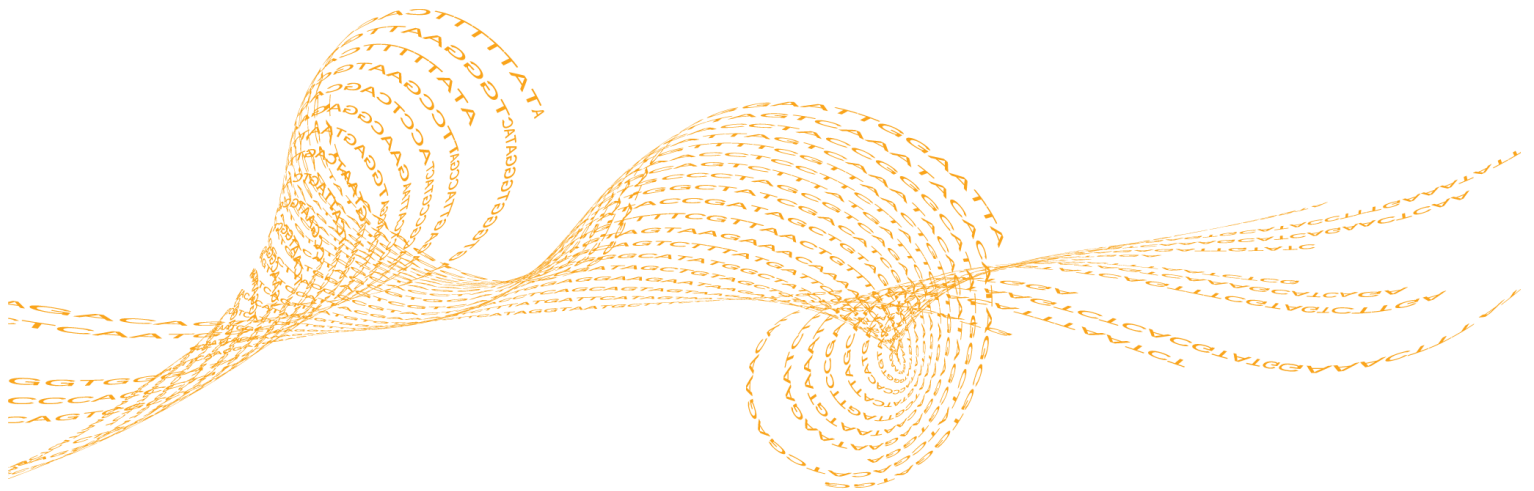


Sequencing Analysis Viewer

User Guide

FOR RESEARCH USE ONLY

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Revision History

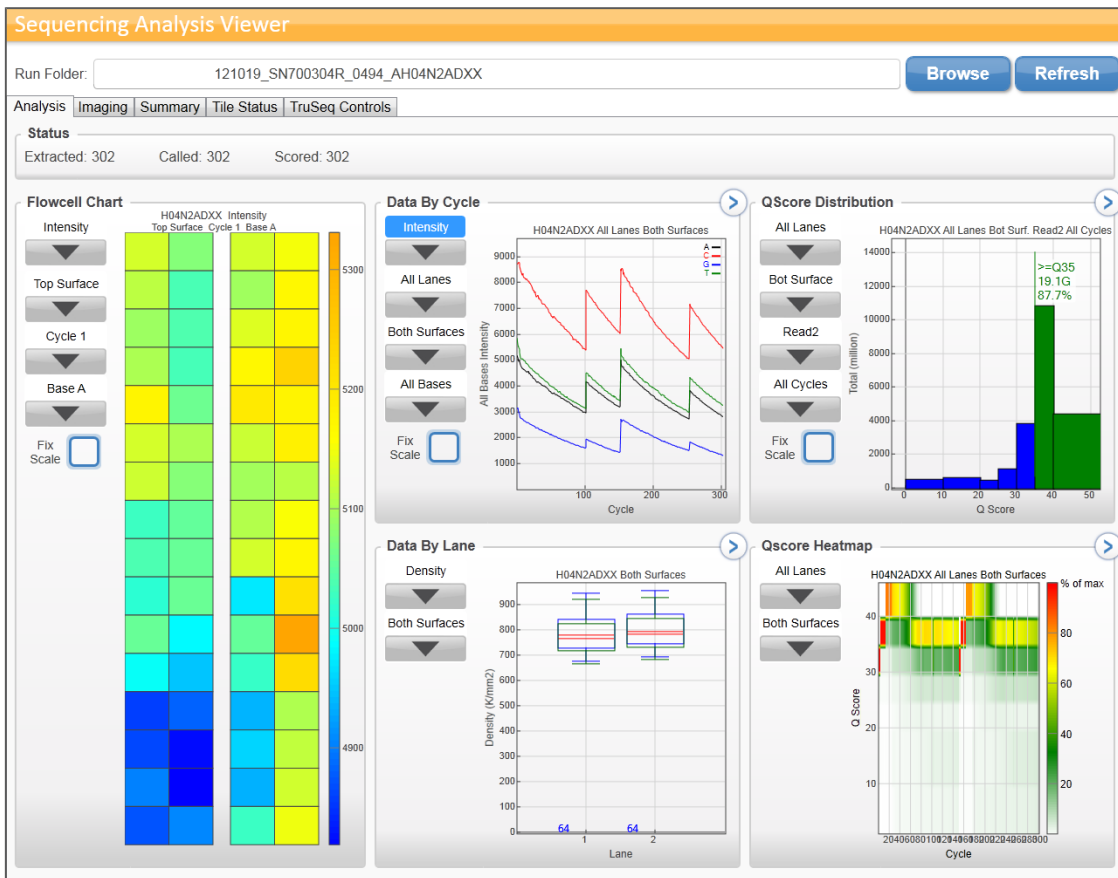
Part #	Revision	Date	Description of Change
15020619	D	January 2013	Added figure of in-line controls in a successful TruSeq DNA PCR-Free run
15020619	C	October 2012	User Guide for Sequencing Analysis Viewer v1.8.20 and up, describes binned Qscores.
15020619	B	October 2011	User Guide for Sequencing Analysis Viewer v1.8, which supports dual indexes and MiSeq metrics.
15020619	A	January 2011	Initial Release

Introduction

The Sequencing Analysis Viewer v1.8 (SAV) is an application that allows you, in real time, to view important quality metrics generated by the real time analysis software on the Illumina sequencing systems.

The Sequencing Analysis Viewer can be installed on a personal workstation, to view quality metrics from a remote location. This booklet provides instructions to install the Sequencing Analysis Viewer on a personal workstation, and describes the various metrics that can be viewed using this tool. Viewing metrics does not interfere with the sequencing run if Sequencing Analysis Viewer is installed on your sequencing instrument.

Figure 1 Sequencing Analysis Viewer



Setting Up Sequencing Analysis Viewer

This section describes setting up of the Sequencing Analysis Viewer on your own personal workstation. You need to have admin privileges to install the Sequencing Analysis Viewer Software.



NOTE

If the Sequencing Analysis Viewer is not installed on your sequencing instrument, contact your FAS. The instructions in this section are not for installing the Sequencing Analysis Viewer on the sequencing instrument.

Requirements

The Sequencing Analysis Viewer software does not need an advanced workstation, since the heavy computational work is done by the instrument computer running the real time analysis software. The following items are required for running the Sequencing Analysis Viewer:

- ▶ Desktop computer running 32 or 64 bit version Windows XP, Windows Vista, or Windows 7
- ▶ Network access to the run data
- ▶ .Net framework 4.0

Downloading Sequencing Analysis Viewer Installer

To download the Sequencing Analysis Viewer installer, perform the following:

- 1 Open your browser, and go to my.illumina.com.
- 2 Enter your MyIllumina username and password.



NOTE

If you do not have an Illumina customer account, register as a new user. It may take up to three business days for initial review of the application.

- 3 Search for Sequencing Analysis Viewer, and select the **Downloads** search filter
- 4 Download the Sequencing Analysis Viewer installer (SAV.x.x.x.x.msi).

Installing Sequencing Analysis Viewer

To install the Sequencing Analysis Viewer, perform the following:

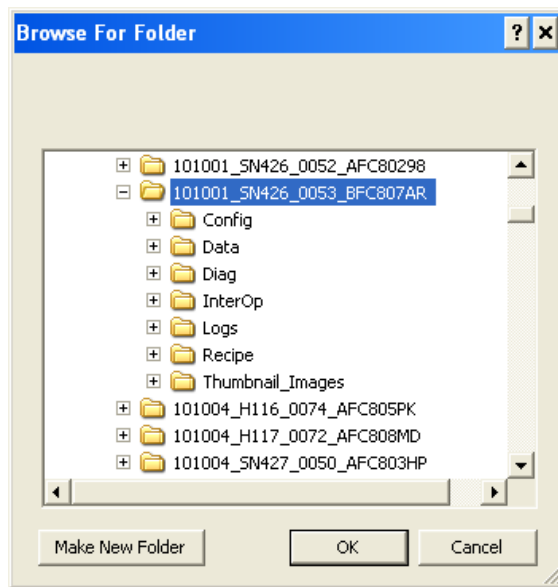
- 1 Navigate to the location where the Sequencing Analysis Viewer is saved.
- 2 Double-click the installer (SAV.x.x.x.x.msi).
The Sequencing Analysis Viewer Setup Wizard opens.
- 3 Click **Next**.
The installation starts, and the progress screen opens. Once the installation is finished, a desktop shortcut is created, and the Set Up Complete screen opens.
- 4 Click **Finish**.

Testing Sequencing Analysis Viewer installation

Once you have installed the Sequencing Analysis Viewer, you should check whether the application was installed properly, and whether you have the proper permissions. Perform the following:

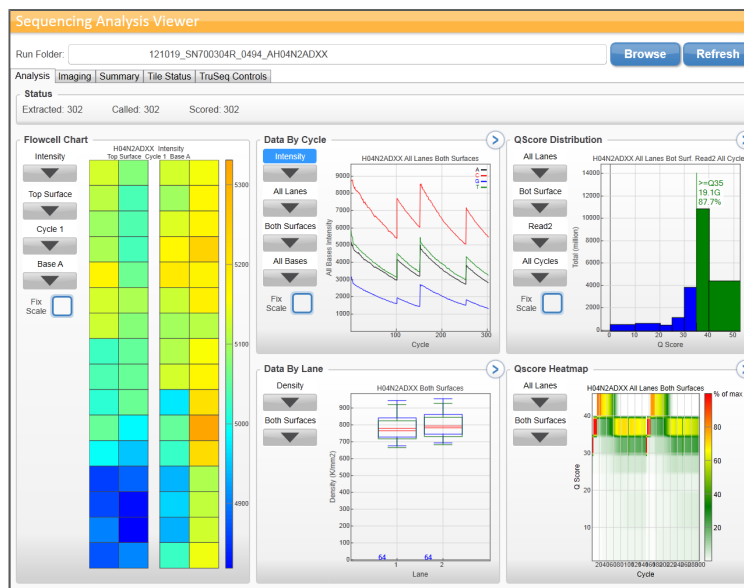
- 1 Double-click the Illumina Sequencing Analysis Viewer desktop shortcut, or go to C:\Illumina\Illumina Sequencing Analysis Viewer and double-click Sequencing Analysis Viewer.exe.
The Sequencing Analysis Viewer opens.
- 2 In the **Run Folder** field, copy the location of or browse to a Run Folder. Make sure to highlight the Run Folder and not the parent folder or any folder/file inside the Run Folder.

Figure 2 Connect to Run Folder



- 3 Click **Refresh**.
The Sequencing Analysis Viewer starts loading data, and once done should display quality metrics for that run.

Figure 3 Sequencing Analysis Viewer with Metrics Loaded



If the Sequencing Analysis Viewer does not display metrics, check whether you pointed the Sequencing Analysis Viewer to a valid Run Folder, and whether you have the proper permissions to view the data. The Sequencing Analysis Viewer will display an error message if there is no valid Run Folder.

Sequencing Analysis Viewer Files

Sequencing Analysis Viewer uses the following files:

- ▶ runinfo.xml files.
- ▶ runparameters.xml (only for HiSeq®/HiScan® runs).
- ▶ The Interop files can be found in the directory: <run directory>\InterOp. The Interop files provide the following information:
 - Extraction Metrics
 - Quality Metrics
 - Error Metrics
 - Tile Metrics
 - Corrected Intensity Metrics
 - Control Metrics
- ▶ Thumbnails (optional).

Data Availability

Not all metrics are available at the early cycles, since some processes need multiple cycles to provide meaningful data. These processes are listed below:

- ▶ Template generation occurs at cycle 4 for most sequencing instruments, or cycle 5 for Genome Analyzer's Sequencing Control Software v2.8 and v2.9; intensities are extracted once the template is generated.



NOTE

The image analysis catches up during cycle four, so cycle 4 takes significantly longer to finish. The analysis waits to end before proceeding with the next cycle. This is normal behavior.

- ▶ The software generates phasing/prephasing estimates after cycle 12. Corrected intensities are generated once phasing and prephasing are calculated. Base calls are generated once corrected intensities are generated.
- ▶ The software generates quality scoring after cycle 25.
- ▶ Alignment to PhiX occurs at cycle 26 – at which point error rates become available, if a PhiX spike-in is being used.



TIP

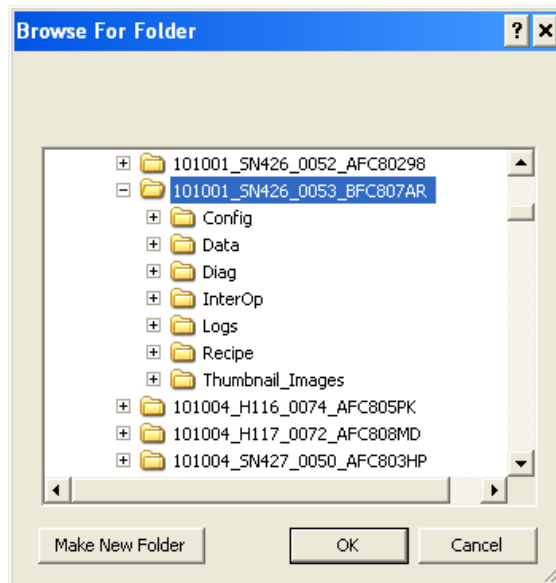
In any screen, click the **Refresh** button to make sure you have the latest metrics.

Loading Data

To load data, perform the following:

- 1 Double-click the Illumina Sequencing Analysis Viewer desktop shortcut, or go to C:\Illumina\Illumina Sequencing Analysis Viewer and double-click Sequencing Analysis Viewer.exe.
The Sequencing Analysis Viewer opens.
- 2 In the **Run Folder** field, copy the location of or browse to a Run Folder. Make sure to highlight the Run Folder and not the parent folder or any folder/file inside the Run Folder.

Figure 4 Connect to Run Folder



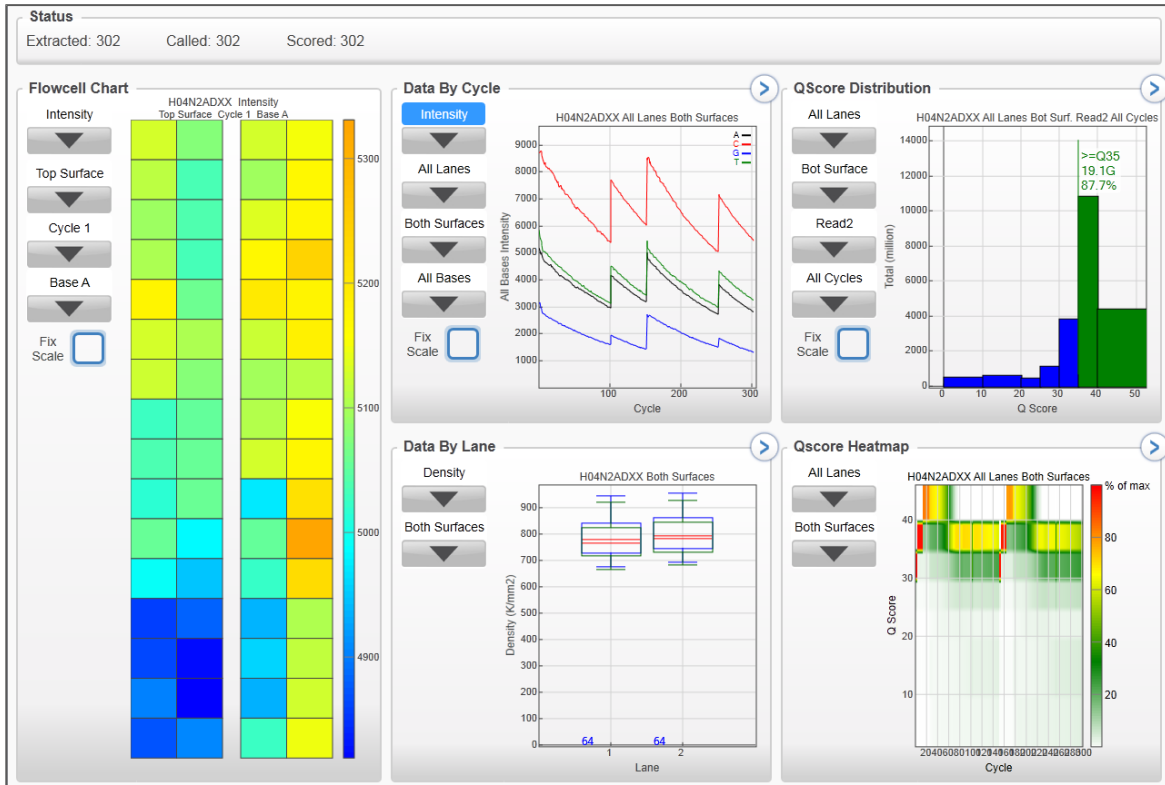
- 3 Click **Refresh**.
The Sequencing Analysis Viewer starts loading data, and once done displays quality metrics for that run.

Analysis Tab

The Analysis tab consists of six panes, which are described below:

- ▶ *Status Pane* on page 10
- ▶ *Flow Cell Chart* on page 11
- ▶ *Cycle Plot* on page 12
- ▶ *Lane Plot* on page 13
- ▶ *QScore Distribution* on page 14
- ▶ *QScore Heatmap* on page 15

Figure 5 Analysis Tab



NOTE

If you are using the Sequencing Analysis Viewer to view metrics from the MiSeq sequencing instrument (which has only one lane), select 1 or All in the **Lane** dropdown list.

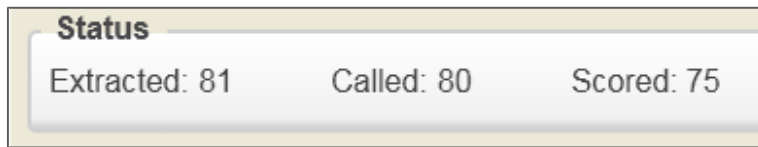
Status Pane

The Status pane reports the progress of the analysis. The following steps are reported:

- ▶ **Extracted:** the last complete cycle for which image analysis has been performed.
- ▶ **Called:** the last complete cycle that has been base-called.
- ▶ **Scored:** the last complete cycle that has been quality scored.

The statistics are updated as soon as there is new analysis information available, and you hit the **Refresh** button.

Figure 6 Status Pane

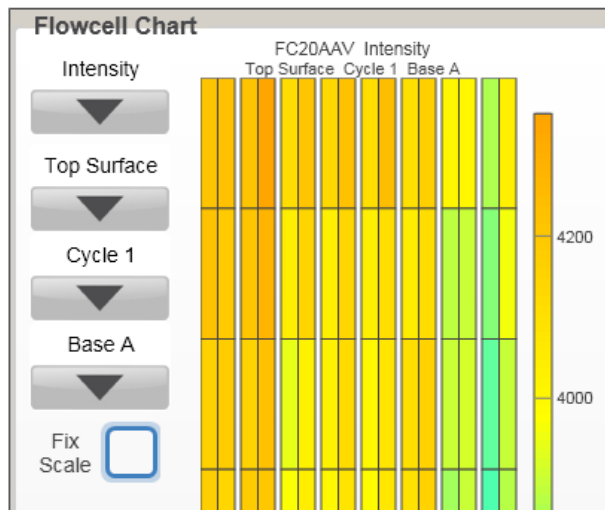


Flow Cell Chart

The Flow Cell Chart displays color-coded quality metrics per tile for the entire flow cell, and has the following features:

- ▶ You can select the displayed metric, surface (if your sequencer scans multiple surfaces), cycle, and base through the dropdown lists.
- ▶ The color bar to the right of the chart indicates the values that the colors represent. The color bar is also a slider: you can adjust the scale values by right-clicking; change the contrast using the mouse-wheel.
- ▶ The chart is displayed with tailored scaling by default, or can be fixed by checking the **Fix Scale** checkbox.
- ▶ Tiles that have not been measured or are not monitored are gray.
- ▶ The interactive tooltips provide the lane, tile and value of the data point.
- ▶ Clicking a tile opens the Imaging Tab with more detailed information for that tile (see *Imaging Tab* on page 17).
- ▶ By right-clicking an image you can copy it to the clipboard.

Figure 7 Flow Cell Chart



You can monitor the following quality metrics with this chart:

- ▶ **Intensity**—This chart displays 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). For a more detailed description, see http://en.wikipedia.org/wiki/Full_width_at_half_maximum.
- ▶ **Corrected Intensity**—The intensity corrected for cross-talk between the color channels and phasing and prephasing.
- ▶ **Called Intensity**—The intensity for the called base.
- ▶ **% Base**—The percentage of clusters for which the selected base has been called.

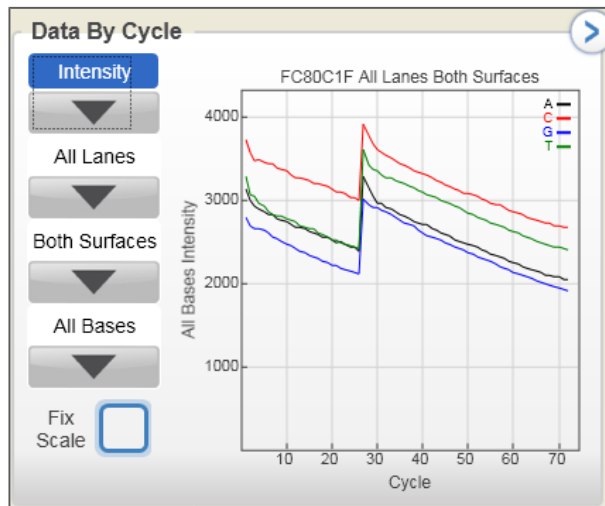
- ▶ **Signal to Noise**—The signal to noise ratio is calculated as mean called intensity divided by standard deviation of non called intensities.
- ▶ **Error Rate**—The calculated error rate, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is 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 not available.
- ▶ **%Q>20, %Q>30**—The percentage of bases with a quality score of 20 or 30 or higher, respectively. These charts are generated after the 25th cycle, and the values represent the current cycle. A check box appears that lets you toggle between cumulative (include previous cycles) or not (just display current cycle).
- ▶ **Median Q-Score**—The median Q-Score over all bases for the current cycle. These charts are generated after the 25th cycle.
- ▶ **Density**—The density of clusters for each tile (in thousands per mm²).
- ▶ **Density PF**—The density of clusters passing filter for each tile (in thousands per mm²).
- ▶ **Clusters**—The number of clusters for each tile (in millions).
- ▶ **Clusters PF**—The number of clusters passing filter for each tile (in millions).
- ▶ **% Phasing, % Prephasing**—The 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 the sample that aligned to the PhiX genome.

Cycle Plot

The Data by Cycle plot displays plots that allow you to follow the progression of quality metrics during a run. These plots have the following features:

- ▶ You can select the displayed metric, lane, surface, and base through the dropdown lists.
- ▶ The plots are displayed with tailored scaling by default, or can be fixed by checking the **Fix Scale** checkbox.
- ▶ The chevron in the top right hand corner toggles the plot between pane view and full screen view.
- ▶ You can pan the graph by clicking-and-dragging, zoom in by using the mousewheel, and zooming in only on a particular axis by using the mouse wheel over that axis.
- ▶ By right-clicking an image you can copy it to the clipboard.

Figure 8 Data by Cycle Plot



You can monitor the following quality metrics with this plot:

- ▶ **Intensity**—This plot displays the intensity by color of the 90% percentile of the data for each cycle.
- ▶ **FWHM**—The average full width of clusters at half maximum (in pixels). For a more detailed description, see http://en.wikipedia.org/wiki/Full_width_at_half_maximum.
- ▶ **Corrected Intensity**—The intensity corrected for cross-talk between the bases and phasing and prephasing.
- ▶ **Called Intensity**—The intensity for the called base.
- ▶ **% Base**—The percentage of clusters for which the selected base has been called.
- ▶ **Signal to Noise**—The signal to noise ratio is calculated as mean called intensity divided by standard deviation of non called intensities.
- ▶ **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 not available.
- ▶ **%Q>20, %Q>30**—The percentage of bases with a quality score of 20 or 30 or higher, respectively. These charts are generated after the 25th cycle, and the values represent the current cycle. A check box appears that lets you toggle between cumulative (include previous cycles) or not (just display current cycle).

Lane Plot

The Data by Lane pane displays plots that allow you to view quality metrics per lane. These plots have the following features:

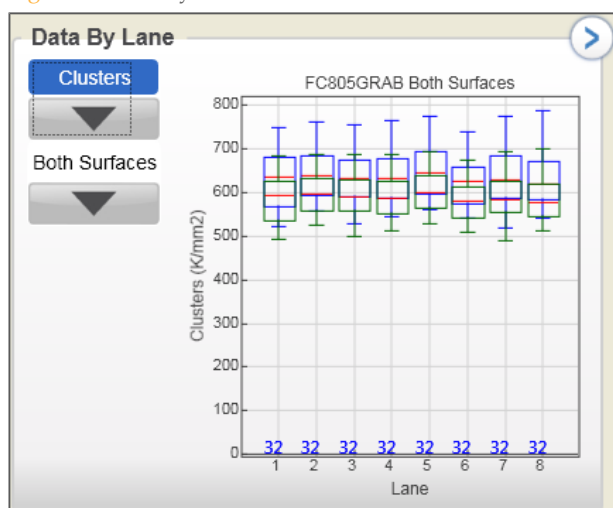
- ▶ You can select the displayed metric, surface, and read (when applicable) through the dropdown lists.
- ▶ The chevron in the top right hand corner toggles the plot between pane view and full screen view.
- ▶ You can zoom in/out and pan the plots.
- ▶ By right-clicking an image you can copy it to the clipboard.

The plots share a number of characteristics.

- The red line indicates the median tile value.

- 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.
- The numbers above the X-axis indicate the number of analyzed tiles used for the data point.

Figure 9 Data by Lane Plot



You can monitor the following quality metrics with this plot (blue boxes are for raw clusters, green boxes for clusters passing filter):

- ▶ **Density**—The density of clusters for each tile (in thousands per mm²).
- ▶ **Clusters**—The number of clusters for each tile (in millions).
- ▶ **% Phasing, % Prephasing**—The percentage of molecules in a cluster for which sequencing falls behind (phasing) or jumps ahead (prephasing) the current cycle within a read. The graphs are split out by read.
- ▶ **% Aligned**—The percentage of the sample that aligned to the PhiX genome. The graphs are split out by read.

QScore Distribution

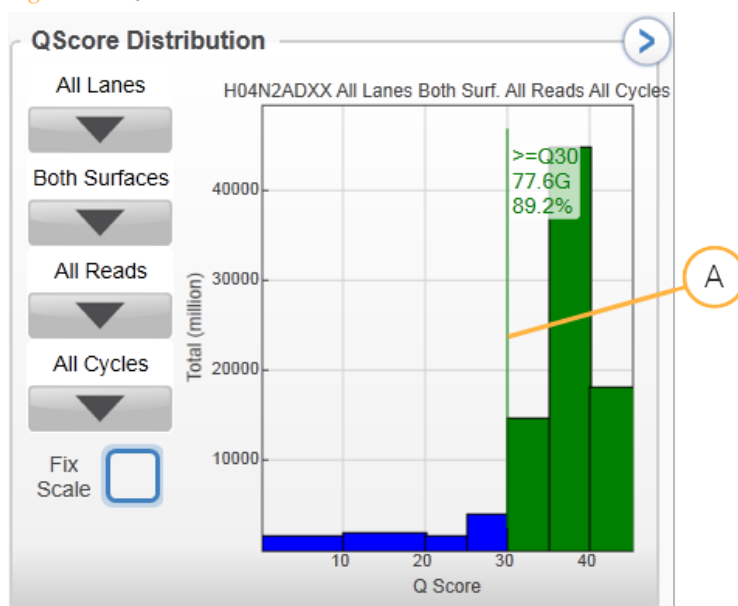
The QScore Distribution pane displays plots that allow you to view the number of reads by quality score. The quality score is cumulative for current cycle and previous cycles, and only reads that pass the quality filter are included. The quality scores are binned in groups: below Qscore 20 in groups of ten, Qscores 20–40 in groups of five, and one group of Qscores above 40.

These plots have the following features:

- ▶ You can select the displayed lane, surface, read, and cycle through the dropdown lists.
- ▶ The **Read** drop-down list is used to select the first cycle for calculating the histogram.
- ▶ The **Cycle** drop-down list is used to determine the last cycle used for calculating the histogram. The last cycle for calculating the histogram is the minimum of the cycle selected in the Cycle drop-down box and the last cycle of read selected in **Read** drop-down list.

- ▶ The cutoff slider allows you to determine how many bases have a minimum QScore or higher. Grab the slider with your mouse pointer, and drop it at the minimum QScore. The Sequencing Analysis Viewer then calculates how many bases have that QScore or higher.
- ▶ The chevron in the top right hand corner toggles the plot between pane view and full screen view.
- ▶ You can pan the graph by clicking-and-dragging, zoom in by using the mousewheel, and zooming in only on a particular axis by using the mouse wheel over that axis.
- ▶ By right-clicking an image you can copy it to the clipboard.

Figure 10 QScore Distribution Plot



A Cutoff Slider

Note that the QScore is based on the Phred scale. The following table list Qscores and the corresponding chance that the base call is wrong:

QScore	Chance of Wrong Base Call
Q10	10 %
Q20	1 %
Q30	0.1 %
Q40	0.01 %

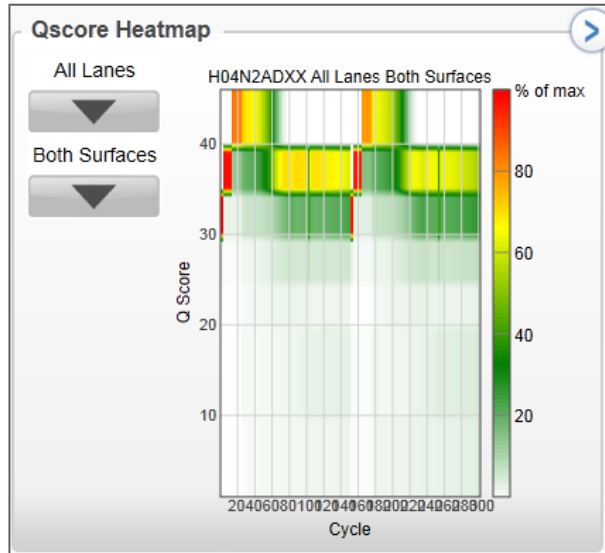
QScore Heatmap

The QScore Heatmap displays plots that allow you to view the QScore by cycle. These plots have the following features:

- ▶ You can select the displayed lane and surface through the dropdown lists.
- ▶ 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. Right-clicking allows you to change the color scheme.
- ▶ The chevron in the top right hand corner toggles the plot between pane view and full screen view.
- ▶ By right-clicking an image you can copy it to the clipboard.

- ▶ The quality score values are binned in groups: below Qscore 20 in groups of ten, Qscores 20–40 in groups of five, and one group of Qscores above 40.

Figure 11 QScore Heatmap



Imaging Tab

The Imaging tab lists detailed data and metrics for the run.

You can select the displayed cycle, lane, surface, swath, and section through the dropdown lists, while the checkboxes can be used to select the displayed bases. In addition, the imaging tab displays available thumbnails for the selected tile, highlighted in blue in the table. You can also use a mouse wheel or the arrow keys on a keyboard to scroll through images. Right-clicking on the top of a column provides more info.

Figure 12 Imaging Tab

Sequencing Analysis Viewer

Run Folder: Y:\101029_P22_0759_BFC805GRAB Browse Refresh

Analysis | **Imaging** | Summary | Tile Status | Controls

Cycle 1 | Lane 1 | Surface Top | Swath All | Section 3

Index	Lane	Tile	Section	Cycle	Surface	Swath	Time	P90 A	P90 C	P90 G	PF
1297	1	1103	3	1	Top	1	10/29/2011...	3326	3944	1729	41%
1298	1	1103	3	2	Top	1	10/29/2011...	3306	3906	1741	41%
1299	1	1103	3	3	Top	1	10/29/2011...	3259	3853	1699	41%
1300	1	1103	3	4	Top	1	10/29/2011...	3211	3806	1720	41%
1301	1	1103	3	5	Top	1	10/30/2011...	3087	3703	1691	37%
1302	1	1103	3	6	Top	1	10/30/2011...	3066	3677	1610	38%
1303	1	1103	3	7	Top	1	10/30/2011...	2959	3554	1577	38%
1304	1	1103	3	8	Top	1	10/30/2011...	2945	3547	1558	38%
1305	1	1103	3	9	Top	1	10/30/2011...	2918	3518	1548	38%
1306	1	1103	3	10	Top	1	10/30/2011...	2906	3491	1536	38%
1307	1	1103	3	11	Top	1	10/30/2011...	2891	3461	1512	38%
1308	1	1103	3	12	Top	1	10/30/2011...	2869	3452	1498	38%
1309	1	1103	3	13	Top	1	10/30/2011...	2835	3423	1510	38%
1310	1	1103	3	14	Top	1	10/30/2011...	2854	3443	1493	38%
1311	1	1103	3	15	Top	1	10/30/2011...	2857	3445	1482	38%
1312	1	1103	3	16	Top	1	10/30/2011...	2811	3392	1452	38%
1313	1	1103	3	17	Top	1	10/30/2011...	2779	3356	1471	38%
1314	1	1103	3	18	Top	1	10/30/2011...	2756	3338	1435	38%
1315	1	1103	3	19	Top	1	10/30/2011...	2778	3364	1419	38%
1316	1	1103	3	20	Top	1	10/30/2011...	2749	3335	1435	38%
1317	1	1103	3	21	Top	1	10/30/2011...	2740	3324	1434	38%
1318	1	1103	3	22	Top	1	10/30/2011...	2692	3271	1441	38%
1319	1	1103	3	23	Top	1	10/30/2011...	2721	3308	1423	38%
1320	1	1103	3	24	Top	1	10/30/2011...	2687	3270	1420	38%
1321	1	1103	3	25	Top	1	10/30/2011...	2656	3229	1398	38%








Rows=20736 Digs=162 Sel=1 Filter

You can track the following metrics:

- ▶ **P90**—This displays the intensity by color of the 90% percentile of the data for each cycle.
- ▶ **Error Rate**—The calculated error rate, as determined by the PhiX alignment.
- ▶ **% Base**—The percentage of clusters for which the selected base has been called. Includes the percentage non-called (%NC).
- ▶ **FWHM**—The average full width of clusters at half maximum (in pixels). For a more detailed description, see http://en.wikipedia.org/wiki/Full_width_at_half_maximum.
- ▶ **Density**—The density of clusters detected by image analysis (in thousands per mm²)
- ▶ **Density PF**—The density of clusters passing filter (in thousands per mm²)
- ▶ **% Aligned**—The percentage of the sample that aligned to the PhiX genome, read 1 and read 2
- ▶ **% Phasing/Prephasing**—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, read 1 and read 2.
- ▶ **Corrected Base**—The intensity corrected for cross-talk between the bases and phasing and prephasing.
- ▶ **Called Base**—The call, by base

- ▶ **Signal to noise**—The signal to noise ratio is calculated as mean called intensity divided by standard deviation of non called intensities.
- ▶ **%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.
- ▶ **%PF**—The percentage of clusters passing filter

The buttons above the data table allow you to do the following:

- ▶ —Select all rows.
- ▶ —Copy selected rows to clipboard.
- ▶ —Sort columns in ascending, descending, or custom fashion.
- ▶ —Create custom scatter or box plot.
- ▶ —Choose columns to display.
- ▶ —Filter columns, or clear filter.
- ▶ —Move thumbnail selection up or down.

Summary Tab

The Summary link leads to tables with basic data quality metrics summarized per lane and per read. All the statistics are given as means and standard deviations over the tiles used in the lane. The following metrics are displayed in the top table, split out by read and total:

- ▶ **Yield Total**—The number of bases sequenced.
- ▶ **Projected Total Yield**—The projected number of bases sequenced at the end of the run.
- ▶ **Yield Perfect**—The number of bases in 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 not available.
- ▶ **Yield <=3 errors**—The number of bases in reads that align with 3 errors or less, as determined by a spiked in PhiX control sample. If no PhiX control sample is run in the lane, this chart is not available.
- ▶ **Aligned**—The percentage of the sample that aligned to the PhiX genome.
- ▶ **% Perfect [Num Cycles]**—The percentage of bases in reads that align perfectly, as determined by a spiked in PhiX control sample, at the cycle indicated in the brackets. If no PhiX control sample is run in the lane, this chart is not available.
- ▶ **% <=3 errors [Num Cycles]**—The percentage of bases in reads that align with 3 errors or less, as determined by a spiked in PhiX control sample, at the indicated cycle. If no PhiX control sample is run in the lane, this chart is not available.
- ▶ **Error Rate**—The calculated error rate, as determined by the PhiX alignment.
- ▶ **Intensity Cycle 1**—The average of the four intensities (one per channel or base type) measured at the first cycle averaged over filtered clusters.
- ▶ **% Intensity Cycle 20**—The corresponding intensity statistic at cycle 20 as a percentage of that at the first cycle.
- ▶ **%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 mm²) detected by image analysis.
- ▶ **Clusters PF**—The percentage of clusters passing filtering.
- ▶ **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.
- ▶ **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.
- ▶ **Cycles Err Rated**—The number of cycles that have been error rated starting at cycle 1.
- ▶ **Aligned**—The percentage of the sample 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 four intensities (one per channel or base type) measured at the first cycle averaged over filtered clusters.

- ▶ **%Intensity Cycle 20**—The corresponding intensity statistic at cycle 20 as a percentage of that at the first cycle.

In addition, at the bottom of the page there are Copy to Clipboard and Generate IVC Plot buttons, so you can copy the data to your computer, and generate your IVC plots at any time during the run.

Tile Status Tab

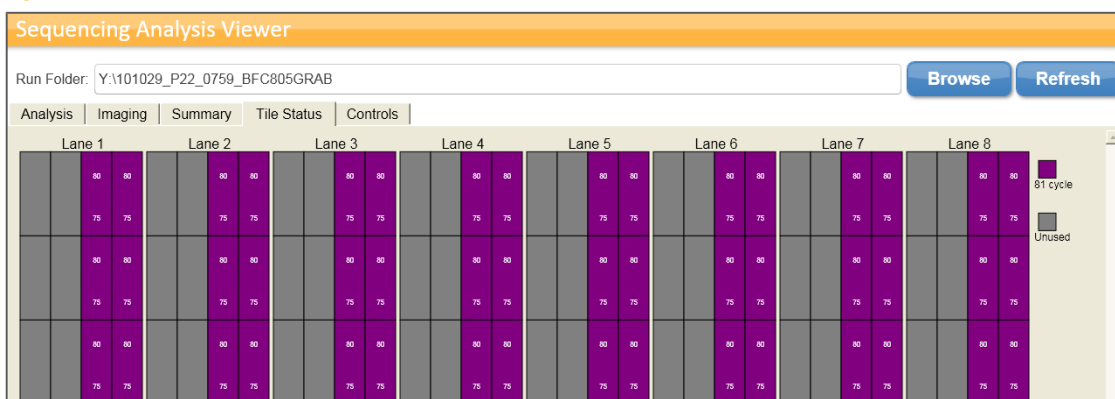
The Tile Status tab shows the progress of the analysis. The colors indicate the status of the image analysis for a tile for a particular cycle, as indicated in the legend to the right.

The tiles are organized by lane, and organized the following way:

- ▶ For the HiSeq System, each lane has four columns. The first two columns are for the top surface of that lane, swath 1 and swath 2 respectively. A swath is a column of tiles in one lane, and there are two swaths per tile surface. The last two columns are for swath 1 and swath 2 of the bottom surface.
- ▶ For the Genome Analyzer System, each lane has two columns.
- ▶ The MiSeq has one lane.

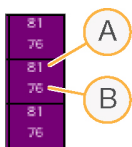
Clicking the **Refresh** button provides the most recent data.

Figure 13 Tile Status



In every tile, two numbers indicate the progress of base calling and quality scoring. For every tile, the top number indicates the last cycle that has been base called, and the bottom number indicates the last cycle for which quality has been scored.

Figure 14 Base Calling and Quality Scoring Progress



- A Last Base-Called Cycle for Tile
- B Last Quality-Scored Cycle for Tile

TruSeq Controls Tab

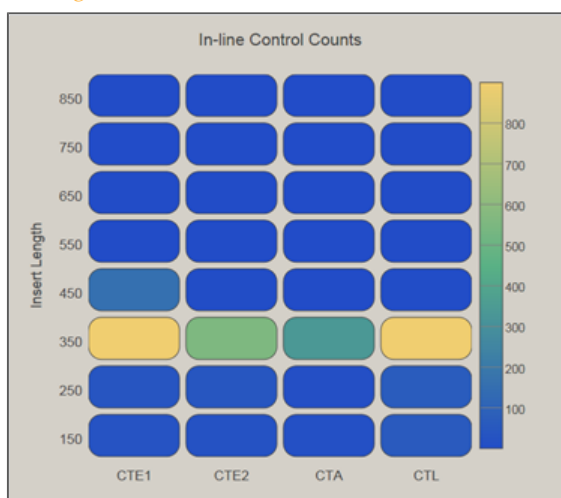
Sequencing Analysis Viewer reports the results of the analysis of the in-line controls on the TruSeq Controls tab. You can select the lane using the dropdown lists.

The in-line controls are added during the sample prep to check whether the sample prep steps are performed properly, and RTA fishes them out of the samples and provides the data that the Sequencing Analysis Viewer can report. The following controls are included:

Step	Reaction	Enzyme	Control
End-Repair	Chew-back of 3' overhangs/ fill-in of 5' overhangs	DNA Polymerase	CTE1
End-Repair	Phosphorylation of 5' OH groups	Polynucleotide Kinase	CTE2
A Tailing	Add 3' A overhang	DNA Polymerase	CTA
Adapter ligation	Join adapters to inserts	DNA Ligase	CTL

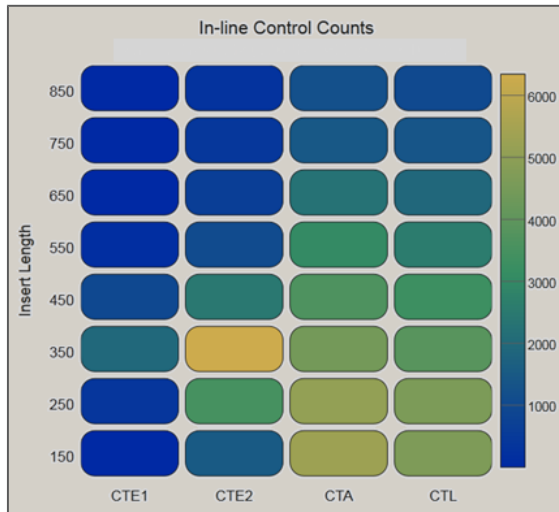
A successful run should have a positive signal for all four controls at one or two adjacent sizes. The reported size should correspond to the excised fragment. The absolute number of reads for each control does not matter much, but there should be a clear peak near the expected size. An example of a successful run for a TruSeq DNA 350 bp gel excised fragment is shown below.

Figure 15 In-Line Controls in a Successful Run



Libraries prepared with the TruSeq DNA PCR-Free Sample Prep kit will show a narrow size distribution for CTE1 and CTE2, however, CTA and CTL size distributions will be broad as the size selection step occurs upstream of the addition of these controls. An example of a successful run with a 350 bp size selected insert is shown below.

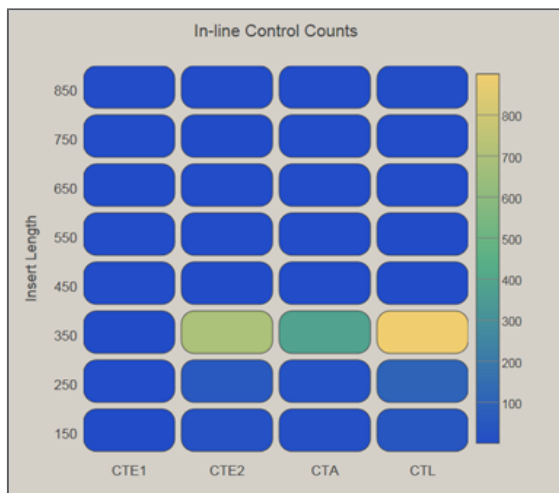
Figure 16 In-Line Controls in a Successful TruSeq DNA PCR-Free Run



If a run was unsuccessful due to an inefficient step in the sample prep, the controls would look like one of the following examples:

Inefficient End-Repair due to a Problem with DNA Polymerase

Figure 17 In-Line Control CTE1 Problem



Inefficient End-Repair due to a Problem with Polynucleotide Kinase

Figure 18 In-Line Control CTE2 Problem



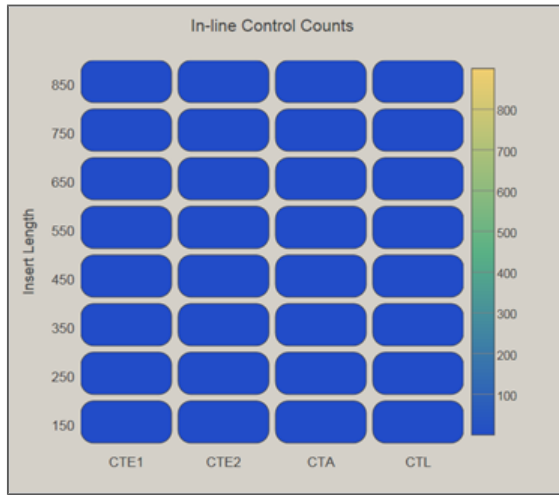
Inefficient A Tailing due to a Problem with DNA Polymerase

Figure 19 In-Line Control CTA Problem



Inefficient Adapter Ligation due to a Problem with DNA Ligase

Figure 20 In-Line Control CTL Problem



Indexing Tab

The Indexing tab lists count information for indices used in the run. Note that the Indexing tab will only be available if a sample sheet was supplied to the instrument control software at the start of the run and the run is an index run.

You can select the displayed lane through the dropdown list.

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

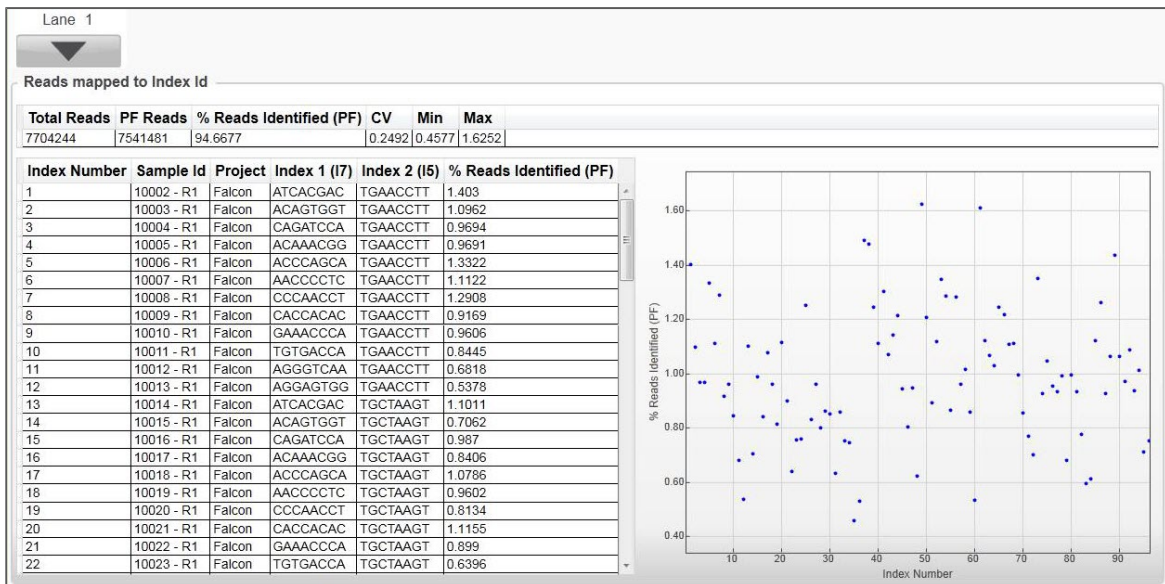
- ▶ **Total Reads**—The total number of reads for this lane.
- ▶ **PF Reads**—The total number of passing filter reads for this lane.
- ▶ **% Reads Identified (PF)**—The total fraction of passing filter reads assigned to an index.
- ▶ **CV**—The coefficient of variation for the number of counts across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

Further information is provided regarding the frequency of individual indices in both table and graph form. The table contains several columns, including

- ▶ **Index Number**—A unique number assigned to each index by Sequencing Analysis Viewer 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 (in terms of % Passing Filter reads) mapped to this index.

This information is also displayed in graphical form. In the graphical display, indices are ordered according to the unique Index Number assigned by Sequencing Analysis Viewer.

Figure 21 Indexing Tab



Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

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

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

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

Product Documentation

Additional product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support and select a product. A MyIllumina login is required. To register for a MyIllumina account, please visit my.illumina.com/Account/Register.



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