



# HiSeq Run Monitoring

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# Session Objectives

- ▶ By the end of this training, you will be able to:
  - List the two options to monitor a run
  - Describe the functionality of SAV

# HiSeq Run Monitoring

## Sequencing Screen in HCS

Monitor:  
On-instrument analysis  
Fluidics  
imaging

## Sequencing Analysis Viewer (SAV)

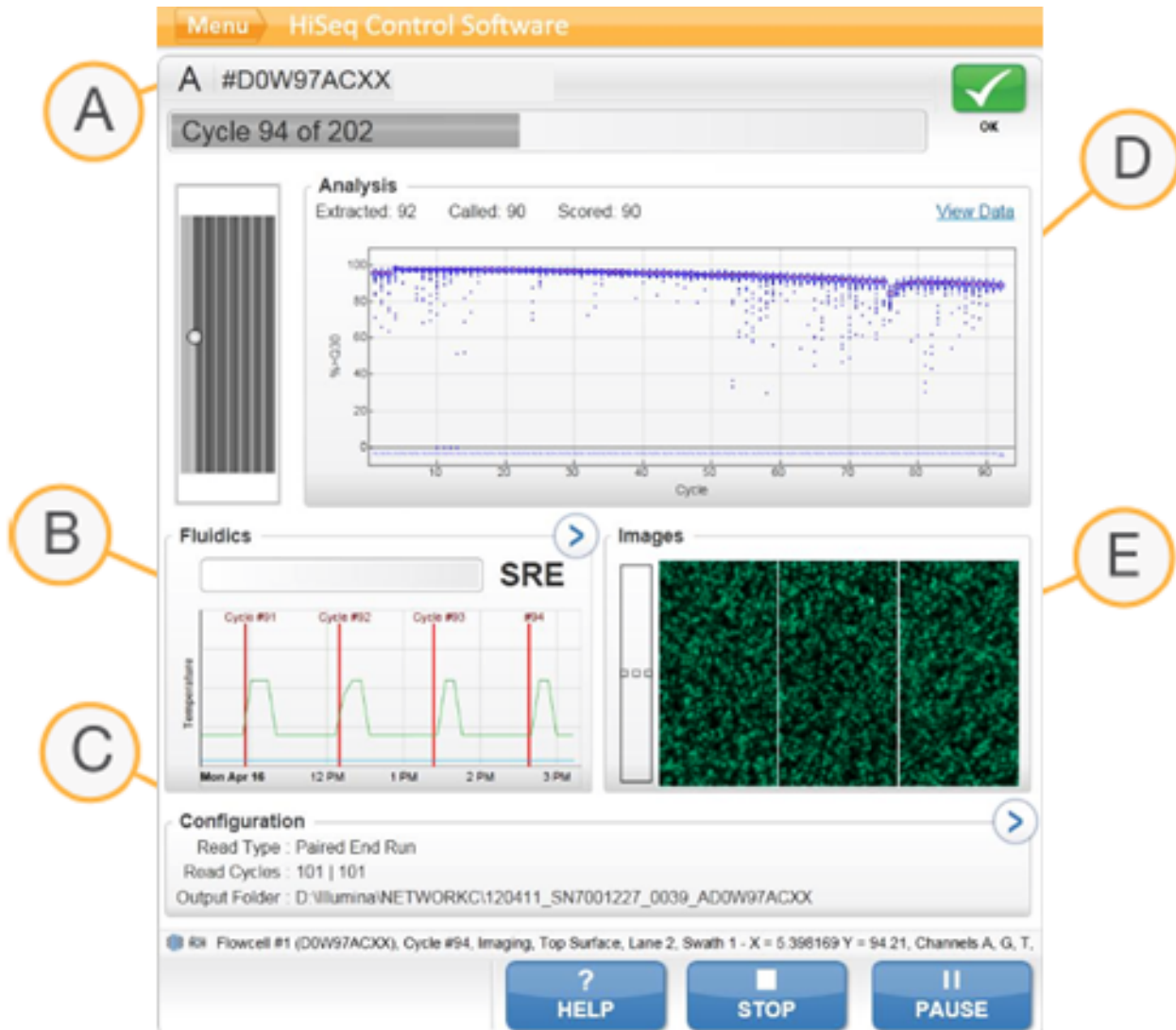
Monitor: Run in greater  
detail

Launches automatically  
after image analysis  
begins and displays the  
status page



# Monitoring a Run using HiSeq Control Software

# Viewing Run Metrics on the Run Overview Screen



- A Run Progress
- B Fluidics and Temperature
- C Run Configuration
- D Analysis
- E Images



# Viewing Run Metrics using Sequencing Analysis Viewer

# Sequencing Analysis Viewer (SAV)

## Files used by SAV



- runinfo.xml
- runparameters.xml
- Interop files
- Thumbnail files (optional)

## Data Availability



- Intensities after Cycle 4
- Phasing/Prephasing after Cycle 12
- Clusters Passing Filter (PF) after Cycle 25
- Quality scoring after Cycle 25
- PhiX alignment and error rates after Cycle 26

# Analysis Tab

## Sequencing Analysis Viewer

Run Folder: E:\Illumina\HiSeqTemp\120411\_SN7001227\_0040\_BC0P14ACXX

Browse

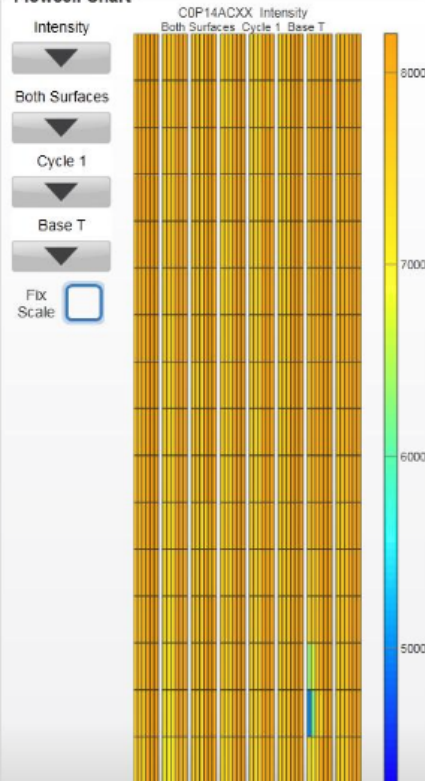
Refresh

Analysis | Inaging | Summary | Tile Status | TruSeq Controls

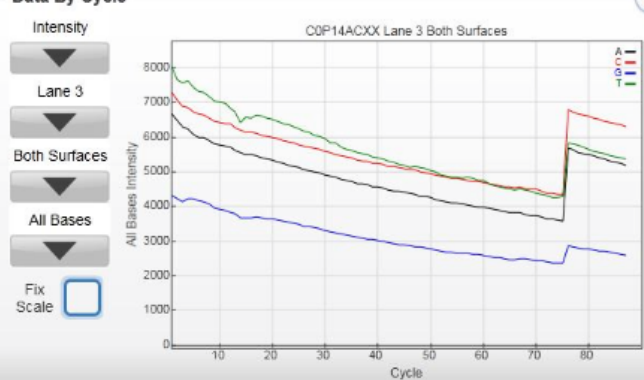
### Status

Extracted: 87    Called: 85    Scored: 85

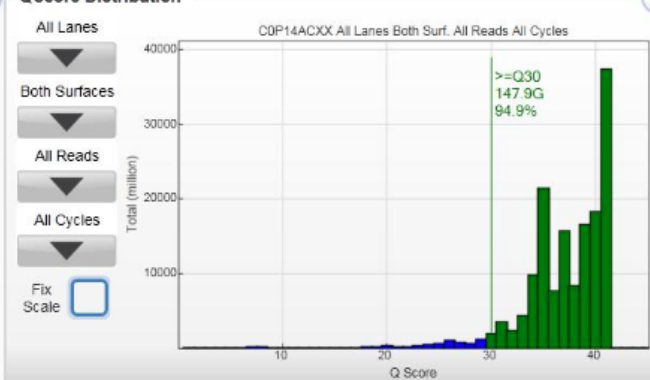
### Flowcell Chart



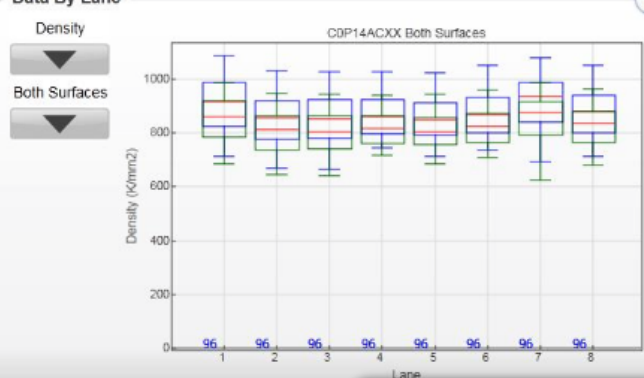
### Data By Cycle



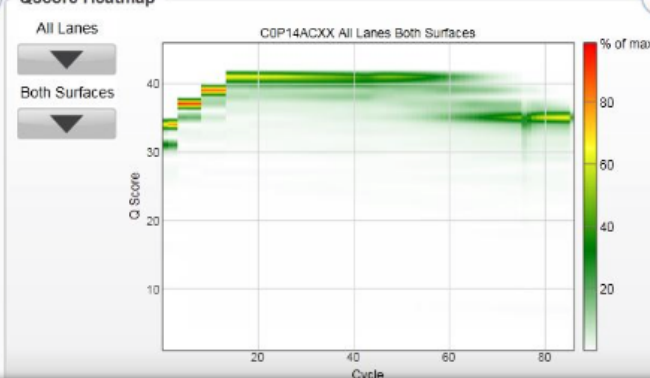
### QScore Distribution



### Data By Lane



### Qscore Heatmap



Run Metrics can also be reviewed offline from any computer with access to the run folder



# Imaging Tab

## Sequencing Analysis Viewer

Run Folder: E:\Illumina\HiSeqTemp\120411\_SN7001227\_0040\_BC0P14ACXX

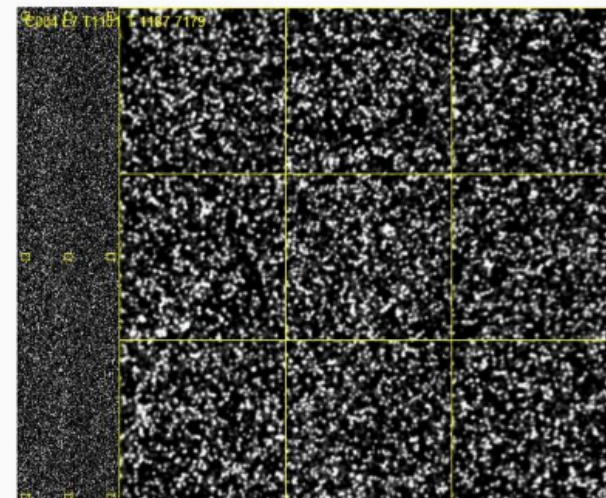
Browse Refresh

Analysis **Imaging** Summary Tile Status TruSeq Controls

Cycle All Lane 7 Surface Top Swath All Section All

A  C  G  T

Index	Lane	Tile	Section	Cycle	Surface	Swath	Time	P90 A	P90 C	P90 G	P90 T	Error Rate	%A	%C	
523	7	1101	1	1	Top	Left	04/12/201...	6681	7293	4234	7761	0.148	30.4	22.1	2
524	7	1101	1	2	Top	Left	04/12/201...	6417	7029	4084	7234	0.131	30.9	20.1	2
525	7	1101	1	3	Top	Left	04/12/201...	6255	6882	4002	7387	0.084	29.3	22.2	2
526	7	1101	1	4	Top	Left	04/12/201...	6246	6880	4093	7446	0.086	29.0	22.2	2
527	7	1101	1	5	Top	Left	04/12/201...	6111	6776	4138	7188	0.074	29.6	21.2	2
528	7	1101	1	6	Top	Left	04/12/201...	6083	6740	4015	7124	0.120	28.8	22.6	2
529	7	1101	1	7	Top	Left	04/12/201...	5996	6658	4018	7050	0.077	29.5	21.8	2
530	7	1101	1	8	Top	Left	04/12/201...	5835	6518	3996	6883	0.069	29.4	22.1	2
531	7	1101	1	9	Top	Left	04/12/201...	5813	6483	3833	6748	0.106	29.0	22.3	2
532	7	1101	1	10	Top	Left	04/12/201...	5850	6508	3856	6833	0.082	29.8	22.3	2
533	7	1101	1	11	Top	Left	04/12/201...	5721	6390	3832	6694	0.091	29.6	22.1	2
534	7	1101	1	12	Top	Left	04/12/201...	5769	6434	3758	6651	0.087	29.2	22.3	2
535	7	1101	1	13	Top	Left	04/12/201...	5640	6311	3680	6460	0.083	29.8	22.1	2
536	7	1101	1	14	Top	Left	04/12/201...	5537	6207	3610	6197	0.070	29.9	22.0	2
537	7	1101	1	15	Top	Left	04/12/201...	5541	6199	3583	6347	0.076	29.2	22.4	2
538	7	1101	1	16	Top	Left	04/12/201...	5526	6185	3564	6302	0.077	29.8	22.1	2
539	7	1101	1	17	Top	Left	04/12/201...	5495	6165	3621	6447	0.088	29.7	22.0	2
540	7	1101	1	18	Top	Left	04/12/201...	5423	6104	3582	6419	0.083	29.1	22.7	2
541	7	1101	1	19	Top	Left	04/12/201...	5444	6112	3546	6361	0.088	29.5	22.4	2
542	7	1101	1	20	Top	Left	04/12/201...	5407	6079	3506	6317	0.086	29.6	22.2	2
543	7	1101	1	21	Top	Left	04/12/201...	5337	6021	3465	6277	0.080	29.1	22.7	2
544	7	1101	1	22	Top	Left	04/13/201...	5313	5984	3442	6187	0.073	29.6	22.3	2
545	7	1101	1	23	Top	Left	04/13/201...	5233	5923	3432	6118	0.083	29.4	22.2	2
546	7	1101	1	24	Top	Left	04/13/201...	5233	5908	3423	6124	0.085	29.1	22.6	2
547	7	1101	1	25	Top	Left	04/13/201...	5183	5866	3376	6044	0.095	29.5	22.4	2
548	7	1101	1	26	Top	Left	04/13/201...	5095	5799	3370	5986	0.108	29.2	22.4	2
549	7	1101	1	27	Top	Left	04/13/201...	5060	5764	3311	5886	0.107	29.0	22.7	2



To create a thumbnail image, HCS combines nine images from left, center, and right sections of the top, center, and bottom regions of a tile

# Summary Tab

## Sequencing Analysis Viewer

Run Folder: E:\Illumina\HiSeqTemp\120411\_SN7001227\_0040\_BC0P14ACXX

[Browse](#) [Refresh](#)

Analysis **Imaging** **Summary** File Status TruSeq Controls

### Run Summary

Level	Yield Total (G)	Projected Total Yield (G)	Yield Perfect (G)	Yield <-3 errors (G)	Aligned (%)	% Perfect [Num Cycles]	% <-3 errors [Num Cycles]	Error Rate (%)	Intensity Cycle 1	% Intensity Cycle 20	% >= Q30
Read 1	157.0	182.6	49.0	54.1	99.22	31.5 [86]	34.7 [86]	0.20	6713	79.6	94.9
Read 2	0.0	182.6	0.0	0.0	0.00	0.0 [0]	0.0 [0]	0.00	0	0.0	NaN
Total	157.0	365.2	49.0	54.1	99.22	31.5	34.7	0.20	6713	79.6	94.9

### Read 1

Lane	Tiles	Density (K/mm2)	Cluster PF (%)	Phas/Prephas (%)	Reads (M)	Reads PF (M)	% >= Q30	Cycles Err Rated	Aligned (%)	Error Rate (%)	Error Rate 35 cycle (%)	Error Rate 75 cycle (%)	Error Rate 100 cycle (%)	Intensity Cycle 1	% Intensity Cycle 20
1	96	913 +/- 94	93.82 +/- 1.37	0.199 / 0.189	252.34	236.40	94.5	85 - 86	99.2 +/- 0.0	0.21 +/- 0.09	0.11 +/- 0.08	0.18 +/- 0.10	0.00 +/- 0.00	6946 +/- 198	79.5 +/- 0.9
2	96	856 +/- 85	94.58 +/- 1.26	0.136 / 0.186	236.79	223.67	95.0	85 - 86	99.2 +/- 0.0	0.18 +/- 0.02	0.10 +/- 0.03	0.16 +/- 0.02	0.00 +/- 0.00	6542 +/- 301	81.1 +/- 1.0
3	96	856 +/- 85	94.43 +/- 1.34	0.202 / 0.192	236.58	223.14	95.1	85 - 86	99.2 +/- 0.1	0.21 +/- 0.08	0.14 +/- 0.14	0.18 +/- 0.09	0.00 +/- 0.00	6682 +/- 223	79.2 +/- 1.1
4	96	865 +/- 75	94.54 +/- 1.12	0.194 / 0.193	239.02	225.75	95.0	85	99.2 +/- 0.4	0.22 +/- 0.11	0.14 +/- 0.13	0.19 +/- 0.12	0.00 +/- 0.00	6645 +/- 232	79.3 +/- 0.9
5	96	856 +/- 76	94.70 +/- 1.06	0.195 / 0.194	236.69	223.94	95.2	85 - 86	99.2 +/- 0.1	0.20 +/- 0.09	0.14 +/- 0.13	0.17 +/- 0.06	0.00 +/- 0.00	6544 +/- 181	79.3 +/- 2.0
6	96	875 +/- 80	94.50 +/- 1.19	0.195 / 0.194	241.80	228.26	94.8	85 - 86	99.2 +/- 0.0	0.22 +/- 0.15	0.11 +/- 0.04	0.19 +/- 0.12	0.00 +/- 0.00	6627 +/- 213	79.5 +/- 0.9
7	96	917 +/- 92	93.58 +/- 1.84	0.195 / 0.188	253.51	236.95	94.5	85 - 86	99.2 +/- 0.0	0.19 +/- 0.07	0.10 +/- 0.01	0.16 +/- 0.06	0.00 +/- 0.00	6858 +/- 521	79.4 +/- 0.8
8	96	874 +/- 87	94.45 +/- 1.16	0.201 / 0.188	241.67	228.00	95.0	85 - 86	99.2 +/- 0.0	0.18 +/- 0.02	0.10 +/- 0.04	0.16 +/- 0.02	0.00 +/- 0.00	6800 +/- 332	79.4 +/- 1.4

### Read 2

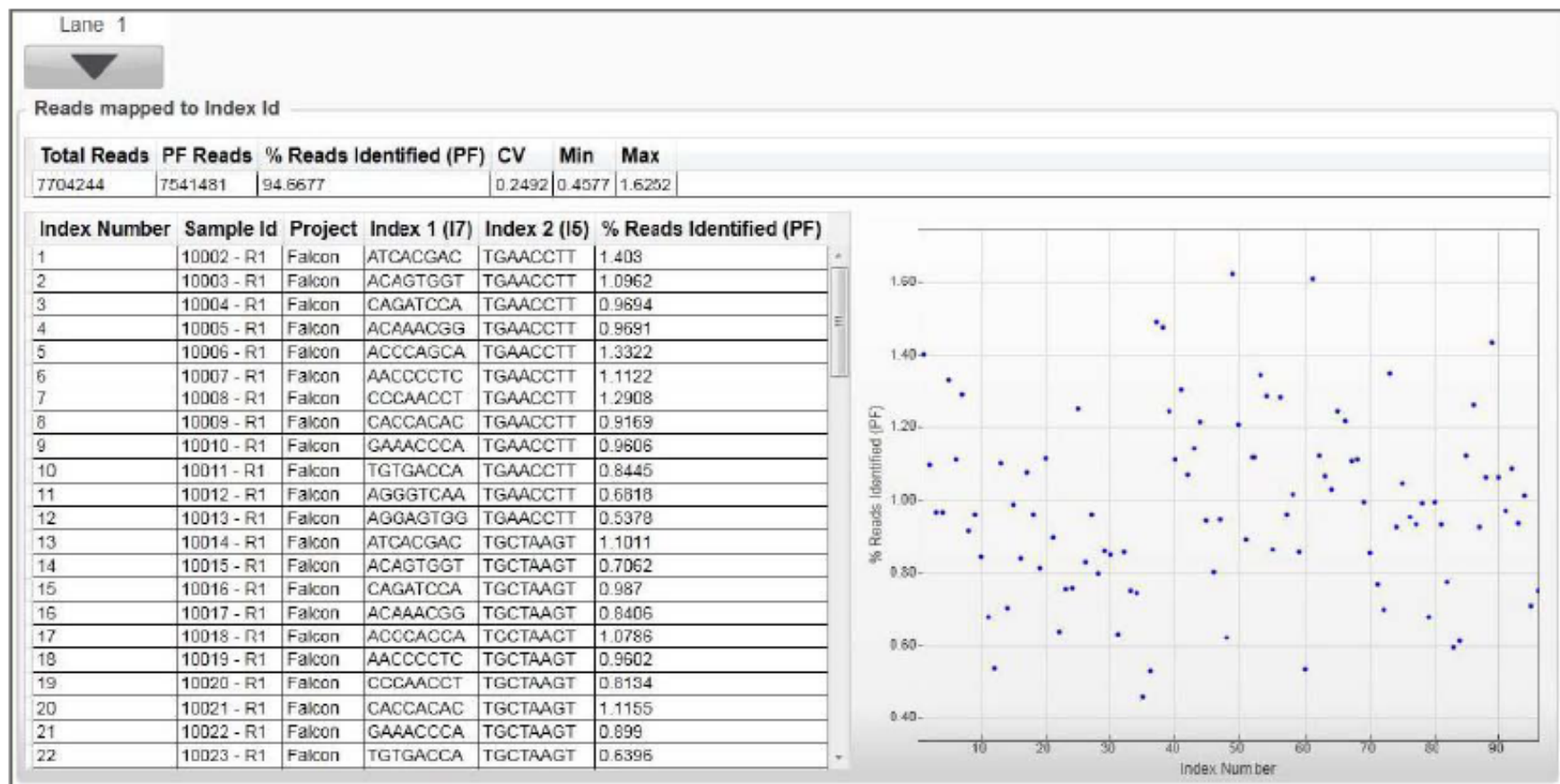
Lane	Tiles	Density (K/mm2)	Cluster PF (%)	Phas/Prephas (%)	Reads (M)	Reads PF (M)	% >= Q30	Cycles Err Rated	Aligned (%)	Error Rate (%)	Error Rate 35 cycle (%)	Error Rate 75 cycle (%)	Error Rate 100 cycle (%)	Intensity Cycle 1	% Intensity Cycle 20
1	96	913 +/- 94	93.82 +/- 1.37	0.000 / 0.000	252.34	236.40	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
2	96	856 +/- 85	94.58 +/- 1.26	0.000 / 0.000	236.79	223.67	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
3	96	856 +/- 85	94.43 +/- 1.34	0.000 / 0.000	236.58	223.14	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
4	96	865 +/- 75	94.54 +/- 1.12	0.000 / 0.000	239.02	225.75	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
5	96	856 +/- 76	94.70 +/- 1.06	0.000 / 0.000	236.69	223.94	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
6	96	875 +/- 80	94.50 +/- 1.19	0.000 / 0.000	241.80	228.26	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
7	96	917 +/- 92	93.58 +/- 1.84	0.000 / 0.000	253.51	236.95	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0
8	96	874 +/- 87	94.45 +/- 1.16	0.000 / 0.000	241.67	228.00	NaN	0	0.0 +/- 0.0	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0.00 +/- 0.00	0 +/- 0	0.0 +/- 0.0

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# Indexing tab



The Indexing tab is only available if a sample sheet was used at the start of the run and the run is an index run

# Summary of Run Monitoring

- ▶ Sequencing run metrics can be viewed:
  - on the HiSeq during a run
  - using SAV at anytime
- ▶ Run metrics are populated at different cycles during a run
  - Intensities after cycle 4
  - Phasing/Prephasing after cycle 12
  - Clusters passing filter after cycle 25
  - Quality scoring after cycle 25
  - PhiX alignment and error rates after cycle 26
- ▶ Absolute or relative values for run metrics are dependent on the type of library
  - Trending of run metrics is highly recommended to identify outliers



Questions?