysis* on page 44.

Manage Users

On the Users page, you see a list of current BaseSpace Onsite accounts, and their roles. You can sort the users by the column headers.

To set up a new user, click **Add User** and fill out the form.

The new user gets an email with a link to set up a new password. If the user does not get an email, the user can go to the BaseSpace Onsite login page and click **Forgot Password** to resend the email.

If you did not configure SMTP during install, be aware of the following items:

- You enter the password for the new user.
- BaseSpace Onsite does not enforce usernames to be in the form of an email. Make sure to enforce that all users create a username in the format of an email.
- If you enable the SMTP support after using BaseSpace Onsite without SMTP support, all users that are logged in must log out and then log back in. Otherwise, they do not receive notifications by email or on the dashboard.

Monitor System Health

On the System Health page, you see the BaseSpace Onsite system health alerts. You can sort the alerts by the column headers.

Many sensors are monitored for health in the BaseSpace Onsite server. If a sensor indicates a failure, BaseSpace Onsite sends an alert to the administrator. When you receive an alert, contact Illumina Support to diagnose the error and, if necessary, arrange a site visit to correct the problem.

You can resolve some of these errors, such as a failure of a disk drive or 1 of the power supplies. See *Replacement Procedures* on page 48 for instructions. Illumina Support can also guide you through the process of replacing the faulty component.

If you want to remove an alert, select the alert and click **Dismiss**.

Unlock Planned Runs

On the Planned Runs page, you see the runs that are currently planned. You can sort the runs by the column headers.

The sequencing system locks planned runs when they are selected. In rare instances, the sequencing system leaves the run in locked state without starting the run. These runs must be manually unlocked to access again. Unlocking runs allows the users to edit the run in the Prep tab, and makes the run available for selection on other sequencing systems. Unlocking also frees up the reserved run storage space on the BaseSpace Onsite system.

To unlock a run, select the planned run and click **Unlock**.

Updates

You can update the server software and upload additional genome content.

1. Plug the external USB drive with the software update into the BaseSpace Onsite head node server.
2. Go to the Updates page.
3. Click **Detect Drive**.
   The Updates wizard leads you through the update.

System Logs

The System Logs page provides a download of the log files. You can download all log files, or packages that contain the system state log files, active analysis log files, or delete log files.

If an issue arises with your BaseSpace Onsite, Illumina support uses these files to troubleshoot your system. To speed up troubleshooting, download the appropriate package before calling Illumina support.

About Page / Licenses

The About page provides a download containing the licenses for third-party software components.

Manage Alarms on the Settings Page

If there are problems with the hard drive, BaseSpace Onsite sounds a loud alarm. The **Disable Alarm** button on the Settings page allows you to turn off a current alarm. For more information, see *Replace Hard Drive* on page 48.

CAUTION

Do not disable the alarm and ignore the warning. Failure to address the warning could lead to irretrievable data loss.

Replacement Procedures

You have received a spare hard drive and power supply module with the instrument. If needed, you can perform the replacement without calling Illumina, using the instructions in this section.

To order more, use the following material numbers.

Part	Nomenclature	Material Number
Power Supply	PSU 750W 1U CRPS 80PLUS PLATINUM	10535S
Hard drive	Hard Drive, 2 TB SATA, 6GB/S	15049450S
	Hard Drive, 4 TB SATA, 6GB/S	20000882

Replace Power Supply Module

1 Remove the power cord from the power supply.

2 Press the green and black tab and slide the power supply out.

Figure 4 Slide Power Supply Out

3 Push the new power supply into the slot and make sure that it is seated properly.

Figure 5 Reseat Power Supply

4 Attach the power cord to the power supply.

Replace Hard Drive

1 Lift the green tab on hard drive tray, and then pull out the drive.

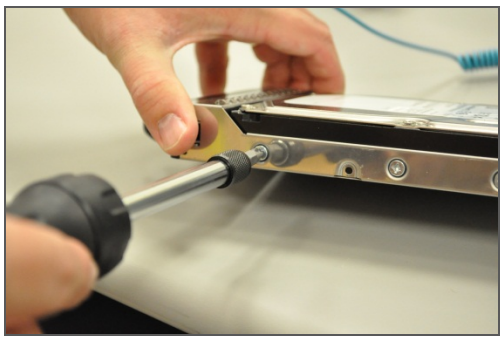
Figure 6 Lift Tab

Figure 7 Pull Drive Out

2 Look at the label on the drive and check whether it is a 2 TB or 4 TB drive.

3 Remove the 3 screws on each side of the carrier, then remove the old drive from the carrier.

Figure 8 Remove Drive From Carrier



4 Attach the new drive to the carrier.

5 Insert the new drive into the bay and make sure the tray latches closed. The new drive is automatically rebuilt.

6 Go to the Settings page in the Admin Panel, and click **Enable Alarm**.

Data Recovery

To recover data, contact Illumina Technical Support.

Error Codes

If there is an issue with your system, BaseSpace Onsite sends an email alert. The possible codes and descriptions are listed in this topic to help you troubleshoot.

Error Code/ Item ID	Item Name	Message	Status
Pwr_Unit_ Status	Power Unit Status	The power unit has detected a shutdown	Error
Pwr_Unit_ Status	Power Unit Status	The power unit has detected that the system has been turned on	OK
Pwr_Unit_ Status	Power Unit Status	The power unit has detected a loss of AC power	Error
Pwr_Unit_ Status	Power Unit Status	The power unit has detected that the AC power has been restored	OK
Pwr_Unit_ Status	Power Unit Status	The power unit has detected a soft-power failure	Error
Pwr_Unit_ Status	Power Unit Status	The power unit has recovered from a soft-power failure	OK
Pwr_Unit_ Status	Power Unit Status	The power unit has detected an unexpected failure	Error
Pwr_Unit_ Status	Power Unit Status	The power unit has recovered from an unexpected failure	OK
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error
PU_$C	Power supply unit number $C	The power supply is no longer redundant due to power unit number $C failing	Error

Error Code/ Item ID	Item Name	Message	Status
PU_$C	Power supply unit number $C	The power supply is redundant again	OK
IPMI	IPMI Watchdog	The hardware monitor has detected an expired timer	Error
IPMI	IPMI Watchdog	The hardware monitor has detected hard reset of the system	Error
IPMI	IPMI Watchdog	The hardware monitor has detected that the system is shutting down	Error
IPMI	IPMI Watchdog	The hardware monitor has detected that the system is restarting	Error
IPMI	IPMI Watchdog	The hardware monitor has detected a timer interrupt	Error
Phy_Sec	Physical Security	The system cover has been opened	Error
Phy_Sec	Physical Security	The system cover has been closed	OK
Phy_Sec	Physical Security	The system has been unplugged from the network	Error
Phy_Sec	Physical Security	The system network connection has been restored	OK
FPB	Front Panel Board	The front panel board has detected a critical interrupt error	Error
SMI	System Board Timeout	The system board has detected a timeout	Error
SMI	System Board Timeout	The system board has recovered from a timeout	OK
SE	System Event	There was a PEF Action detected	Error
SB	System Board	The system board has detected that the power button has been pressed	OK
SB	System Board	The system board has detected that the reset button has been pressed	OK
VR	Voltage Watchdog	The voltage sensor has detected that the voltage is not within normal range	Error

Error Code/ Item ID	Item Name	Message	Status
VR	Voltage Watchdog	The voltage sensor has detected that the voltage is back within normal range	OK
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are no longer redundant due to fan number $C failing	Error
F_$C	Fan number $C	The fans are redundant again	OK
TEMP	System Board Temperature	The system board temperature has exceeded the normal range	Error
TEMP	System Board Temperature	The system board temperature is back within normal range	OK
BMC_FW	BMC Firmware Health	The BMC board has detected a sensor failure	Error
F1	Fan 1	Fan number 1 is not functioning	Error
F1	Fan 1	Fan number 1 is functioning	OK
F2	Fan 2	Fan number 2 is not functioning	Error
F2	Fan 2	Fan number 2 is functioning	OK
F3	Fan 3	Fan number 3 is not functioning	Error
F3	Fan 3	Fan number 3 is functioning	OK
F4	Fan 4	Fan number 4 is not functioning	Error
F4	Fan 4	Fan number 4 is functioning	OK
F5	Fan 5	Fan number 5 is not functioning	Error
F5	Fan 5	Fan number 5 is functioning	OK

Error Code/ Item ID	Item Name	Message	Status
PS_$C	Power supply number $C	The number $C AC power supply is no longer available	Error
PS_$C	Power supply number $C	The number $C AC power supply has been connected	OK
PS_$C	Power supply number $C	The number $C AC power supply has recovered from failure	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected a failure	Error
PS_$C	Power supply number $C	The number $C AC power supply has recovered from a predictive failure	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected a predictive failure	Error
PS_$C	Power supply number $C	The number $C AC power supply has been lost	Error
PS_$C	Power supply number $C	The number $C AC power supply has been restored	OK
PS_$C	Power supply number $C	The number $C AC power supply has resolved the configuration error for a vendor mismatch	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected a configuration error for a vendor mismatch	Error
PS_$C	Power supply number $C	The number $C AC power supply has resolved the configuration error for a revision mismatch	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected a configuration error for a revision mismatch	Error
PS_$C	Power supply number $C	The number $C AC power supply has resolved the configuration error for a missing processor	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected a configuration error for a missing processor	Error
PS_$C	Power supply number $C	The number $C AC power supply has resolved an unexpected configuration error	OK
PS_$C	Power supply number $C	The number $C AC power supply has detected an unexpected configuration error	Error

Error Code/ Item ID	Item Name	Message	Status
CBPB	Chassis Back Panel Board	The panel board in the back of the chassis has changed to being offline	Error
CBPB	Chassis Back Panel Board	The panel board in the back of the chassis is back online	OK
P_$C	Processor number $C	The processor number $C has exceeded the normal range	Error
P_$C	Processor number $C	The processor number $C is back within the normal range	OK
P_$C	Processor number $C	The processor number $C has been detected	OK
P_$C	Processor number $C	The processor number $C is not being detected	OK
P_ERR_$C	Processor number $C digital state error	The processor number $C has detected a digital state error	Error
P_ERR_$C	Processor number $C digital state error	The processor number $C has recovered from a digital state error	OK
P_TEMP_$C	System temperature	The system temperature has exceeded its threshold	Error
P_TEMP_$C	System temperature	The system temperature is back within normal operating range	OK
PS_FAN_$C	Power Supply Fan $C	The power supply fan number $C has failed	Error
PS_FAN_$C	Power Supply Fan $C	The power supply fan number $C has been restored	OK
M_$C	Memory Sensor number $C	The thermal sensor at memory location $C has exceeded its threshold	Error
M_$C	Memory Sensor number $C	The thermal sensor at memory location $C is back within normal range	OK
HD_$C	Disk in slot $C	Disk rebuild in progress at slot $C	Error
HD_$C	Disk in slot $C	Disk rebuild at slot $C has completed	OK
HD_$C	Disk in slot $C	Disk at slot $C is no longer online	Error
HD_$C	Disk in slot $C	Disk at slot $C is back online	OK

Error Code/ Item ID	Item Name	Message	Status
HD_$C	Disk in slot $C	Disk at slot $C is no longer working	
HD_$C	Disk in slot $C	Disk at slot $C is no functioning correctly again	
LV_$B	Logical volume $B with capacity $C $D	The logical volume at $B size $C $D is not currently active	Error

Troubleshooting

Appendix 1

Fix Sample Sheet / Rerun Workflow

The Fix Sample Sheet page lets you correct errors in your sample sheet or set up a new analysis to requeue. Use this feature for the following reasons:

1. Open the Fix Sample Sheet page using the following methods:
   - If a run has a Needs Attention state, open the run, and then click **Fix Sample Sheet**.
   - Go to a run, click **More**, and then select **Fix Sample Sheet**.

   If BaseSpace Onsite has detected an error, it shows the issue above the black sample sheet editor.
2. Make changes using the following methods:
   - Edit the sample sheet in the sample sheet editor. BaseSpace Onsite keeps validating the sample sheet as you edit. Any remaining issues are displayed above the sample sheet editor.
   - Use Illumina Experiment Manager (IEM) to create a sample sheet.

     a. If necessary, install IEM and open the program.
     b. Import the original sample sheet from your system in IEM and edit it, or generate a new sample sheet. For more information, see the *Illumina Experiment Manager User Guide (document # 15031335)*.
     c. Copy and paste the sample sheet into the Sample Sheet Editor in BaseSpace Onsite.
     BaseSpace Onsite validates the sample sheet; any issues are displayed above the sample sheet editor.
3. When you are done editing and the sample sheet is valid, click **Queue Analysis**.

NOTE
If your edits result in an invalid sample sheet, the **Queue Analysis** button is not available. You can return to the original using the **Load Original** button.

Common Sample Sheet Fixes

If a sample sheet is invalid, it could be because the genome path is not set up correctly. This situation is indicated through the *Genome Path Unknown Genome* warning (as in the example). The paths of the standard BaseSpace Onsite genomes have to conform to the following relative paths:

```
Arabidopsis_thaliana\NCBI\build9.1\Sequence\WholeGenomeFASTA
Bos_taurus\Ensembl\UMD3.1\Sequence\WholeGenomeFASTA
Escherichia_coli_K_12_DH10B\NCBI\2008-03-
   17\Sequence\WholeGenomeFASTA
Homo_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA
Mus_musculus\UCSC\mm9\Sequence\WholeGenomeFASTA
PhiX\Illumina\RTA\Sequence\WholeGenomeFASTA
Rattus_norvegicus\UCSC\rn4\Sequence\WholeGenomeFASTA
Saccharomyces_cerevisiae\UCSC\sacCer2\Sequence\WholeGenomeFASTA
Staphylococcus_aureus_NCTC_8325\NCBI\2006-02-
   13\Sequence\WholeGenomeFASTA
```

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

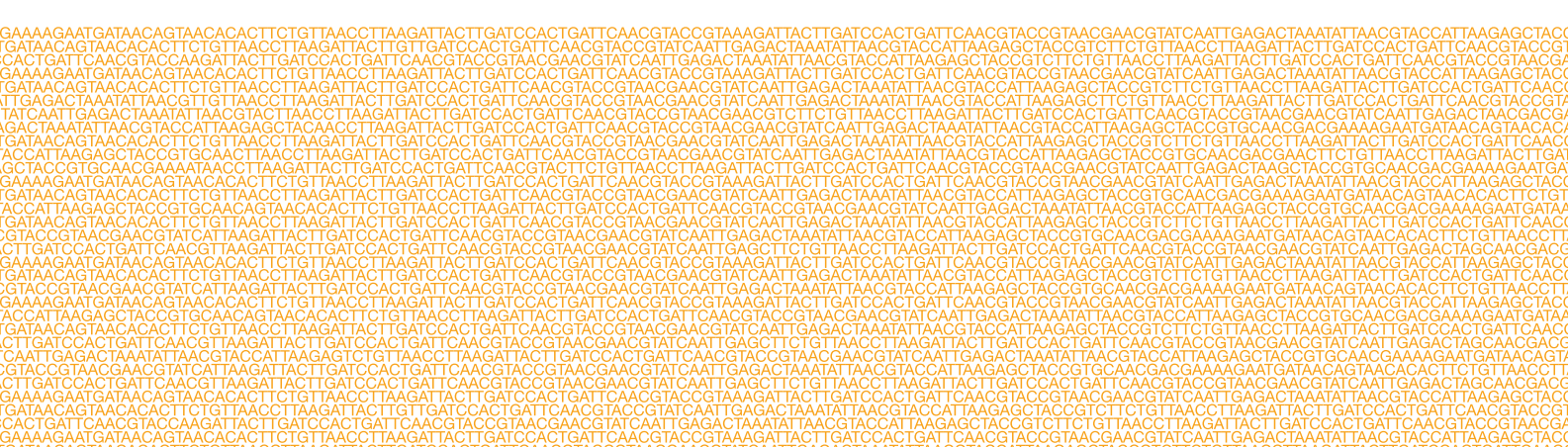
Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

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

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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