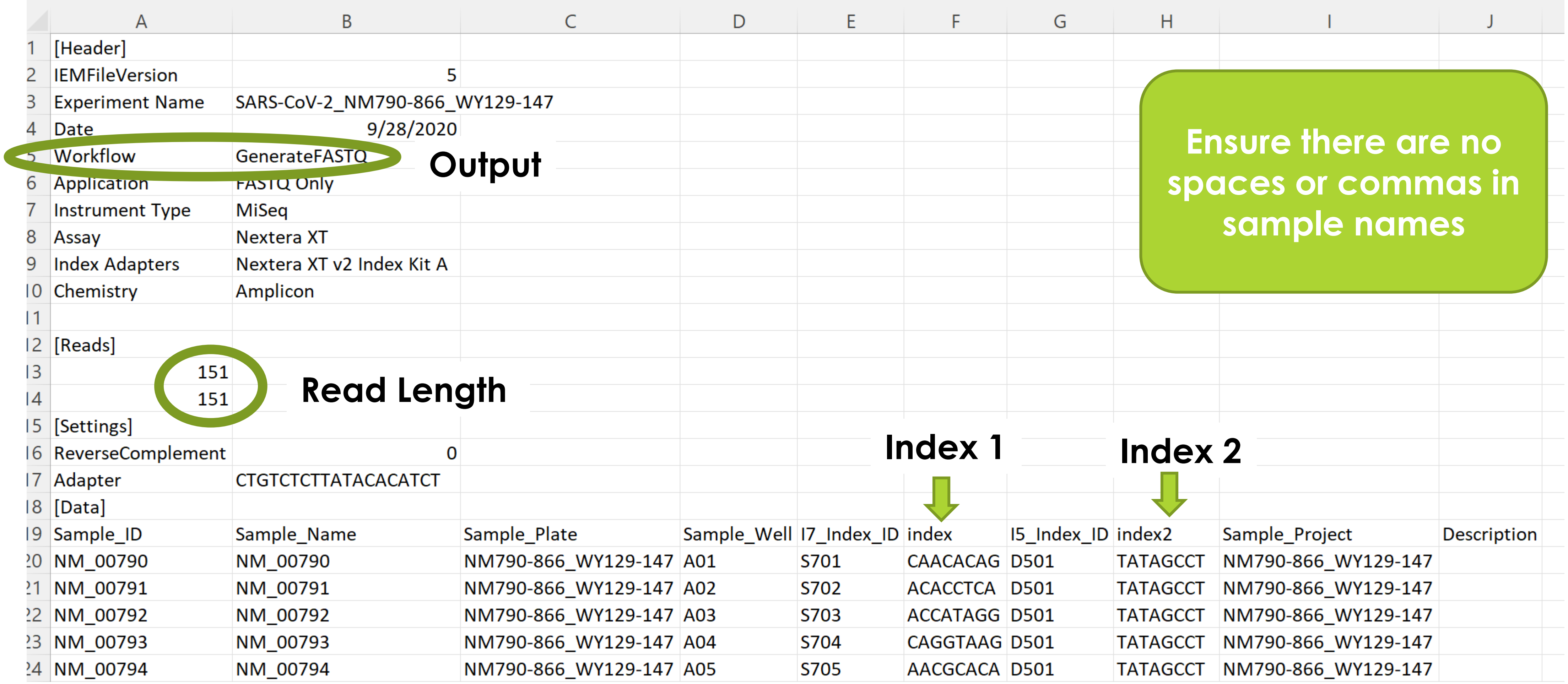
5- Illumina Sample Sheets, Sequencing Analysis Viewer & Troubleshooting

DARRELL L. DINWIDDIE, PHD
DARYL B. DOMMAN, PHD

Illumina Sample Sheet

Illumina Sample Sheet



	A	B	C	D	E	F	G	H	I	J
1	[Header]									
2	IEMFileVersion	5								
3	Experiment Name	SARS-CoV-2_NM790-866_WY129-147								
4	Date	9/28/2020								
5	Workflow	GenerateFASTQ								
6	Application	FASTQ Only								
7	Instrument Type	MiSeq								
8	Assay	Nextera XT								
9	Index Adapters	Nextera XT v2 Index Kit A								
10	Chemistry	Amplicon								
11										
12	[Reads]									
13	151									
14	151									
15	[Settings]									
16	ReverseComplement	0								
17	Adapter	CTGTCTCTTATACACATCT								
18	[Data]									
19	Sample_ID	Sample_Name	Sample_Plate	Sample_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
20	NM_00790	NM_00790	NM790-866_WY129-147	A01	S701	CAACACAG	D501	TATAGCCT	NM790-866_WY129-147	
21	NM_00791	NM_00791	NM790-866_WY129-147	A02	S702	ACACCTCA	D501	TATAGCCT	NM790-866_WY129-147	
22	NM_00792	NM_00792	NM790-866_WY129-147	A03	S703	ACCATAGG	D501	TATAGCCT	NM790-866_WY129-147	
23	NM_00793	NM_00793	NM790-866_WY129-147	A04	S704	CAGGTAAG	D501	TATAGCCT	NM790-866_WY129-147	
24	NM_00794	NM_00794	NM790-866_WY129-147	A05	S705	AACGCACA	D501	TATAGCCT	NM790-866_WY129-147	

Output

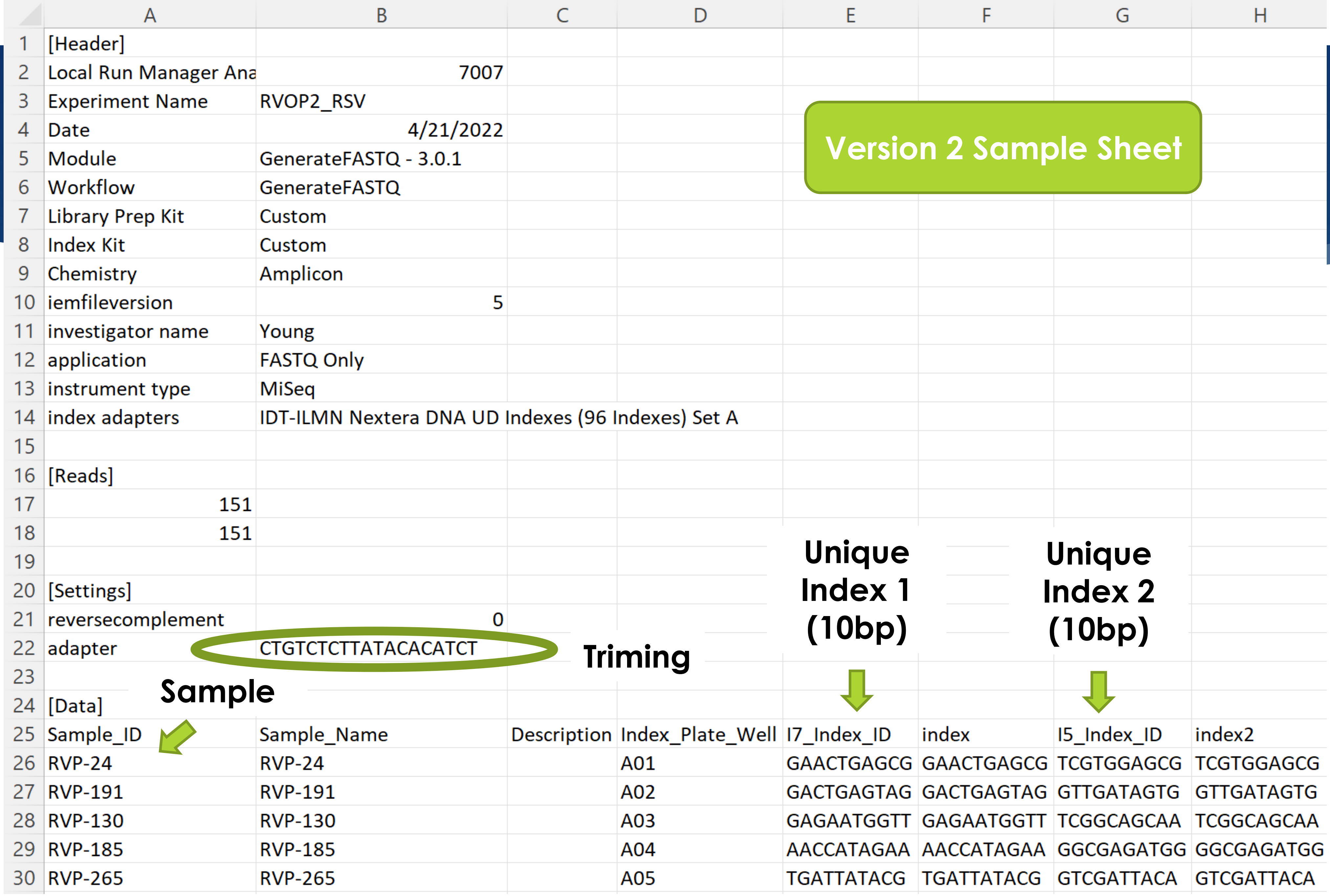
Read Length

Index 1

Index 2

Ensure there are no spaces or commas in sample names

Version 2 Sample Sheet



	A	B	C	D	E	F	G	H
1	[Header]							
2	Local Run Manager Ana	7007						
3	Experiment Name	RVOP2_RSV						
4	Date	4/21/2022						
5	Module	GenerateFASTQ - 3.0.1						
6	Workflow	GenerateFASTQ						
7	Library Prep Kit	Custom						
8	Index Kit	Custom						
9	Chemistry	Amplicon						
10	iemfileversion	5						
11	investigator name	Young						
12	application	FASTQ Only						
13	instrument type	MiSeq						
14	index adapters	IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A						
15								
16	[Reads]							
17	151							
18	151							
19								
20	[Settings]							
21	reversecomplement	0						
22	adapter	CTGTCTCTTATACACATCT						
23								
24	[Data]							
25	Sample_ID	Sample_Name	Description	Index_Plate_Well	I7_Index_ID	index	I5_Index_ID	index2
26	RVP-24	RVP-24		A01	GAACTGAGCG	GAACTGAGCG	TCGTGGAGCG	TCGTGGAGCG
27	RVP-191	RVP-191		A02	GACTGAGTAG	GACTGAGTAG	GTTGATAGTG	GTTGATAGTG
28	RVP-130	RVP-130		A03	GAGAATGGTT	GAGAATGGTT	TCGGCAGCAA	TCGGCAGCAA
29	RVP-185	RVP-185		A04	AACCATAGAA	AACCATAGAA	GGCGAGATGG	GGCGAGATGG
30	RVP-265	RVP-265		A05	TGATTATACG	TGATTATACG	GTCGATTACA	GTCGATTACA

Triming

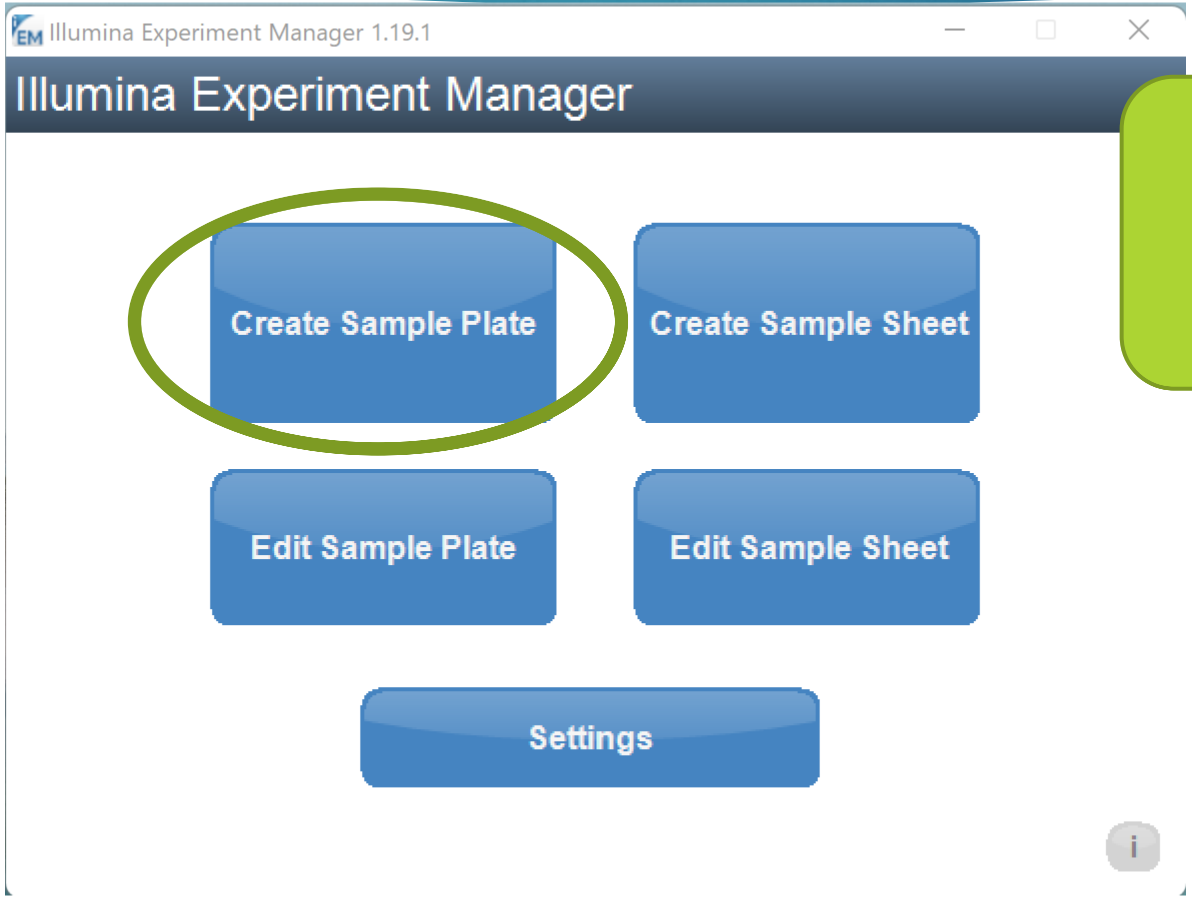
Sample

Unique Index 1 (10bp)

Unique Index 2 (10bp)

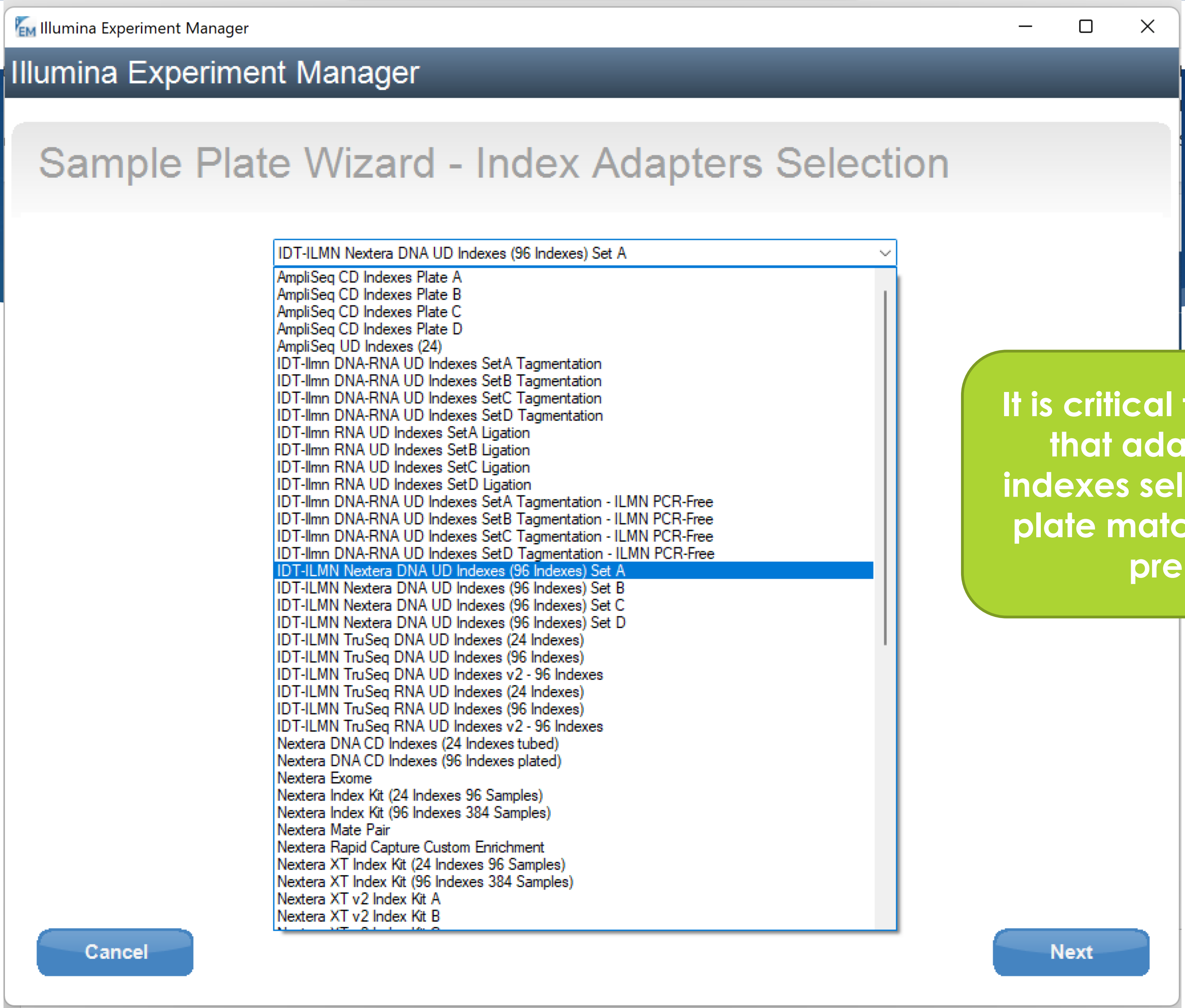
Illumina Sample Sheet- Experiment Manager

Illumina Experiment Manager



A “Sample Plate” can be created before a sample sheet

https://support.illumina.com/sequencing/sequencing_software/experiment_manager/downloads.html

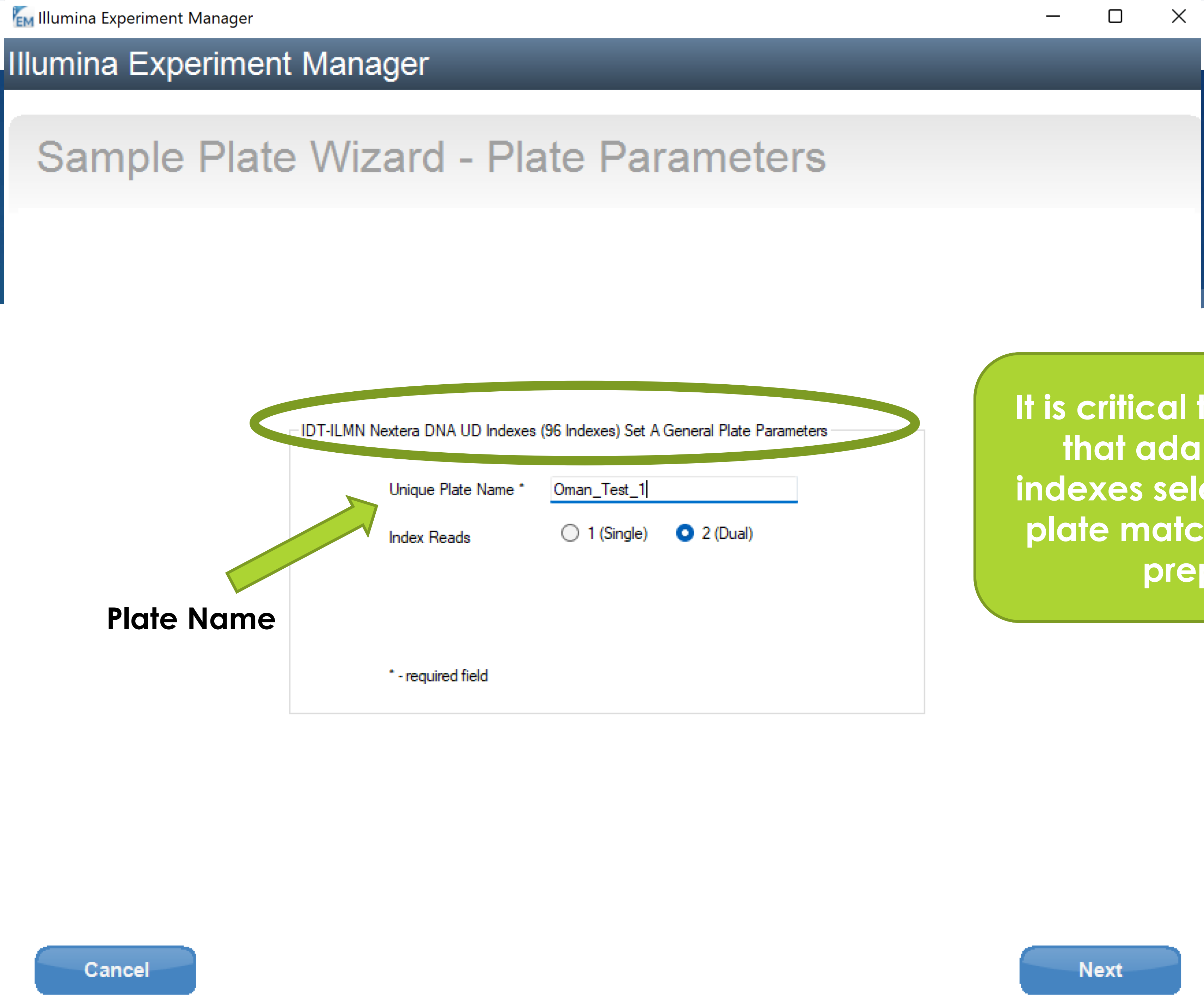


Sample Plate Wizard - Index Adapters Selection

IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A

- AmpliSeq CD Indexes Plate A
- AmpliSeq CD Indexes Plate B
- AmpliSeq CD Indexes Plate C
- AmpliSeq CD Indexes Plate D
- AmpliSeq UD Indexes (24)
- IDT-Ilmn DNA-RNA UD Indexes SetA Tagmentation
- IDT-Ilmn DNA-RNA UD Indexes SetB Tagmentation
- IDT-Ilmn DNA-RNA UD Indexes SetC Tagmentation
- IDT-Ilmn DNA-RNA UD Indexes SetD Tagmentation
- IDT-Ilmn RNA UD Indexes SetA Ligation
- IDT-Ilmn RNA UD Indexes SetB Ligation
- IDT-Ilmn RNA UD Indexes SetC Ligation
- IDT-Ilmn RNA UD Indexes SetD Ligation
- IDT-Ilmn DNA-RNA UD Indexes SetA Tagmentation - ILMN PCR-Free
- IDT-Ilmn DNA-RNA UD Indexes SetB Tagmentation - ILMN PCR-Free
- IDT-Ilmn DNA-RNA UD Indexes SetC Tagmentation - ILMN PCR-Free
- IDT-Ilmn DNA-RNA UD Indexes SetD Tagmentation - ILMN PCR-Free
- IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A
- IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set B
- IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set C
- IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set D
- IDT-ILMN TruSeq DNA UD Indexes (24 Indexes)
- IDT-ILMN TruSeq DNA UD Indexes (96 Indexes)
- IDT-ILMN TruSeq DNA UD Indexes v2 - 96 Indexes
- IDT-ILMN TruSeq RNA UD Indexes (24 Indexes)
- IDT-ILMN TruSeq RNA UD Indexes (96 Indexes)
- IDT-ILMN TruSeq RNA UD Indexes v2 - 96 Indexes
- Nextera DNA CD Indexes (24 Indexes tubed)
- Nextera DNA CD Indexes (96 Indexes plated)
- Nextera Exome
- Nextera Index Kit (24 Indexes 96 Samples)
- Nextera Index Kit (96 Indexes 384 Samples)
- Nextera Mate Pair
- Nextera Rapid Capture Custom Enrichment
- Nextera XT Index Kit (24 Indexes 96 Samples)
- Nextera XT Index Kit (96 Indexes 384 Samples)
- Nextera XT v2 Index Kit A
- Nextera XT v2 Index Kit B

It is critical to ensure that adapter & indexes selected for plate match library prep



Sample Plate Wizard - Plate Parameters

IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A General Plate Parameters

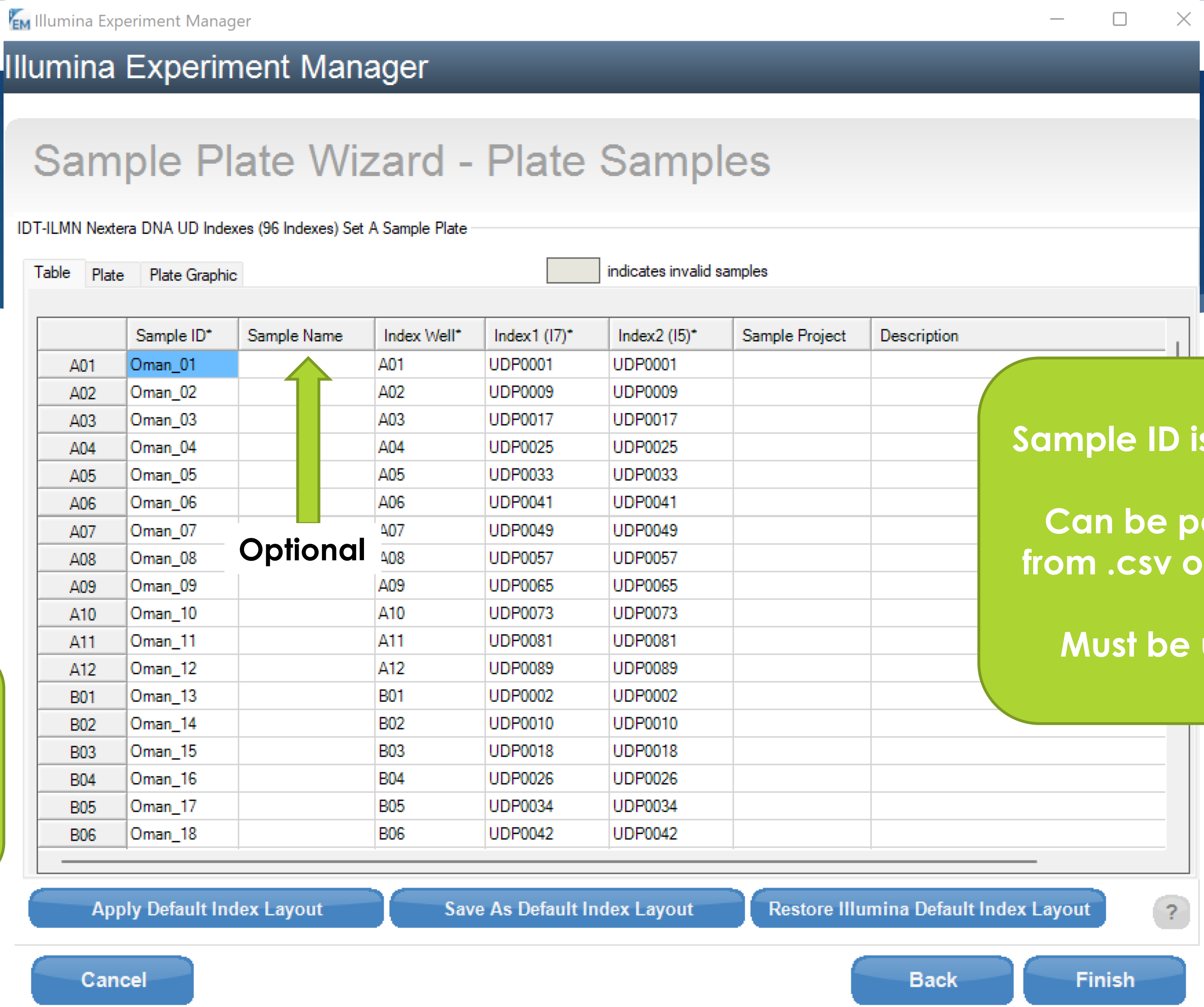
Unique Plate Name * Oman_Test_1

Index Reads ○ 1 (Single) ● 2 (Dual)

* - required field

Plate Name

It is critical to ensure that adapter & indexes selected for plate match library prep



Sample Plate Wizard - Plate Samples

IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A Sample Plate

▭ indicates invalid samples

	Sample ID*	Sample Name	Index Well*	Index1 (I7)*	Index2 (I5)*	Sample Project	Description
A01	Oman_01		A01	UDP0001	UDP0001		
A02	Oman_02		A02	UDP0009	UDP0009		
A03	Oman_03		A03	UDP0017	UDP0017		
A04	Oman_04		A04	UDP0025	UDP0025		
A05	Oman_05		A05	UDP0033	UDP0033		
A06	Oman_06		A06	UDP0041	UDP0041		
A07	Oman_07		A07	UDP0049	UDP0049		
A08	Oman_08		A08	UDP0057	UDP0057		
A09	Oman_09		A09	UDP0065	UDP0065		
A10	Oman_10		A10	UDP0073	UDP0073		
A11	Oman_11		A11	UDP0081	UDP0081		
A12	Oman_12		A12	UDP0089	UDP0089		
B01	Oman_13		B01	UDP0002	UDP0002		
B02	Oman_14		B02	UDP0010	UDP0010		
B03	Oman_15		B03	UDP0018	UDP0018		
B04	Oman_16		B04	UDP0026	UDP0026		
B05	Oman_17		B05	UDP0034	UDP0034		
B06	Oman_18		B06	UDP0042	UDP0042		

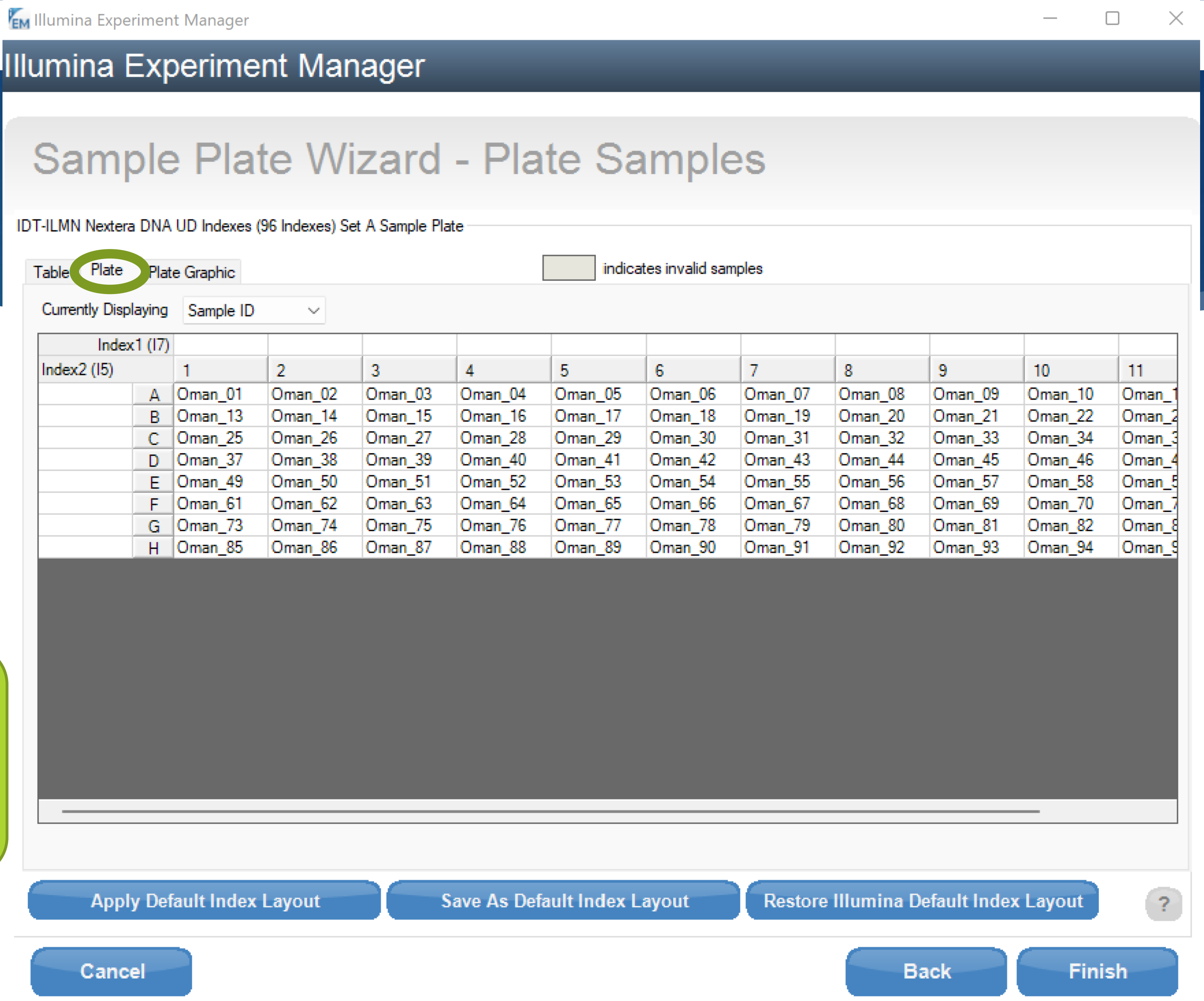
Optional

Sample ID is required

Can be pasted in from .csv or .xlsx file

Must be unique

Ensure Default Layout Matches Plate



Sample Plate Wizard - Plate Samples

IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A Sample Plate

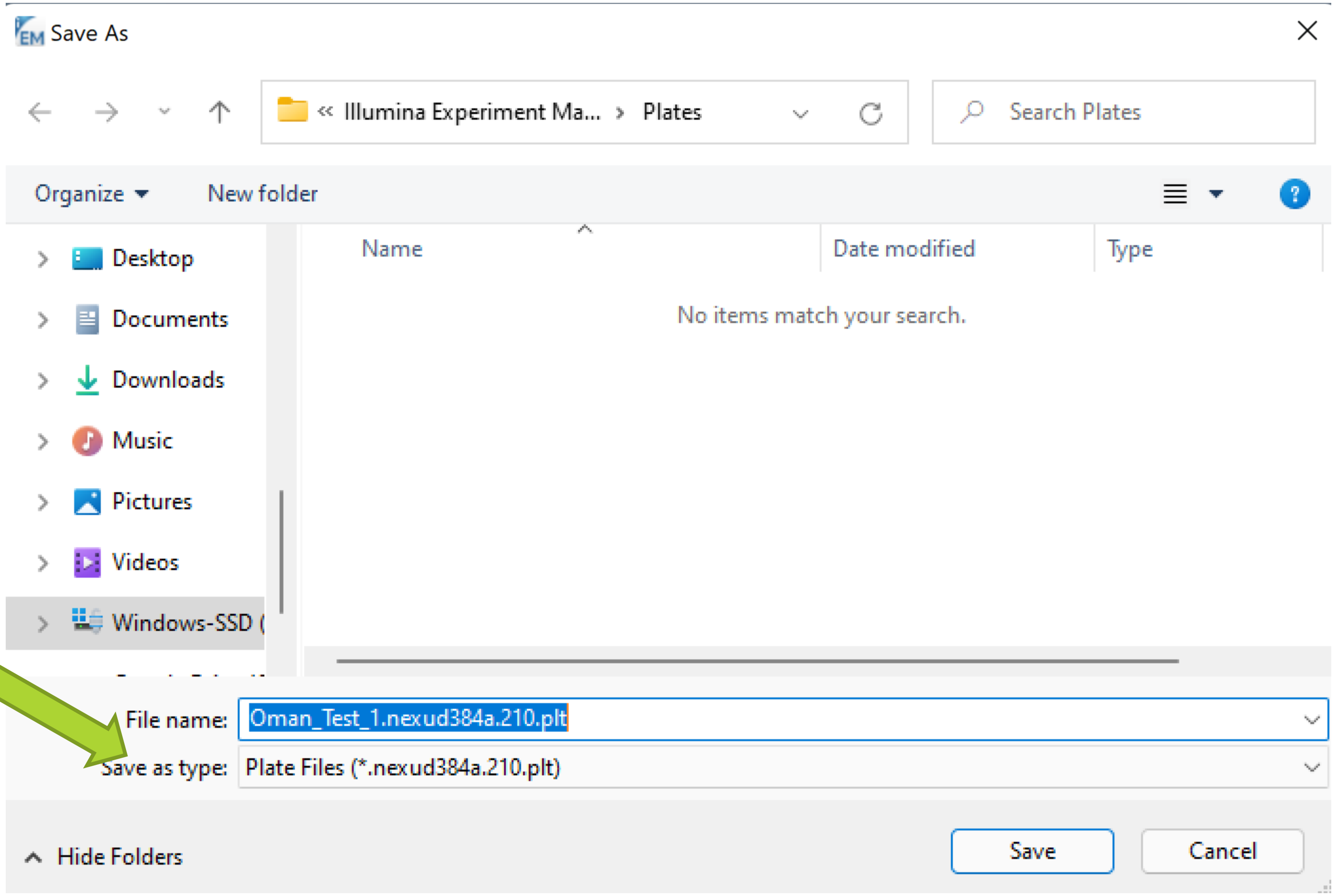
▭ indicates invalid samples

Currently Displaying: Sample ID

Index1 (I7)											
Index2 (I5)	1	2	3	4	5	6	7	8	9	10	11
A	Oman_01	Oman_02	Oman_03	Oman_04	Oman_05	Oman_06	Oman_07	Oman_08	Oman_09	Oman_10	Oman_1
B	Oman_13	Oman_14	Oman_15	Oman_16	Oman_17	Oman_18	Oman_19	Oman_20	Oman_21	Oman_22	Oman_2
C	Oman_25	Oman_26	Oman_27	Oman_28	Oman_29	Oman_30	Oman_31	Oman_32	Oman_33	Oman_34	Oman_3
D	Oman_37	Oman_38	Oman_39	Oman_40	Oman_41	Oman_42	Oman_43	Oman_44	Oman_45	Oman_46	Oman_4
E	Oman_49	Oman_50	Oman_51	Oman_52	Oman_53	Oman_54	Oman_55	Oman_56	Oman_57	Oman_58	Oman_5
F	Oman_61	Oman_62	Oman_63	Oman_64	Oman_65	Oman_66	Oman_67	Oman_68	Oman_69	Oman_70	Oman_7
G	Oman_73	Oman_74	Oman_75	Oman_76	Oman_77	Oman_78	Oman_79	Oman_80	Oman_81	Oman_82	Oman_8
H	Oman_85	Oman_86	Oman_87	Oman_88	Oman_89	Oman_90	Oman_91	Oman_92	Oman_93	Oman_94	Oman_9

96 Well Plate View

Plate Files



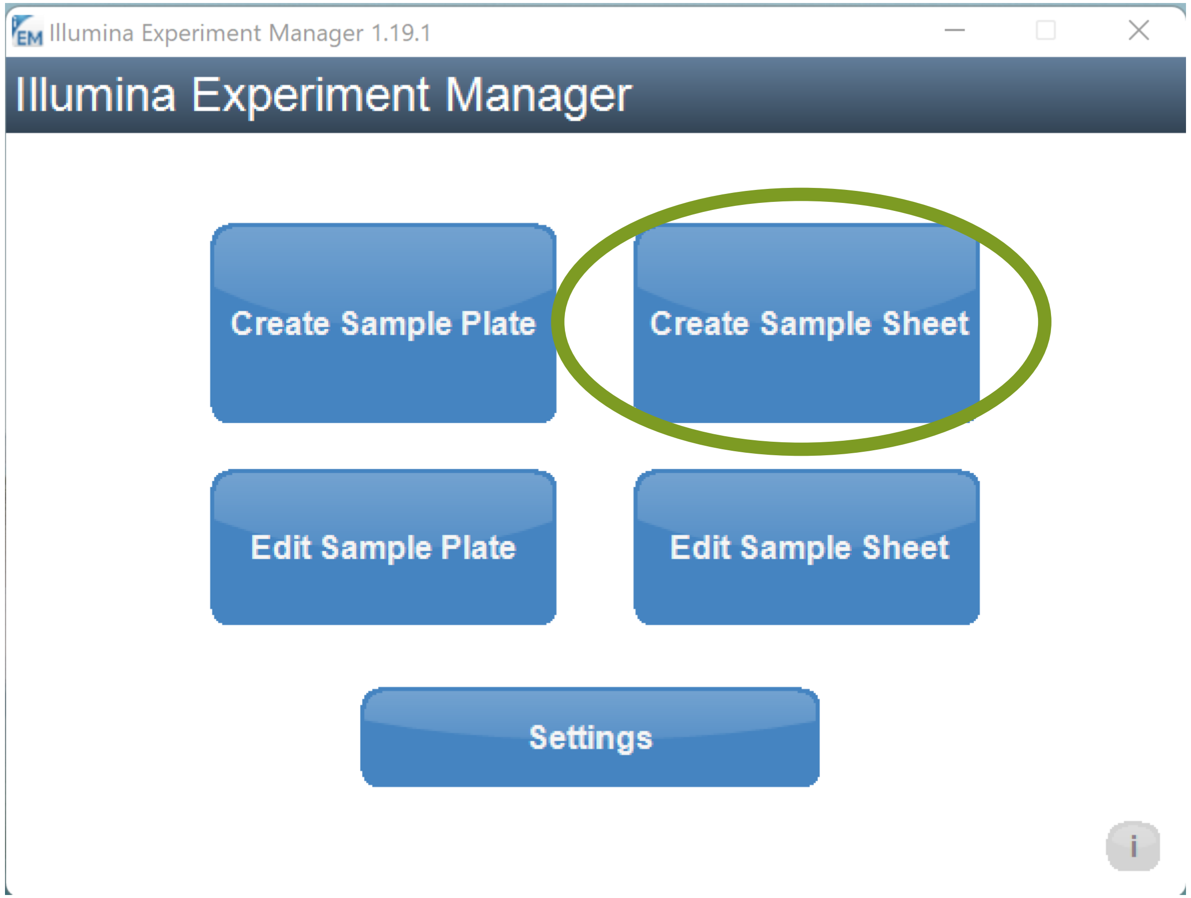
No items match your search.

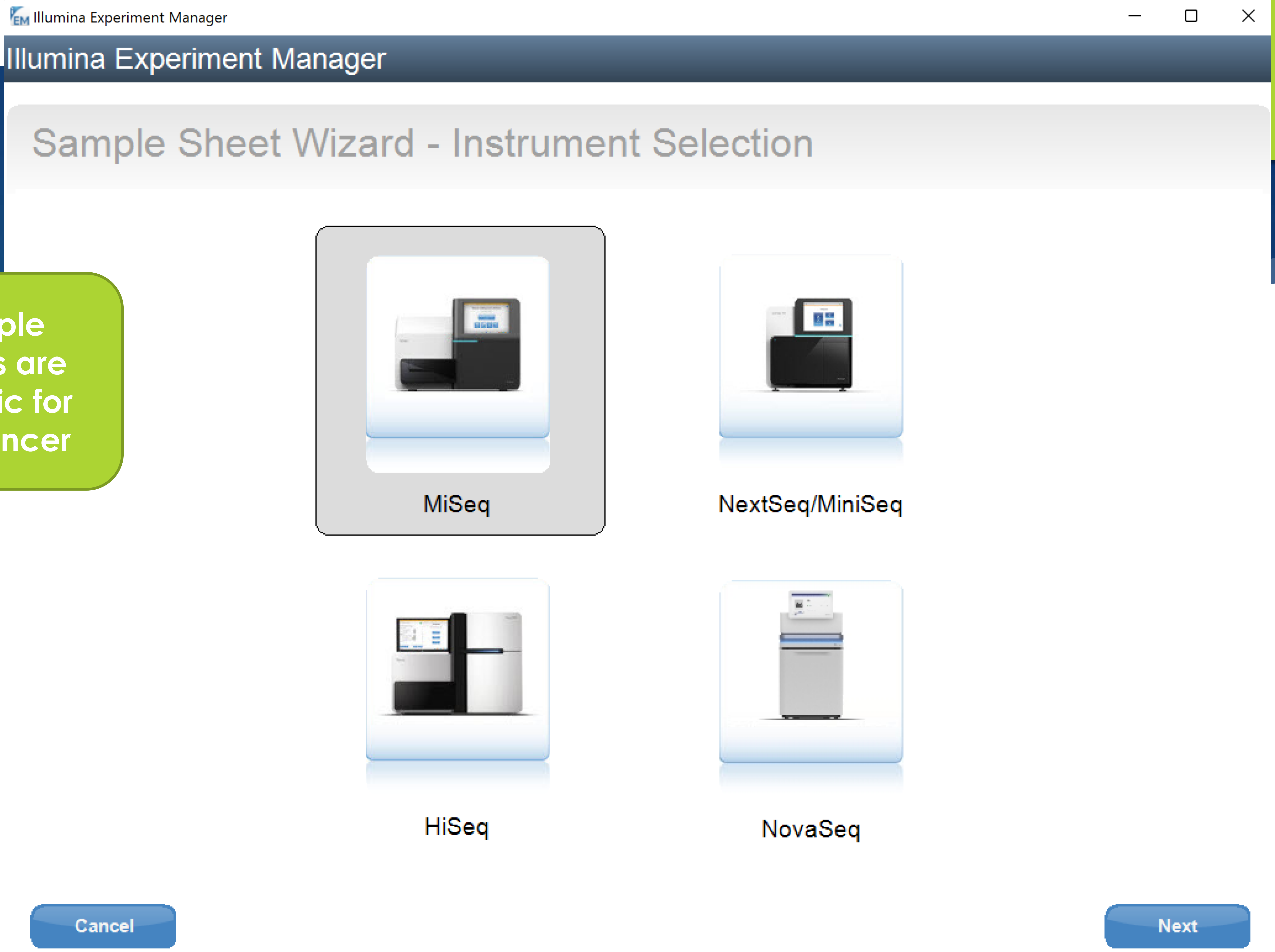
File name: Oman_Test_1.nexud384a.210.plt

Save as type: Plate Files (*.nexud384a.210.plt)

Do not change file type

Illumina Experiment Manager



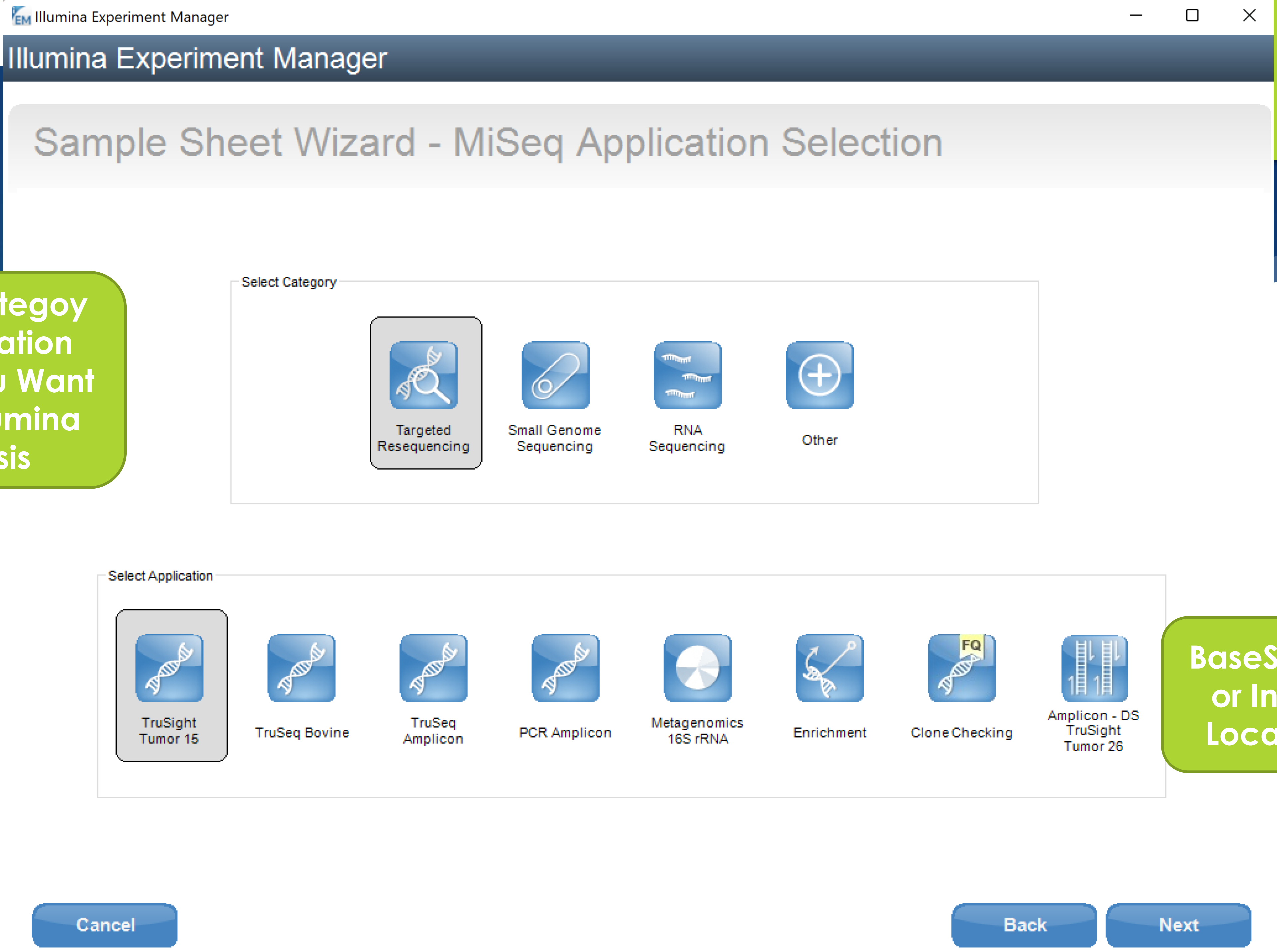


Illumina Experiment Manager

Illumina Experiment Manager

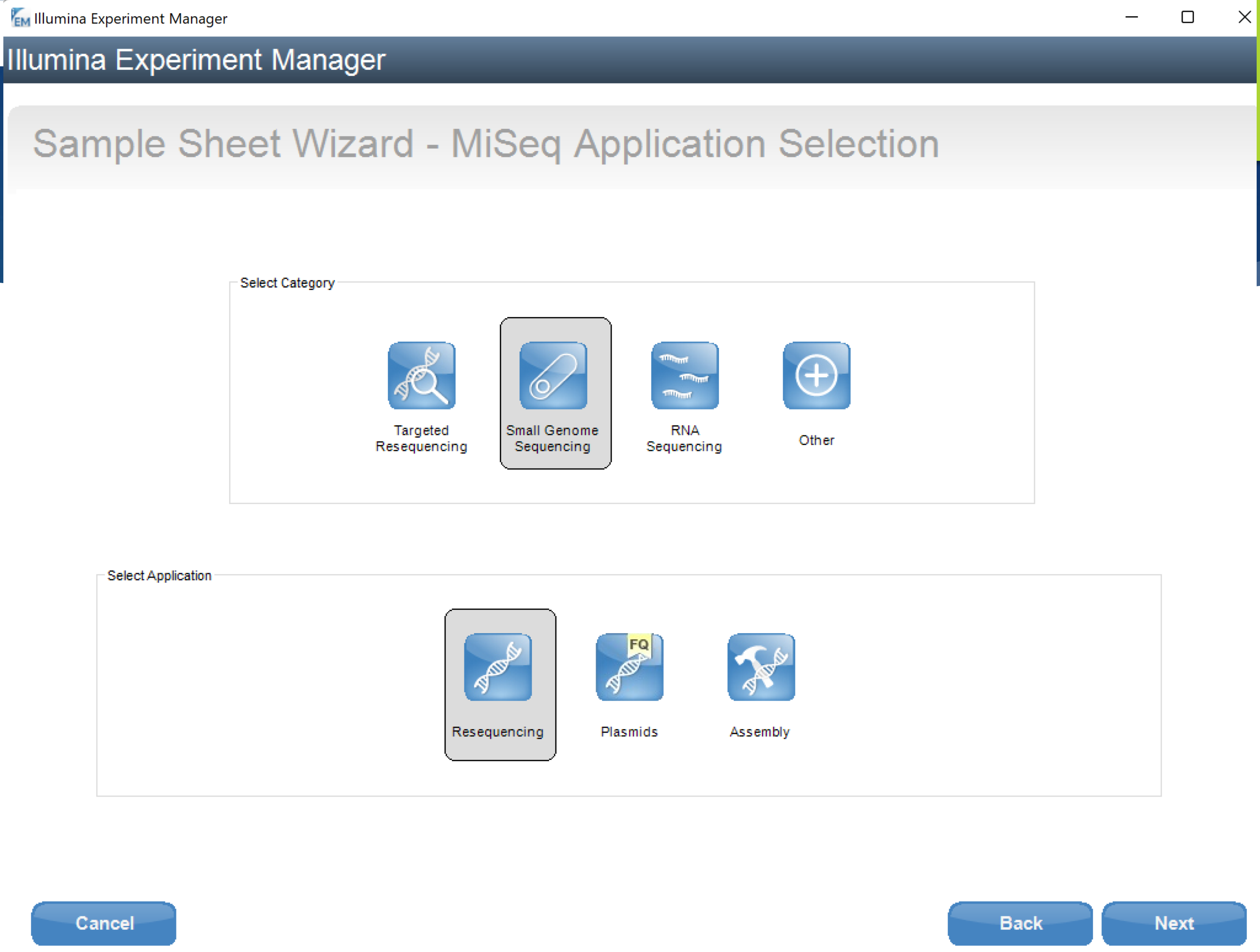
Sample Sheet Wizard - Instrument Selection

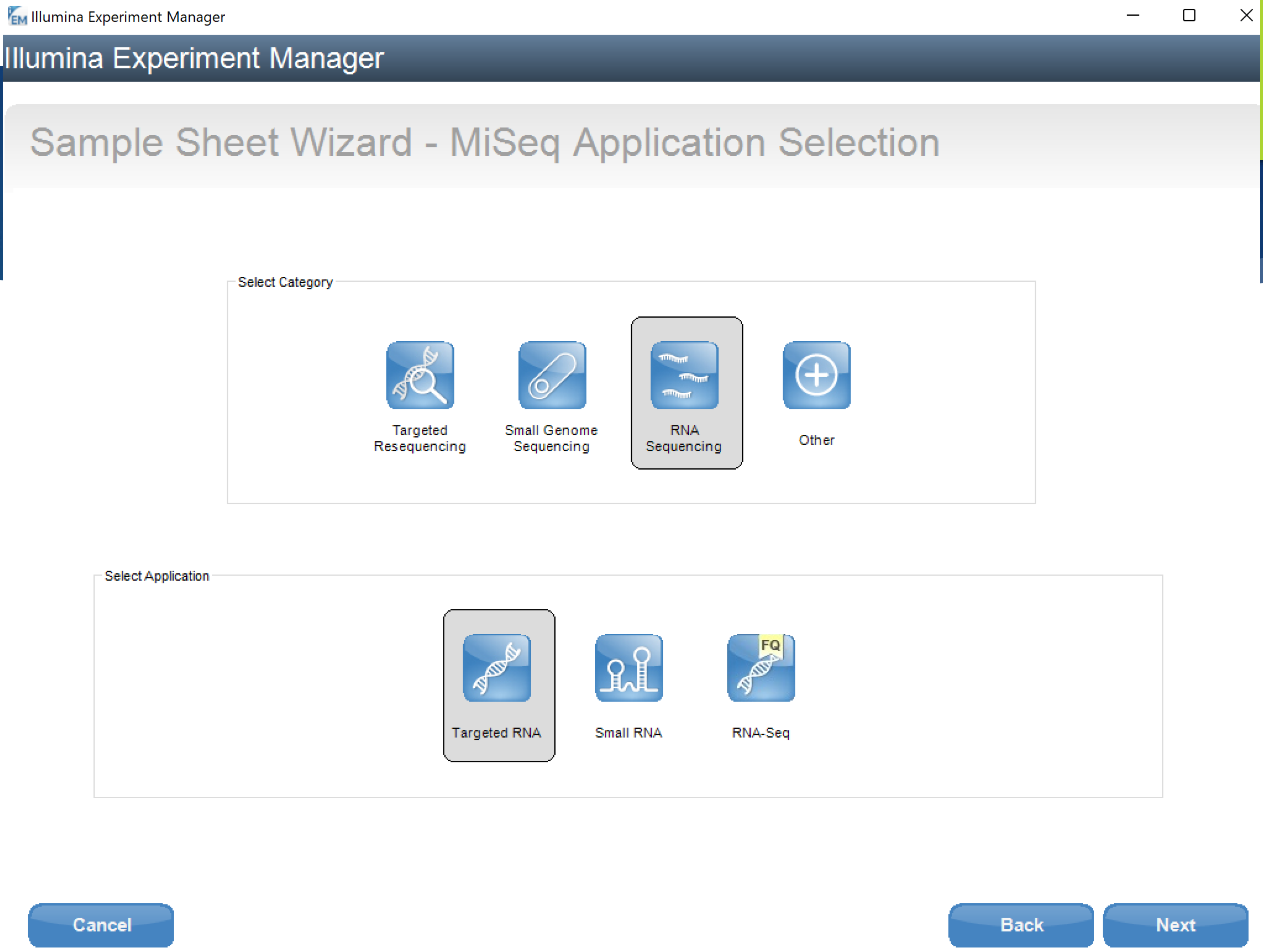
Sample Sheets are Specific for Sequencer



Select Categoy & Application Only if You Want to Run Illumina Analysis

BaseSpace Apps or Installed on Local Machine

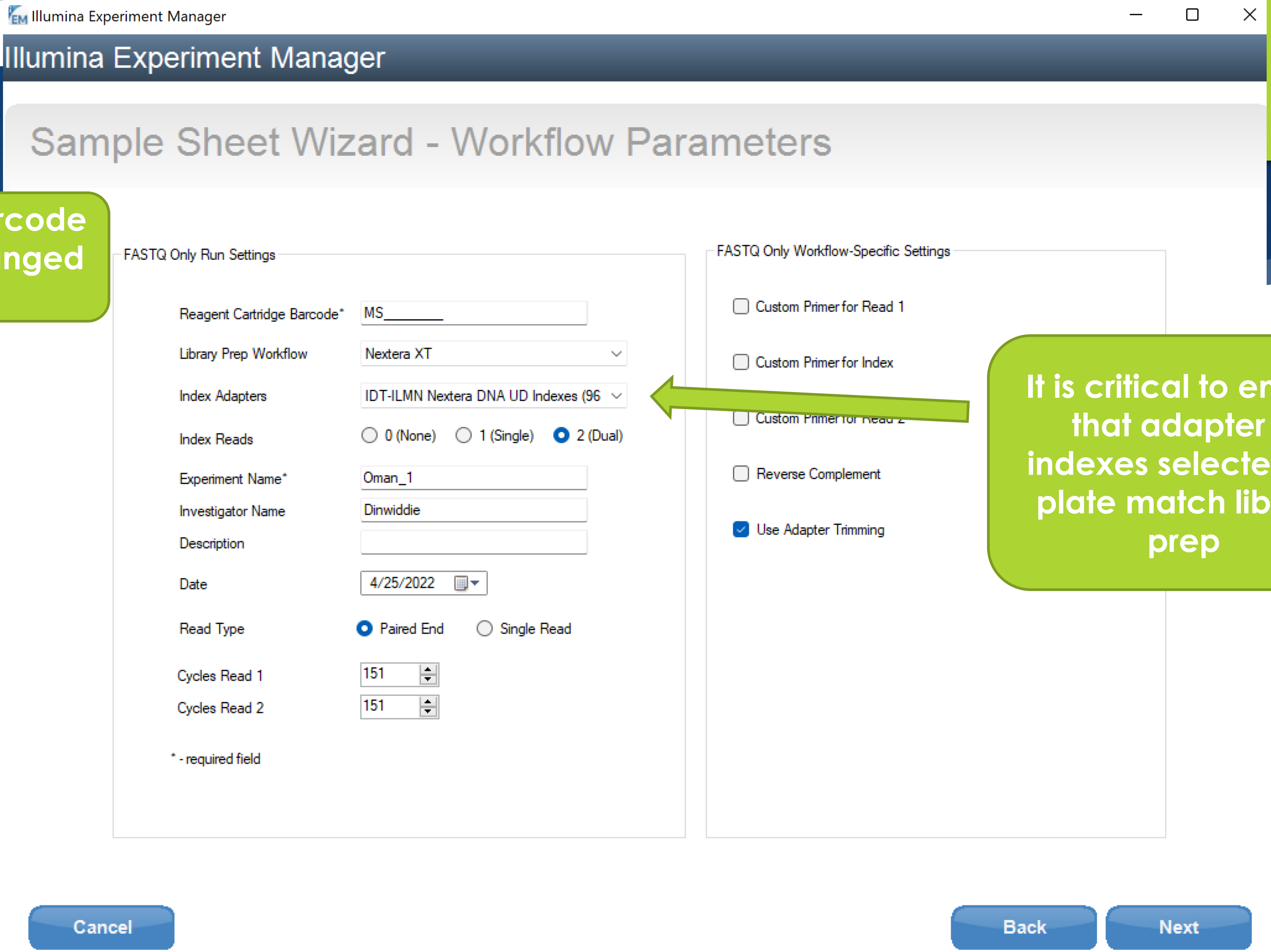

Illumina Experiment Manager
Illumina Experiment Manager
Sample Sheet Wizard - MiSeq Application Selection
Select Category
Targeted Resequencing
Small Genome Sequencing
RNA Sequencing
Other
Select Application
Resequencing
FQ
Plasmids
Assembly
Cancel
Back
Next


Illumina Experiment Manager
Illumina Experiment Manager
Sample Sheet Wizard - MiSeq Application Selection
Select Category
Targeted Resequencing
Small Genome Sequencing
RNA Sequencing
Other
Select Application
Targeted RNA
Small RNA
FQ
RNA-Seq
Cancel
Back
Next

If Completing Custom Analysis Select Other & FASTQ Only

Other & FASTQ Only Most Common Combination

Reagent Barcode can be Changed Later



Sample Sheet Wizard - Workflow Parameters

FASTQ Only Run Settings

Reagent Cartridge Barcode* MS_______

Library Prep Workflow: Nextera XT

Index Adapters: IDT-ILMN Nextera DNA UD Indexes (96

Index Reads: ○ 0 (None) ○ 1 (Single) ● 2 (Dual)

Experiment Name* Oman_1

Investigator Name: Dinwiddie

Description:

Date: 4/25/2022

Read Type: ● Paired End ○ Single Read

Cycles Read 1: 151

Cycles Read 2: 151

* - required field

FASTQ Only Workflow-Specific Settings

☐ Custom Primer for Read 1

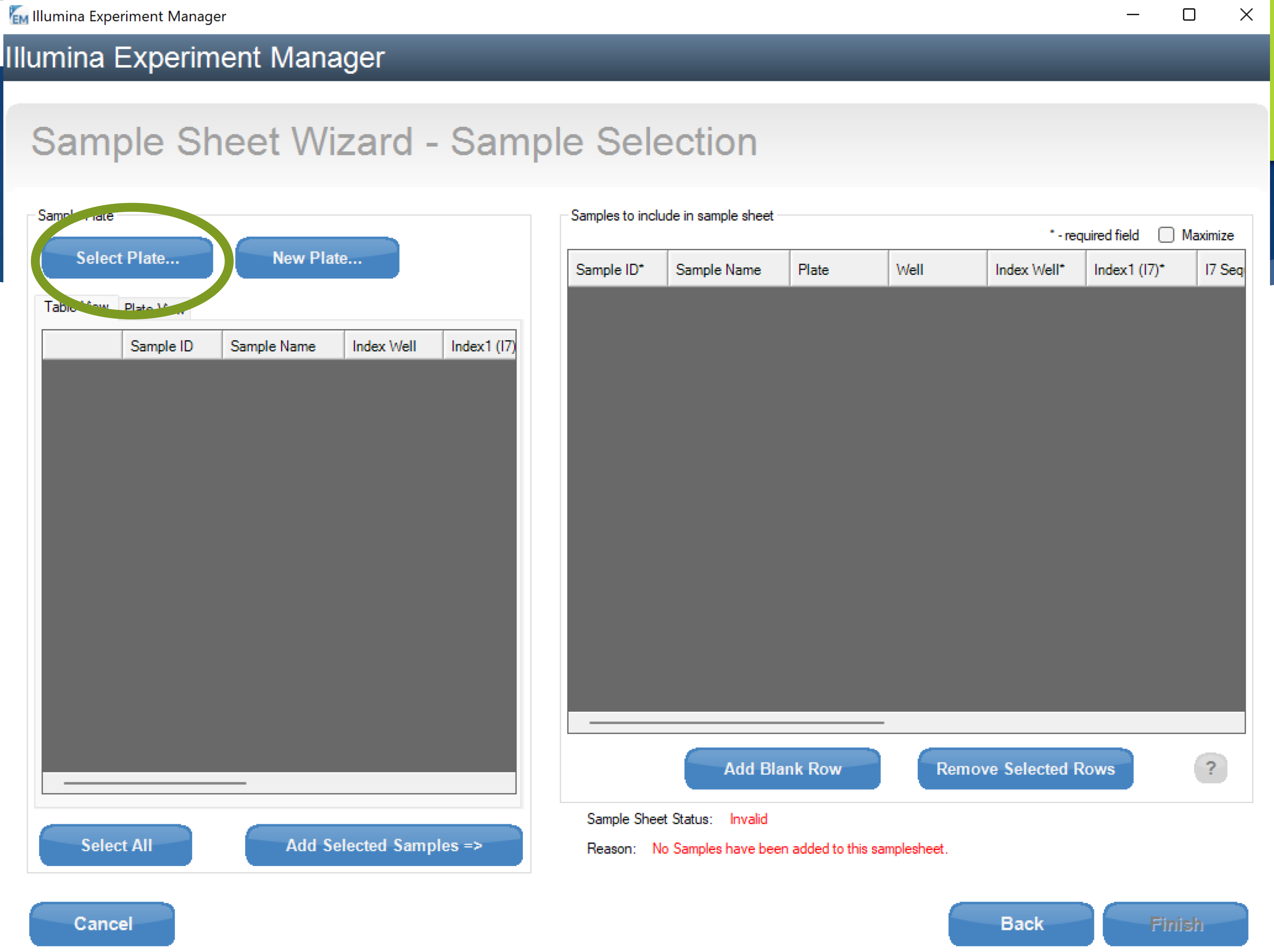
☐ Custom Primer for Index

☐ Custom Primer for Read 2

☐ Reverse Complement

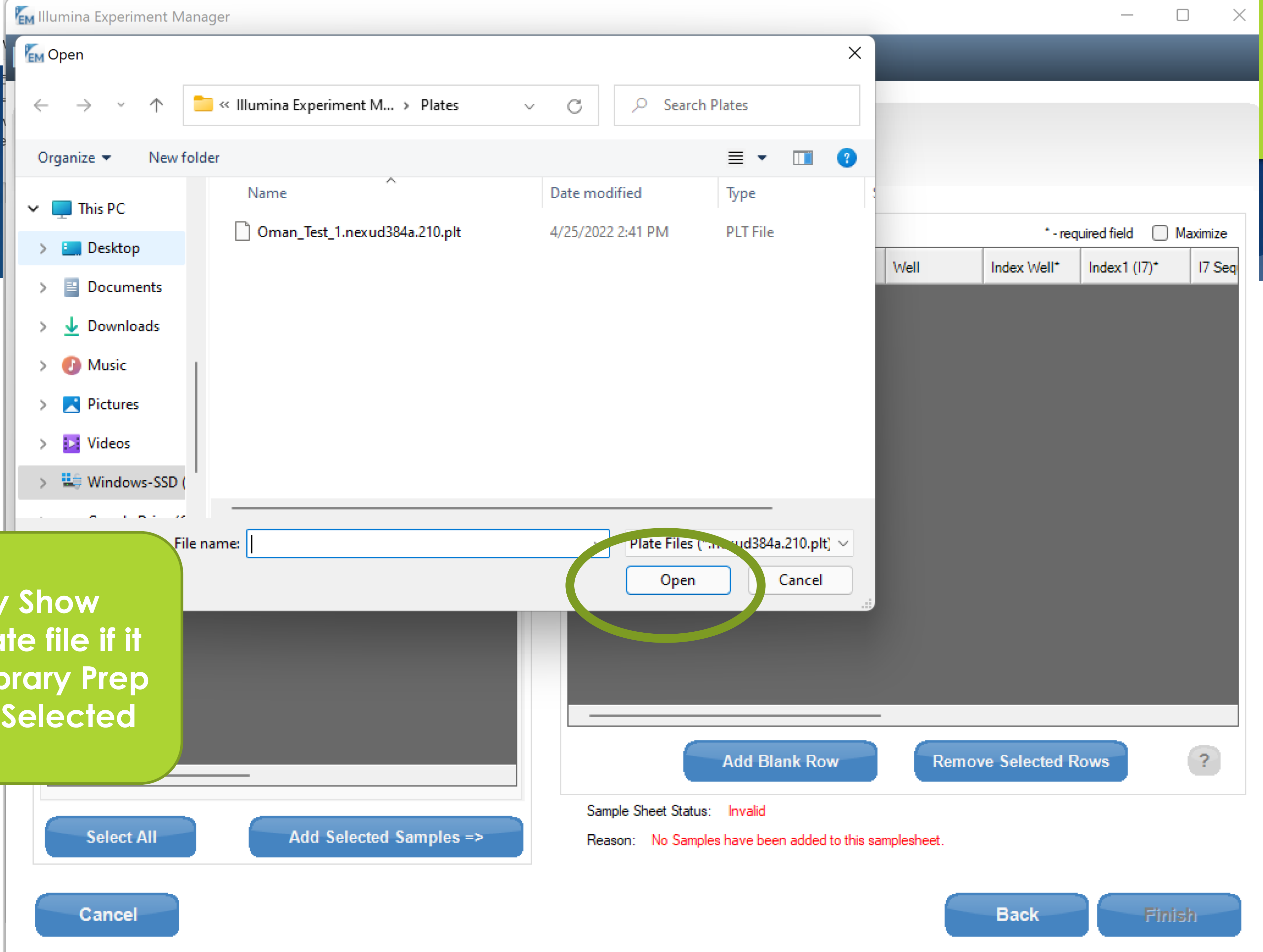
☑ Use Adapter Trimming

It is critical to ensure that adapter & indexes selected for plate match library prep



Sample Sheet Status: Invalid

Reason: No Samples have been added to this samplesheet.



Illumina Experiment Manager

Open

« Illumina Experiment M... › Plates

Search Plates

Name	Date modified	Type
Oman_Test_1.nexud384a.210.plt	4/25/2022 2:41 PM	PLT File

File name:

Plate Files (*.nexud384a.210.plt)

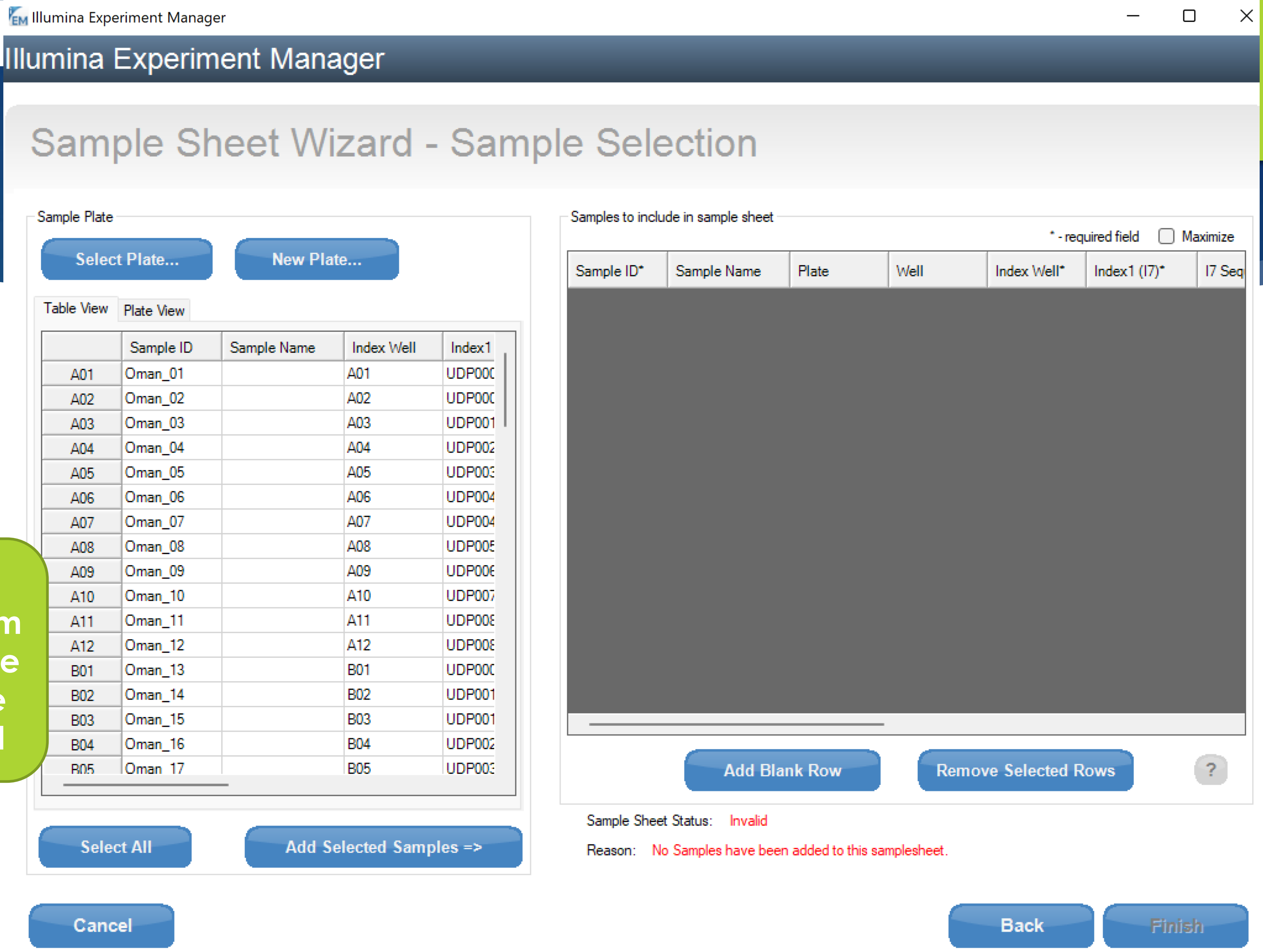
* - required field ☐ Maximize

Well	Index Well*	Index1 (I7)*	I7 Seq

Sample Sheet Status: Invalid

Reason: No Samples have been added to this samplesheet.

Will Only Show Sample Plate file if it Matches Library Prep & Indexes Selected



Sample Sheet Wizard - Sample Selection

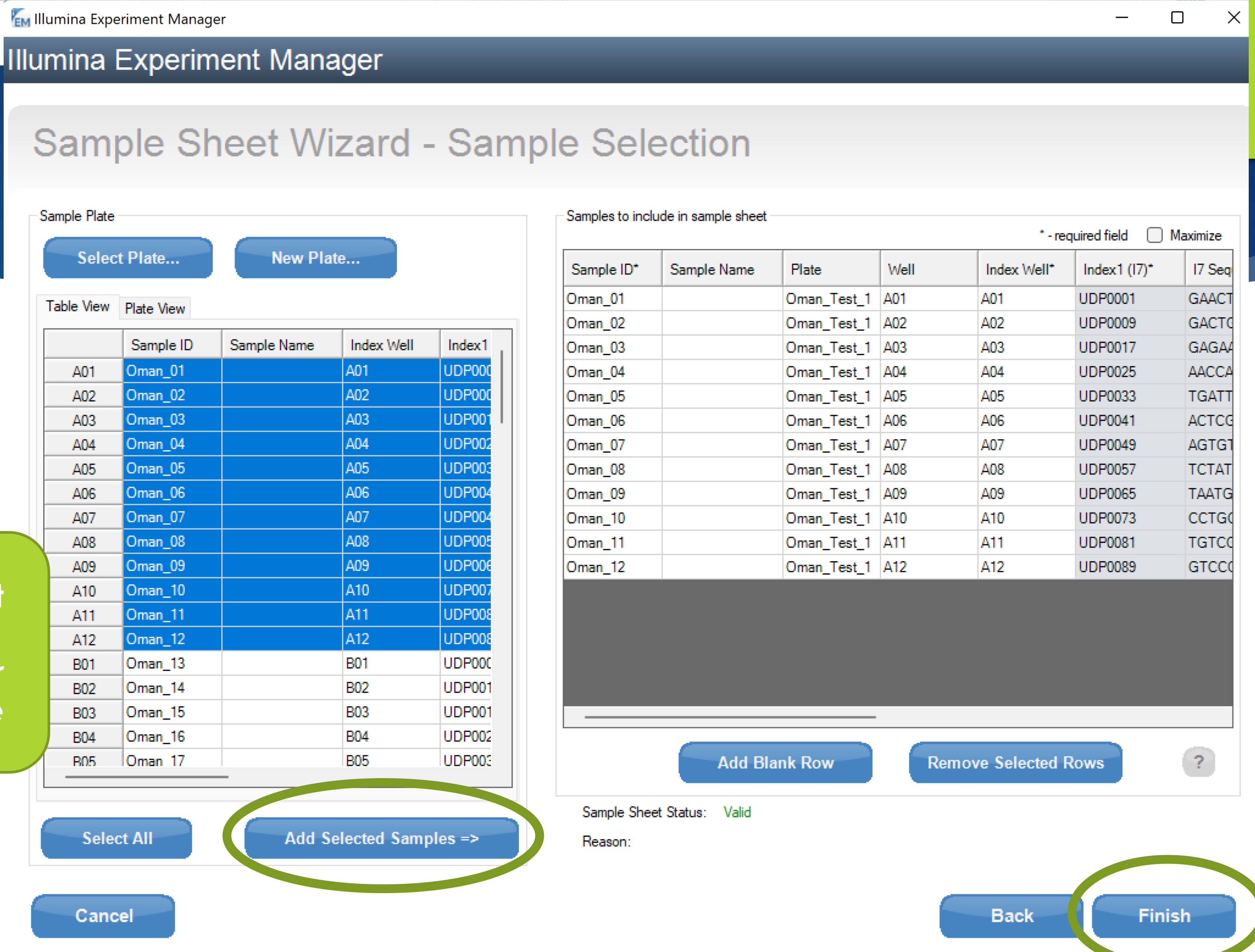
	Sample ID	Sample Name	Index Well	Index1
A01	Oman_01		A01	UDP000
A02	Oman_02		A02	UDP000
A03	Oman_03		A03	UDP001
A04	Oman_04		A04	UDP002
A05	Oman_05		A05	UDP003
A06	Oman_06		A06	UDP004
A07	Oman_07		A07	UDP004
A08	Oman_08		A08	UDP005
A09	Oman_09		A09	UDP006
A10	Oman_10		A10	UDP007
A11	Oman_11		A11	UDP008
A12	Oman_12		A12	UDP008
B01	Oman_13		B01	UDP000
B02	Oman_14		B02	UDP001
B03	Oman_15		B03	UDP001
B04	Oman_16		B04	UDP002
B05	Oman_17		B05	UDP003

Sample ID*	Sample Name	Plate	Well	Index Well*	Index1 (I7)*	I7 Seq

Sample Sheet Status: Invalid

Reason: No Samples have been added to this samplesheet.

Select Samples from Plate that are going to be sequenced



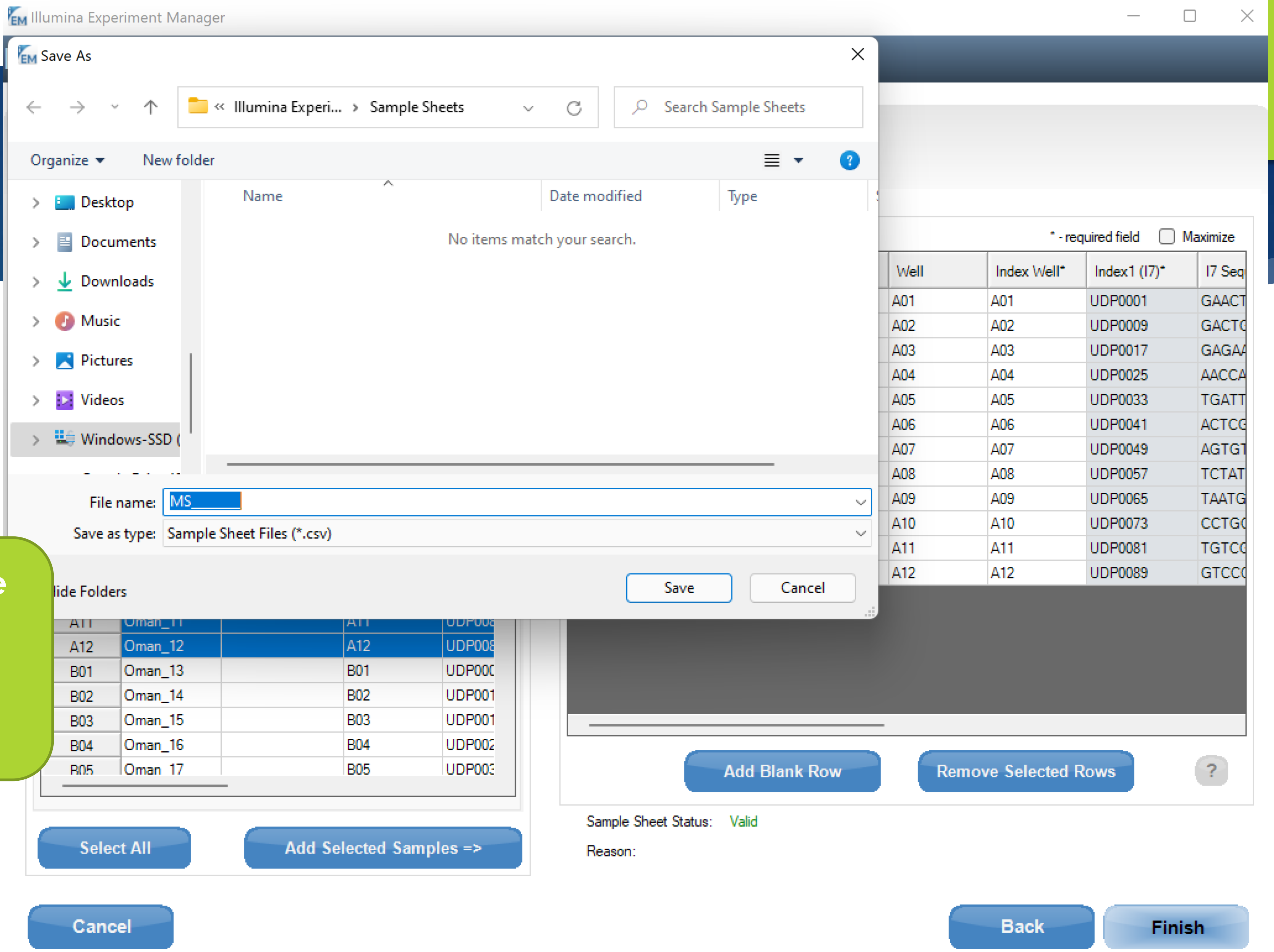
	Sample ID	Sample Name	Index Well	Index1
A01	Oman_01		A01	UDP000
A02	Oman_02		A02	UDP000
A03	Oman_03		A03	UDP001
A04	Oman_04		A04	UDP002
A05	Oman_05		A05	UDP003
A06	Oman_06		A06	UDP004
A07	Oman_07		A07	UDP004
A08	Oman_08		A08	UDP005
A09	Oman_09		A09	UDP006
A10	Oman_10		A10	UDP007
A11	Oman_11		A11	UDP008
A12	Oman_12		A12	UDP008
B01	Oman_13		B01	UDP000
B02	Oman_14		B02	UDP001
B03	Oman_15		B03	UDP001
B04	Oman_16		B04	UDP002
B05	Oman_17		B05	UDP003

Sample ID*	Sample Name	Plate	Well	Index Well*	Index1 (I7)*	I7 Seq
Oman_01		Oman_Test_1	A01	A01	UDP0001	GAACT
Oman_02		Oman_Test_1	A02	A02	UDP0009	GACT
Oman_03		Oman_Test_1	A03	A03	UDP0017	GAGA
Oman_04		Oman_Test_1	A04	A04	UDP0025	AACCA
Oman_05		Oman_Test_1	A05	A05	UDP0033	TGATT
Oman_06		Oman_Test_1	A06	A06	UDP0041	ACTCG
Oman_07		Oman_Test_1	A07	A07	UDP0049	AGTGT
Oman_08		Oman_Test_1	A08	A08	UDP0057	TCTAT
Oman_09		Oman_Test_1	A09	A09	UDP0065	TAATG
Oman_10		Oman_Test_1	A10	A10	UDP0073	CCTG
Oman_11		Oman_Test_1	A11	A11	UDP0081	TGTC
Oman_12		Oman_Test_1	A12	A12	UDP0089	GTCC

Sample Sheet Status: Valid

Reason:

Can Select Subset of Samples or Entire Plate



No items match your search.

File name: MS_____

Save as type: Sample Sheet Files (*.csv)

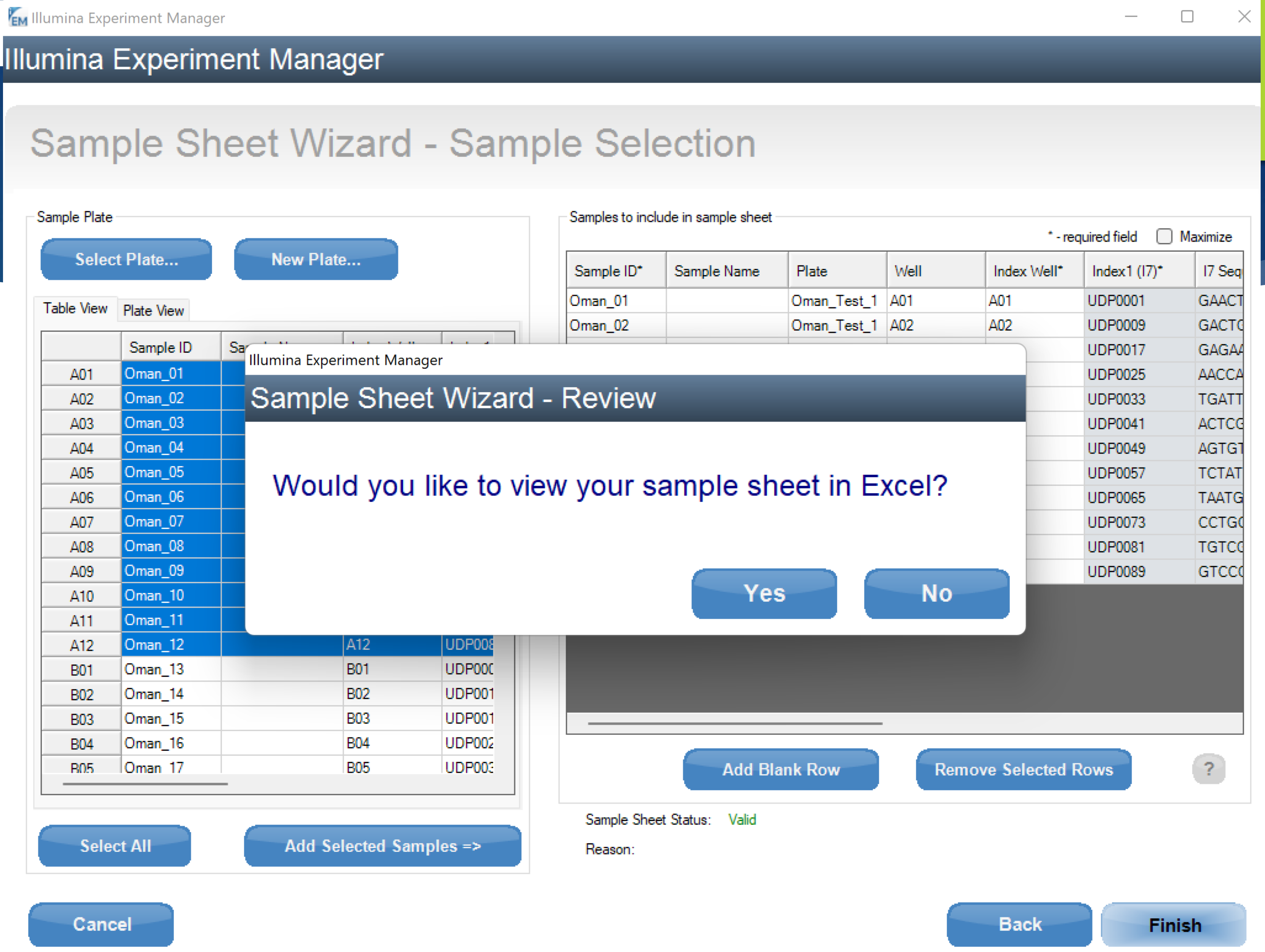
A11	Oman_11	A11	UDP008
A12	Oman_12	A12	UDP008
B01	Oman_13	B01	UDP000
B02	Oman_14	B02	UDP001
B03	Oman_15	B03	UDP001
B04	Oman_16	B04	UDP002
B05	Oman_17	B05	UDP003

Well	Index Well*	Index1 (I7)*	I7 Seq
A01	A01	UDP0001	GAACT
A02	A02	UDP0009	GACTC
A03	A03	UDP0017	GAGAA
A04	A04	UDP0025	AACCA
A05	A05	UDP0033	TGATT
A06	A06	UDP0041	ACTCG
A07	A07	UDP0049	AGTGT
A08	A08	UDP0057	TCTAT
A09	A09	UDP0065	TAATG
A10	A10	UDP0073	CCTGC
A11	A11	UDP0081	TGTCC
A12	A12	UDP0089	GTCCC

Sample Sheet Status: Valid

Reason:

Default File Name is Reagent Cartridge Barcode



Illumina Experiment Manager

Illumina Experiment Manager

Sample Sheet Wizard - Sample Selection

Sample Plate

	Sample ID	Sa		
A01	Oman_01			
A02	Oman_02			
A03	Oman_03			
A04	Oman_04			
A05	Oman_05			
A06	Oman_06			
A07	Oman_07			
A08	Oman_08			
A09	Oman_09			
A10	Oman_10			
A11	Oman_11			
A12	Oman_12		A12	UDP008
B01	Oman_13		B01	UDP000
B02	Oman_14		B02	UDP001
B03	Oman_15		B03	UDP001
B04	Oman_16		B04	UDP002
B05	Oman_17		B05	UDP003

Samples to include in sample sheet

* - required field ☐ Maximize

Sample ID*	Sample Name	Plate	Well	Index Well*	Index1 (I7)*	I7 Seq
Oman_01		Oman_Test_1	A01	A01	UDP0001	GAACT
Oman_02		Oman_Test_1	A02	A02	UDP0009	GACT
					UDP0017	GAGA
					UDP0025	AACCA
					UDP0033	TGATT
					UDP0041	ACTC
					UDP0049	AGTG
					UDP0057	TCTAT
					UDP0065	TAATG
					UDP0073	CCTG
					UDP0081	TGTC
					UDP0089	GTCC

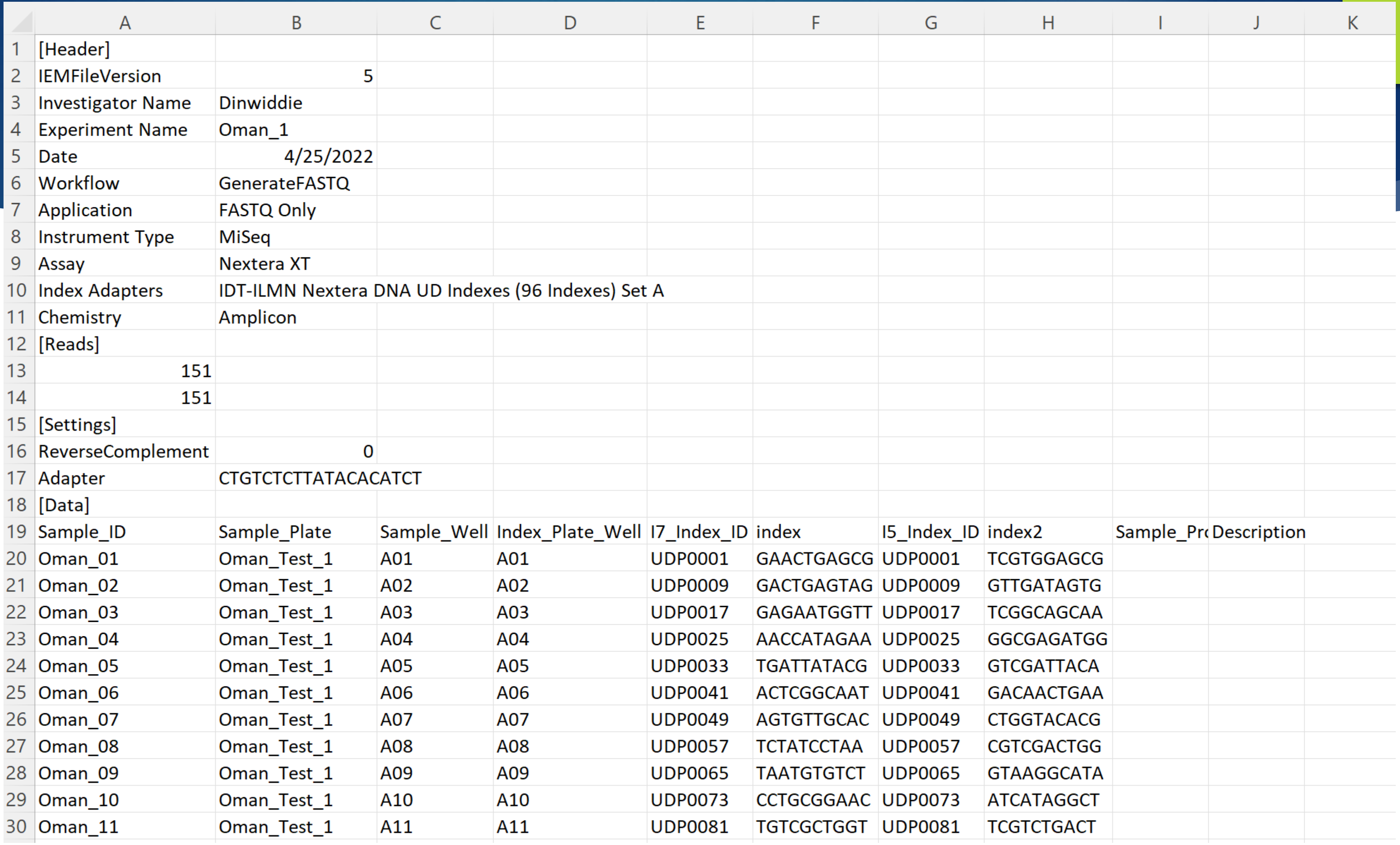
Sample Sheet Status: Valid

Reason:

Illumina Experiment Manager

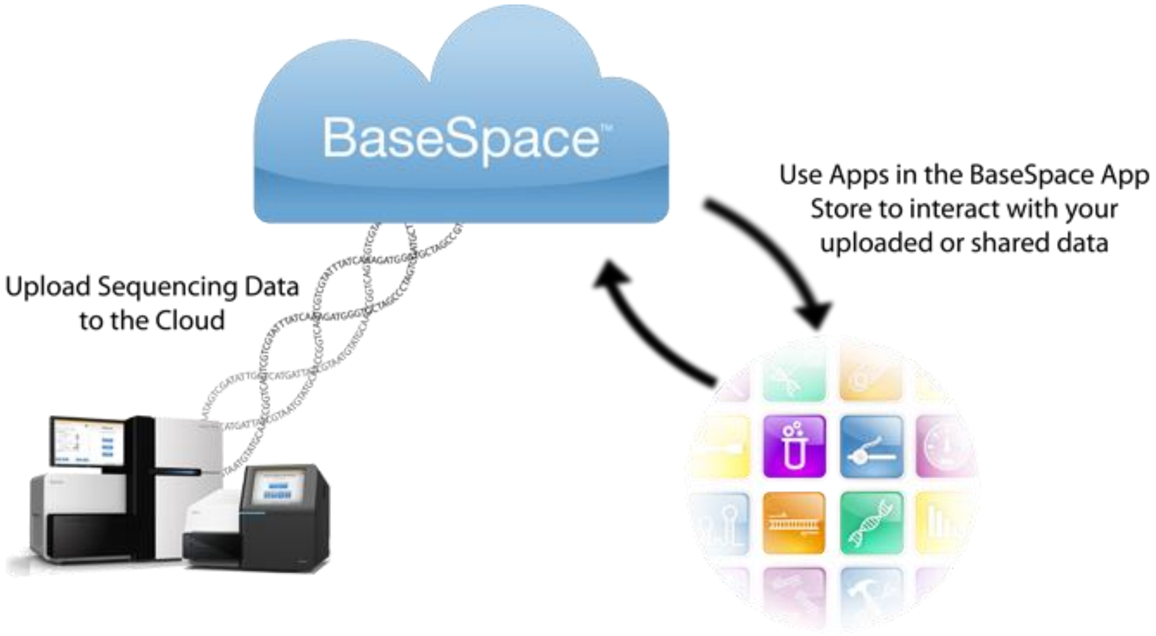
Sample Sheet Wizard - Review

Would you like to view your sample sheet in Excel?

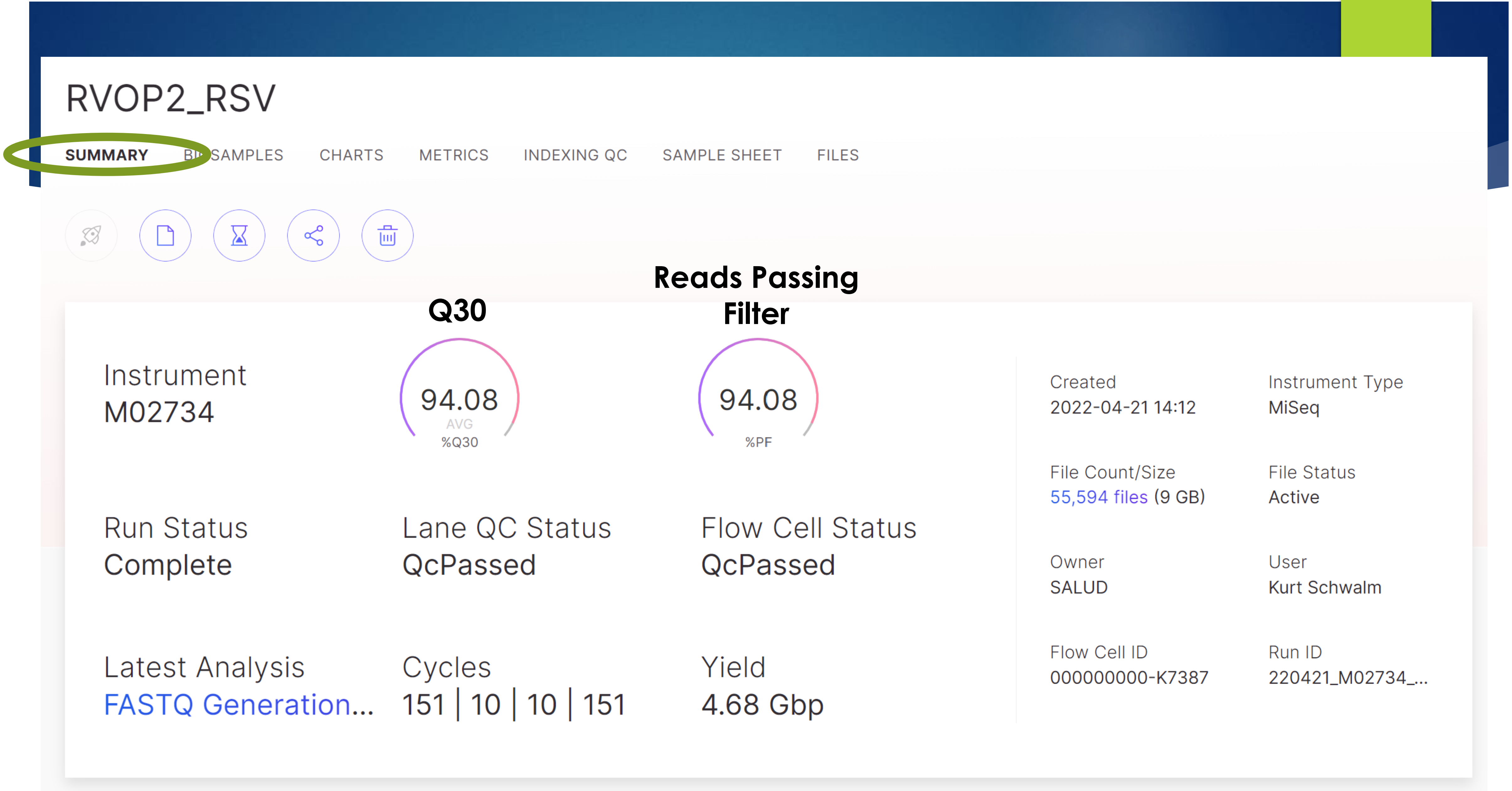


	A	B	C	D	E	F	G	H	I	J	K
1	[Header]										
2	IEMFileVersion	5									
3	Investigator Name	Dinwiddie									
4	Experiment Name	Oman_1									
5	Date	4/25/2022									
6	Workflow	GenerateFASTQ									
7	Application	FASTQ Only									
8	Instrument Type	MiSeq									
9	Assay	Nextera XT									
10	Index Adapters	IDT-ILMN Nextera DNA UD Indexes (96 Indexes) Set A									
11	Chemistry	Amplicon									
12	[Reads]										
13	151										
14	151										
15	[Settings]										
16	ReverseComplement	0									
17	Adapter	CTGTCTCTTATACACATCT									
18	[Data]										
19	Sample_ID	Sample_Plate	Sample_Well	Index_Plate_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Pr	Description	
20	Oman_01	Oman_Test_1	A01	A01	UDP0001	GAACTGAGCG	UDP0001	TCGTGGAGCG			
21	Oman_02	Oman_Test_1	A02	A02	UDP0009	GACTGAGTAG	UDP0009	GTTGATAGTG			
22	Oman_03	Oman_Test_1	A03	A03	UDP0017	GAGAATGGTT	UDP0017	TCGGCAGCAA			
23	Oman_04	Oman_Test_1	A04	A04	UDP0025	AACCATAGAA	UDP0025	GGCGAGATGG			
24	Oman_05	Oman_Test_1	A05	A05	UDP0033	TGATTATACG	UDP0033	GTCGATTACA			
25	Oman_06	Oman_Test_1	A06	A06	UDP0041	ACTCGGCAAT	UDP0041	GACAACTGAA			
26	Oman_07	Oman_Test_1	A07	A07	UDP0049	AGTGTTGCAC	UDP0049	CTGGTACACG			
27	Oman_08	Oman_Test_1	A08	A08	UDP0057	TCTATCCTAA	UDP0057	CGTCGACTGG			
28	Oman_09	Oman_Test_1	A09	A09	UDP0065	TAATGTGTCT	UDP0065	GTAAGGCATA			
29	Oman_10	Oman_Test_1	A10	A10	UDP0073	CCTGCGGAAC	UDP0073	ATCATAGGCT			
30	Oman_11	Oman_Test_1	A11	A11	UDP0081	TGTCGCTGGT	UDP0081	TCGTCTGACT			

Illumina Sequencing Analysis Viewer (BaseSpace)



Sequencing Analysis Viewer (SAV) is Installed on Machine As Well



RVOP2_RSV

Q30

Reads Passing Filter

Instrument
M02734

94.08
AVG
%Q30

94.08
%PF

Run Status
Complete

Lane QC Status
QcPassed

Flow Cell Status
QcPassed

Latest Analysis
FASTQ Generation...

Cycles
151 | 10 | 10 | 151

Yield
4.68 Gbp

Created
2022-04-21 14:12

Instrument Type
MiSeq

File Count/Size
55,594 files (9 GB)

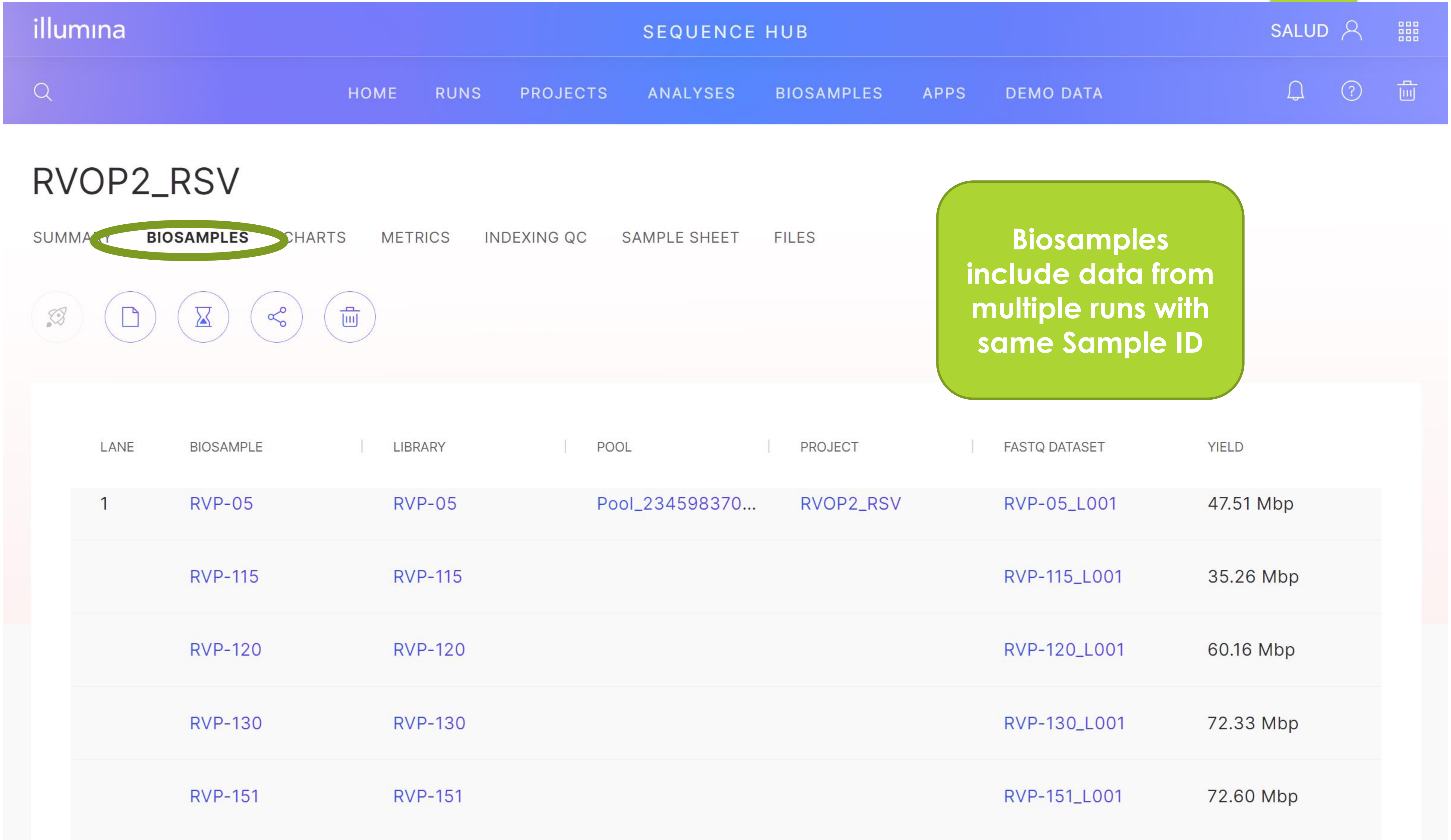
File Status
Active

Owner
SALUD

User
Kurt Schwalm

Flow Cell ID
000000000-K7387

Run ID
220421_M02734_...

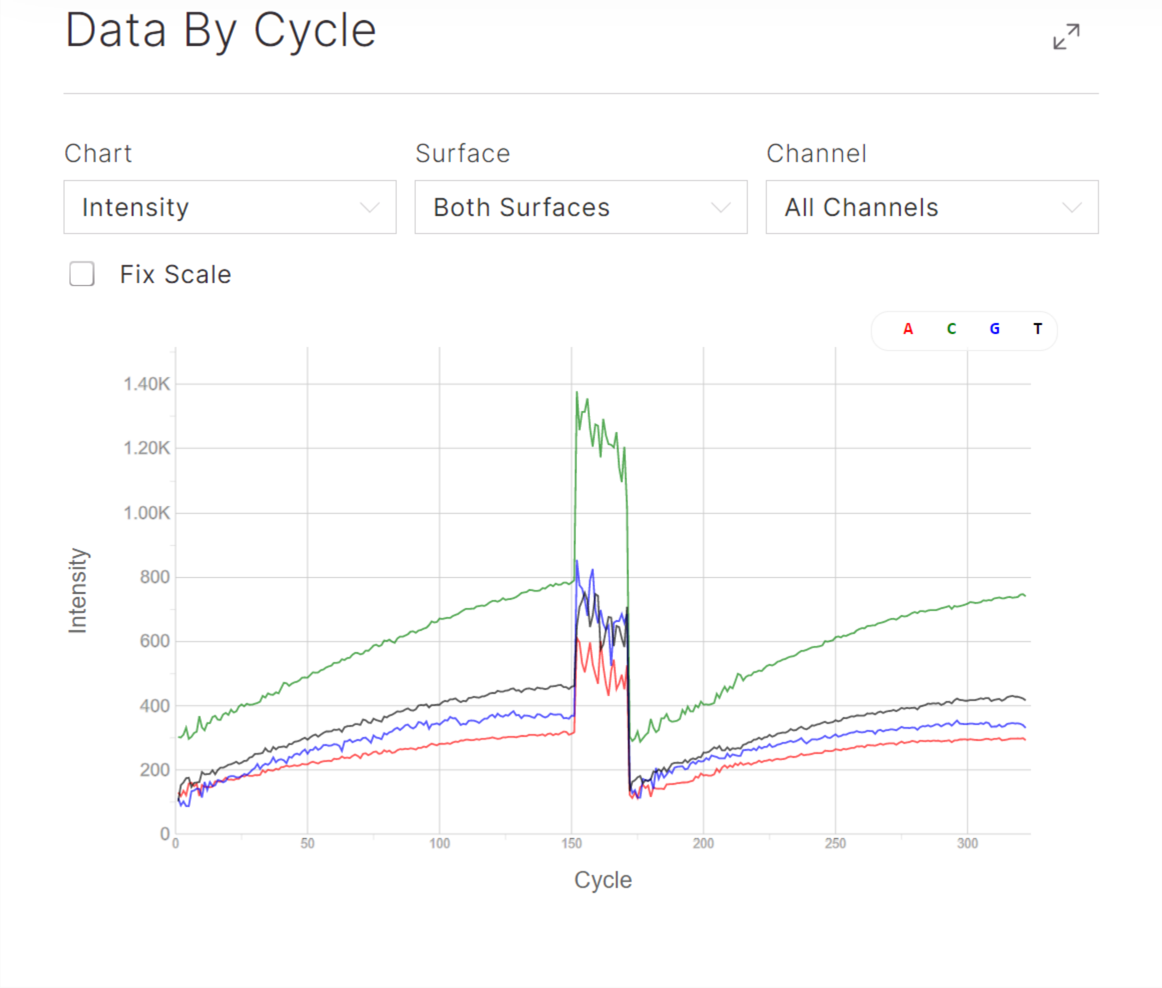


RVOP2_RSV

LANE	BIOSAMPLE	LIBRARY	POOL	PROJECT	FASTQ DATASET	YIELD
1	RVP-05	RVP-05	Pool_234598370...	RVOP2_RSV	RVP-05_L001	47.51 Mbp
	RVP-115	RVP-115			RVP-115_L001	35.26 Mbp
	RVP-120	RVP-120			RVP-120_L001	60.16 Mbp
	RVP-130	RVP-130			RVP-130_L001	72.33 Mbp
	RVP-151	RVP-151			RVP-151_L001	72.60 Mbp

Biosamples include data from multiple runs with same Sample ID

MiSeq Intensity Generally Increase During Sequencing Run



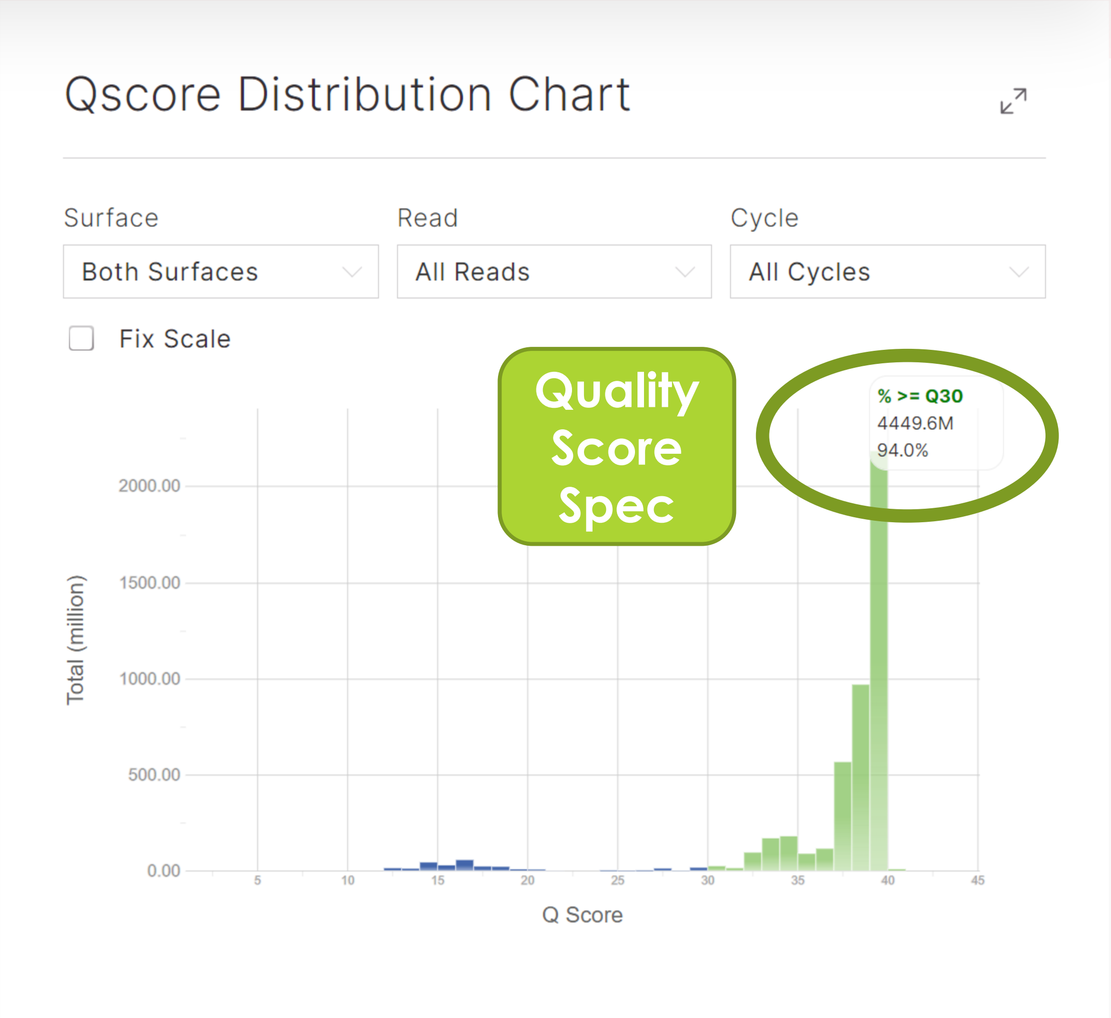
Data By Cycle

Chart: Intensity

Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale



Qscore Distribution Chart

Surface: Both Surfaces

Read: All Reads

Cycle: All Cycles

☐ Fix Scale

% >= Q30
4449.6M
94.0%

Quality Score Spec

Illumina MiSeq Specifications

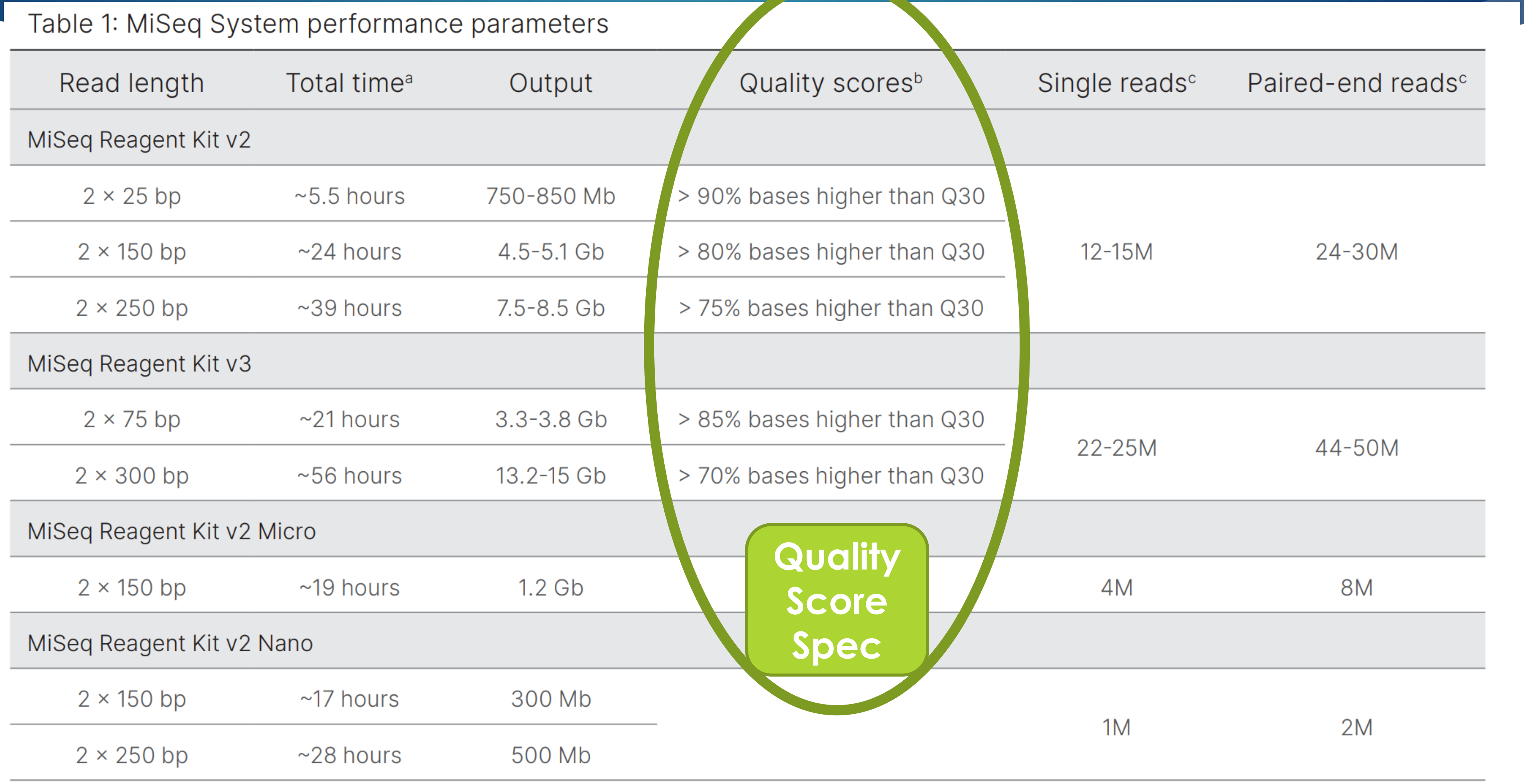
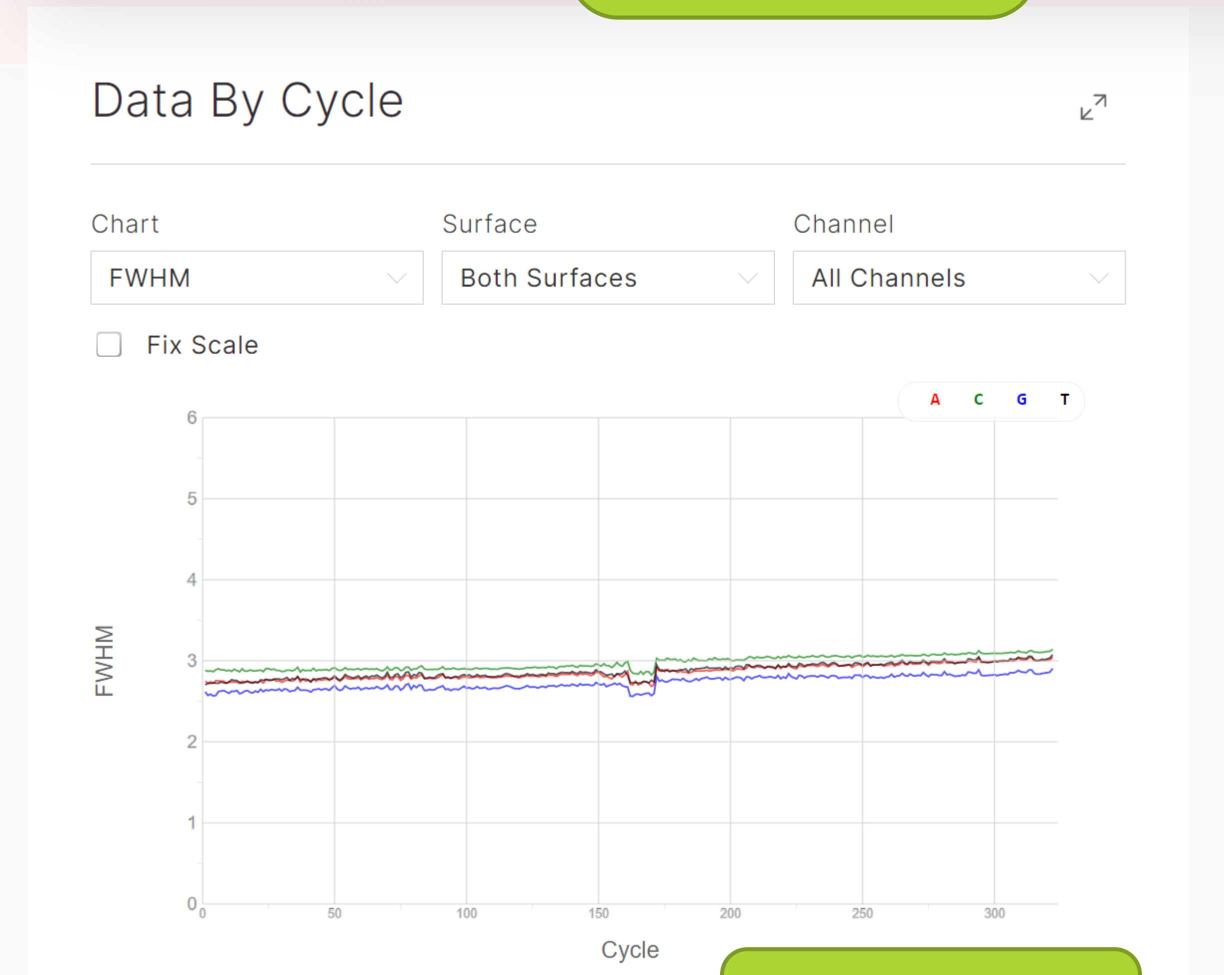


Table 1: MiSeq System performance parameters

Read length	Total time[a]	Output	Quality scores[b]	Single reads[c]	Paired-end reads[c]
MiSeq Reagent Kit v2					
2 × 25 bp	~5.5 hours	750-850 Mb	> 90% bases higher than Q30	12-15M	24-30M
2 × 150 bp	~24 hours	4.5-5.1 Gb	> 80% bases higher than Q30		
2 × 250 bp	~39 hours	7.5-8.5 Gb	> 75% bases higher than Q30		
MiSeq Reagent Kit v3					
2 × 75 bp	~21 hours	3.3-3.8 Gb	> 85% bases higher than Q30	22-25M	44-50M
2 × 300 bp	~56 hours	13.2-15 Gb	> 70% bases higher than Q30		
MiSeq Reagent Kit v2 Micro					
2 × 150 bp	~19 hours	1.2 Gb		4M	8M
MiSeq Reagent Kit v2 Nano					
2 × 150 bp	~17 hours	300 Mb		1M	2M
2 × 250 bp	~28 hours	500 Mb			

Quality Score Spec

FWHM- Average Full Width of Clusters at Half Maximum



Data By Cycle

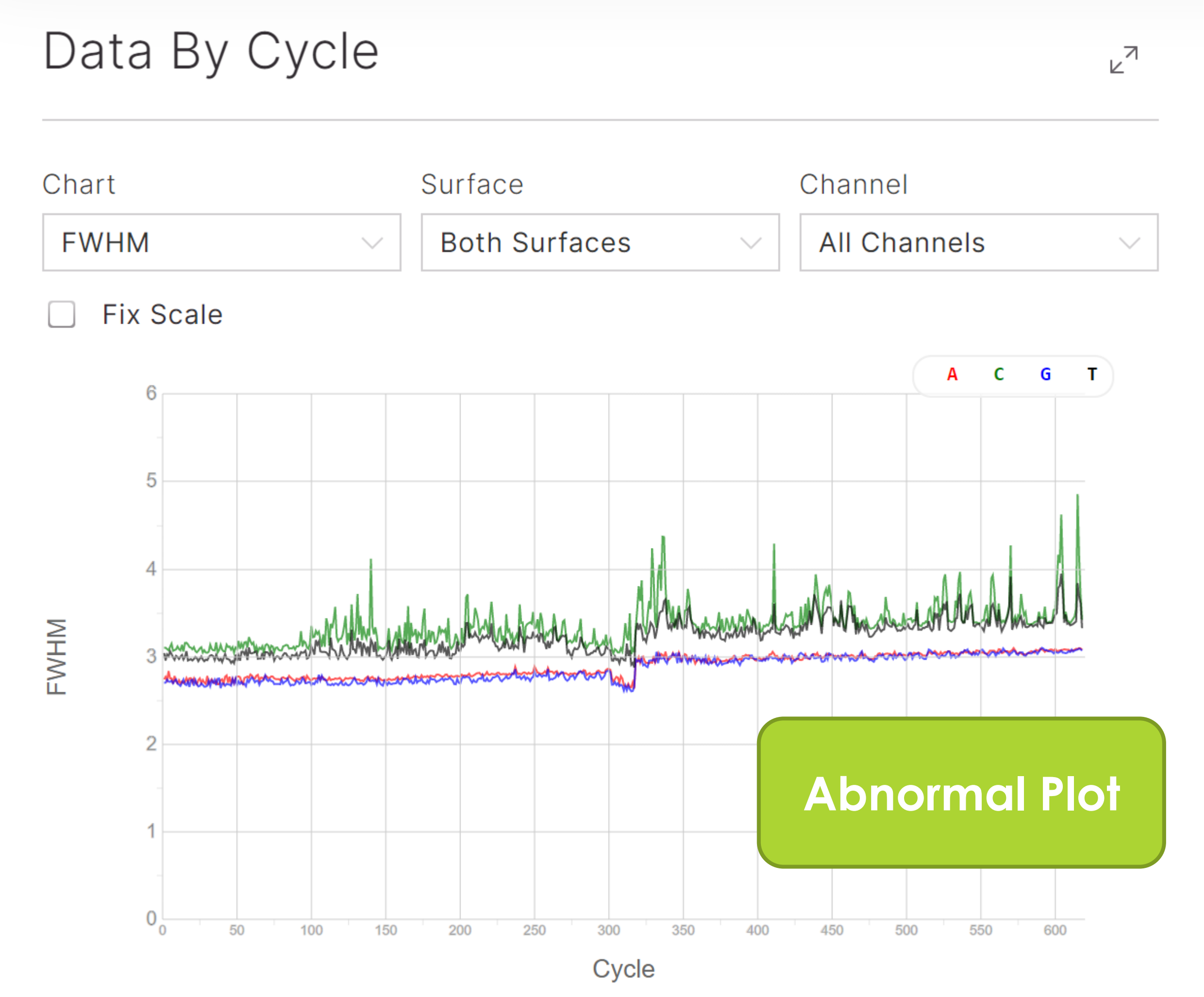
Chart: FWHM

Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale

Normally Mostly Flat



Data By Cycle

Chart: FWHM

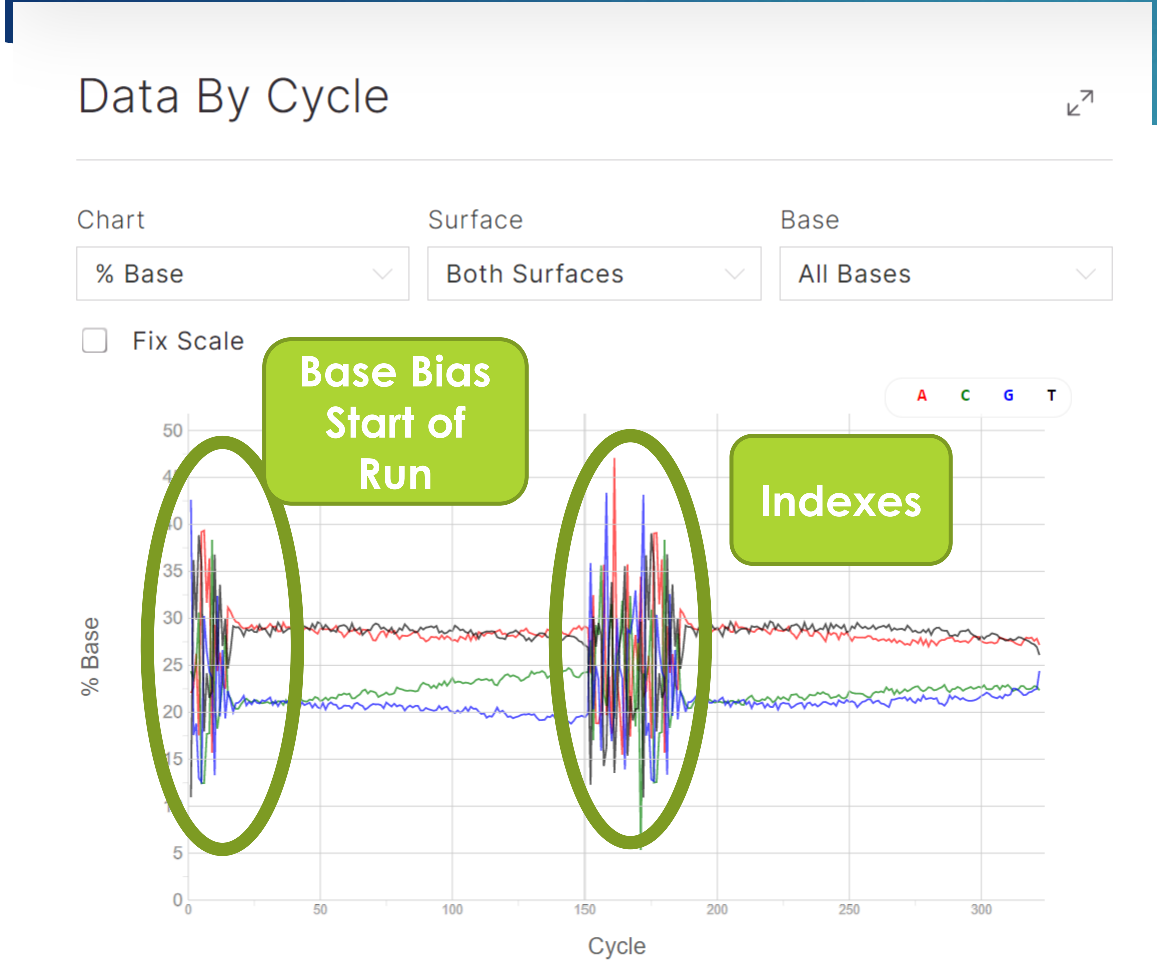
Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale

Abnormal Plot

% Base by Cycle



Data By Cycle

Chart: % Base

Surface: Both Surfaces

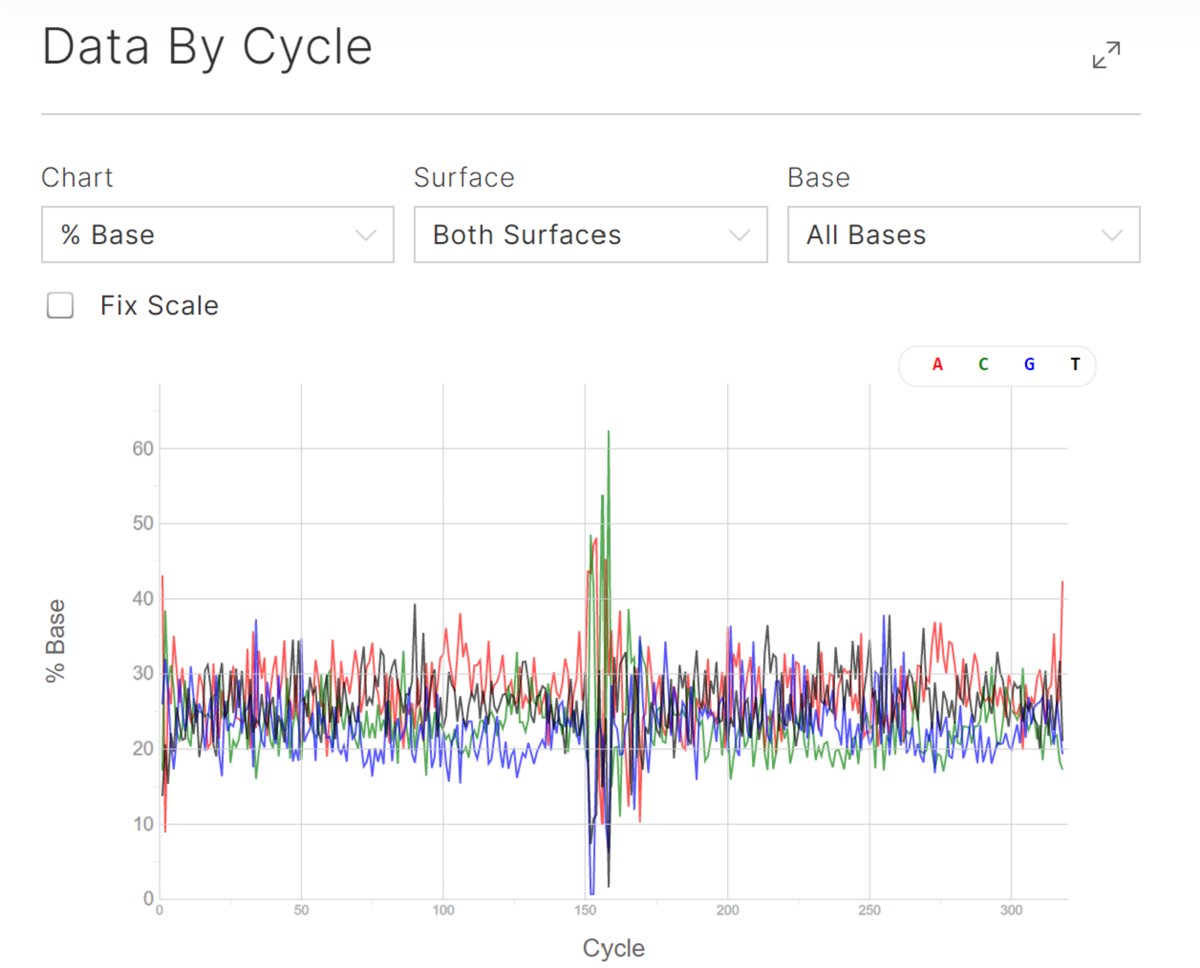
Base: All Bases

☐ Fix Scale

Base Bias Start of Run

Indexes

Nextera XT



Data By Cycle

Chart: % Base

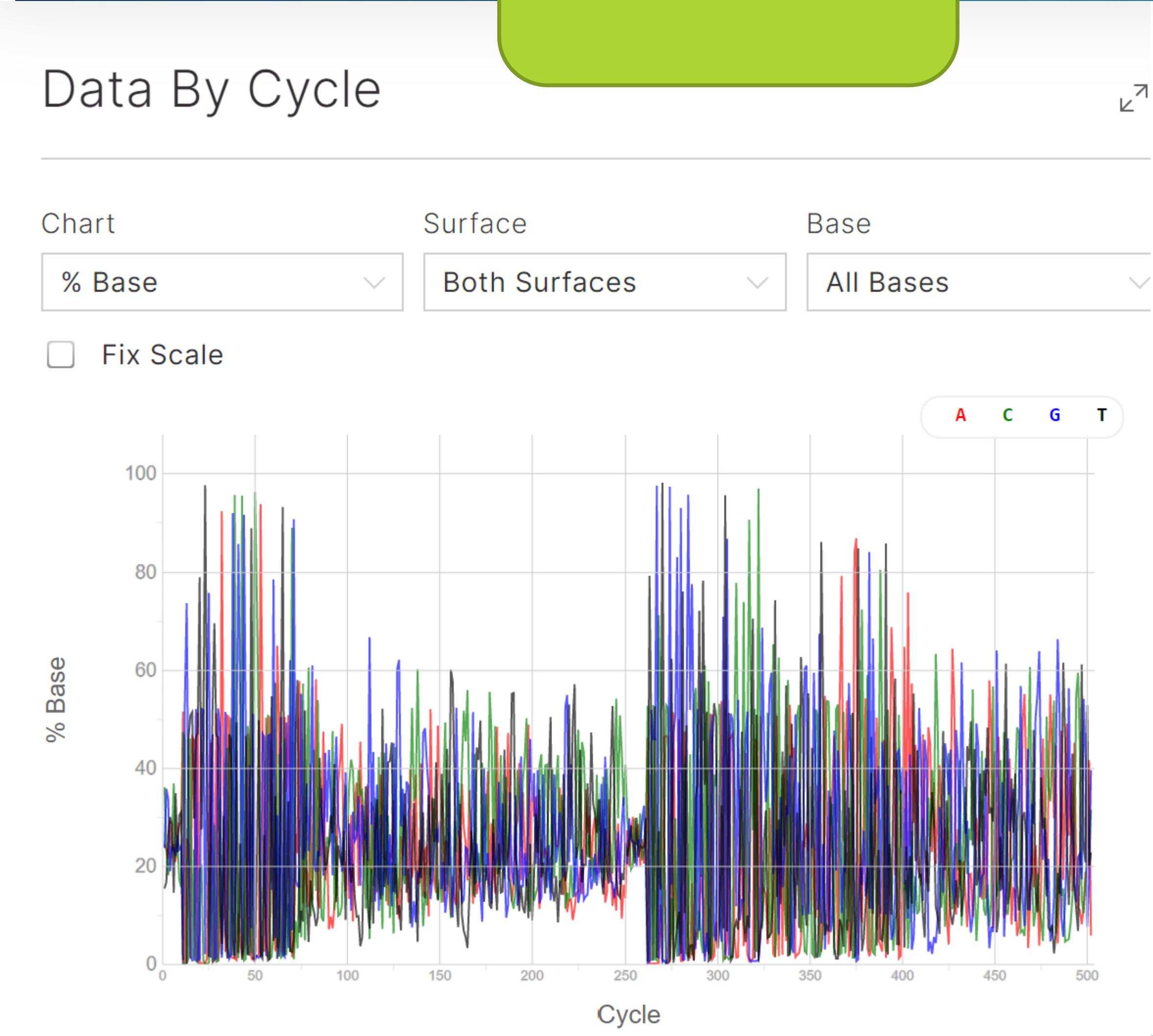
Surface: Both Surfaces

Base: All Bases

☐ Fix Scale

RNASeq

% Base by Cycle



Data By Cycle

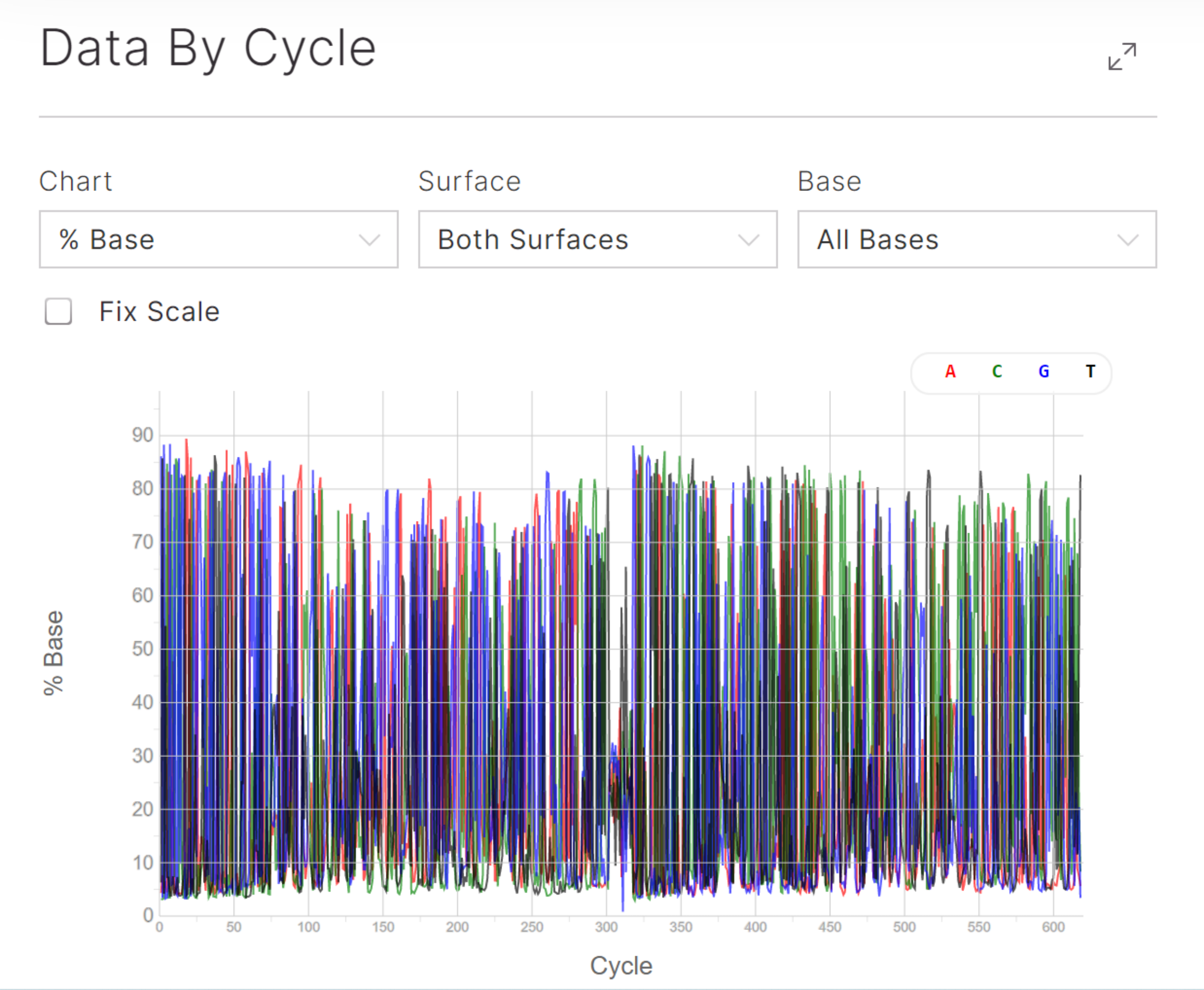
Chart: % Base

Surface: Both Surfaces

Base: All Bases

☐ Fix Scale

PCR Amplicon



Data By Cycle

Chart: % Base

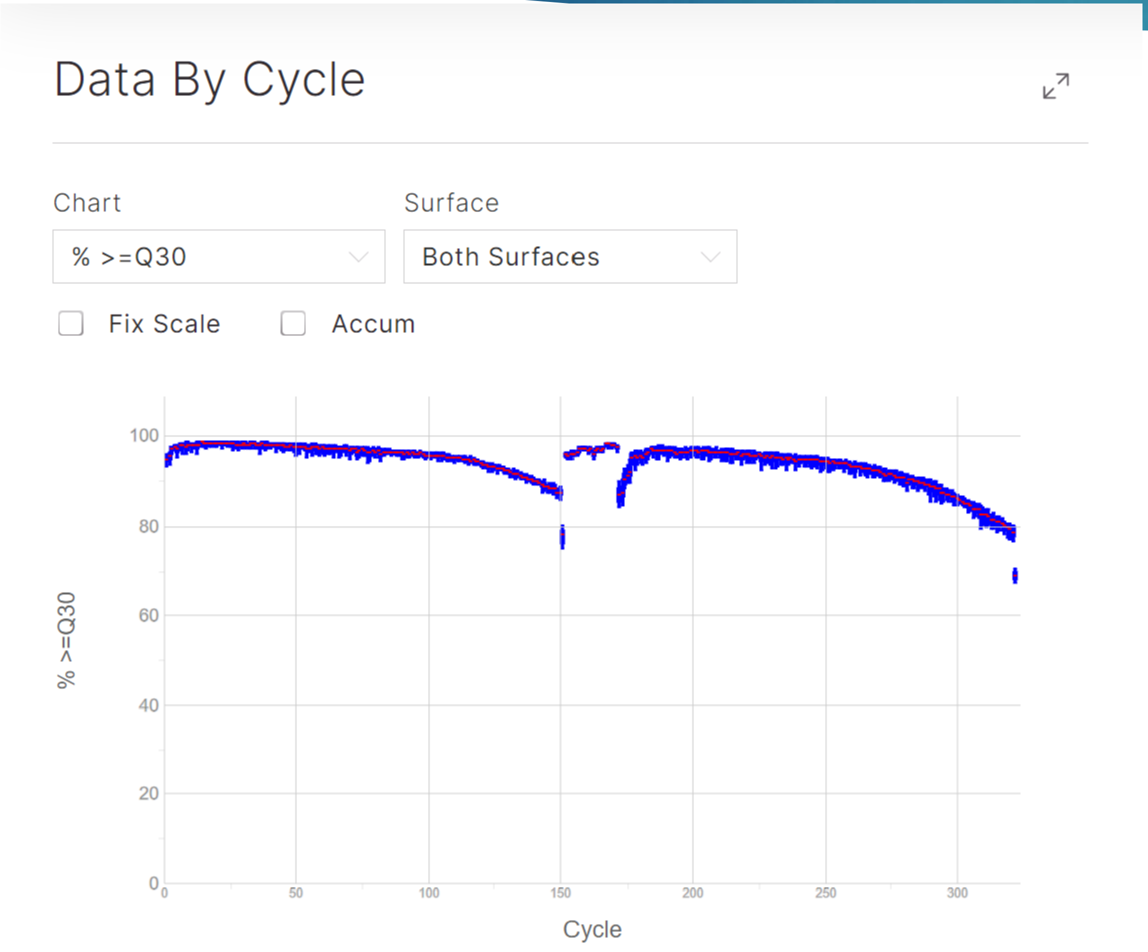
Surface: Both Surfaces

Base: All Bases

☐ Fix Scale

16S rRNA PCR

Q30 by Cycle

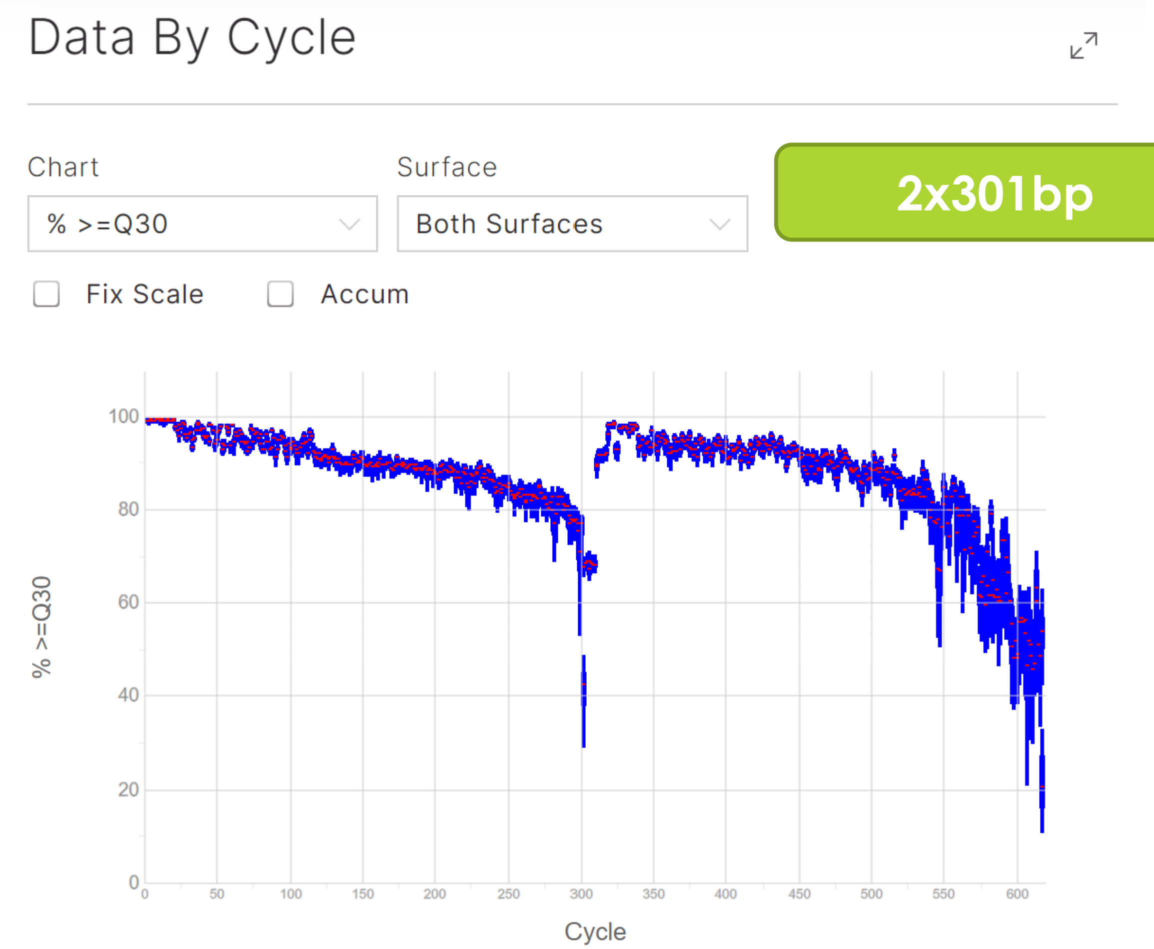


Data By Cycle

Chart: % >=Q30

Surface: Both Surfaces

☐ Fix Scale ☐ Accum



Data By Cycle

Chart: % >=Q30

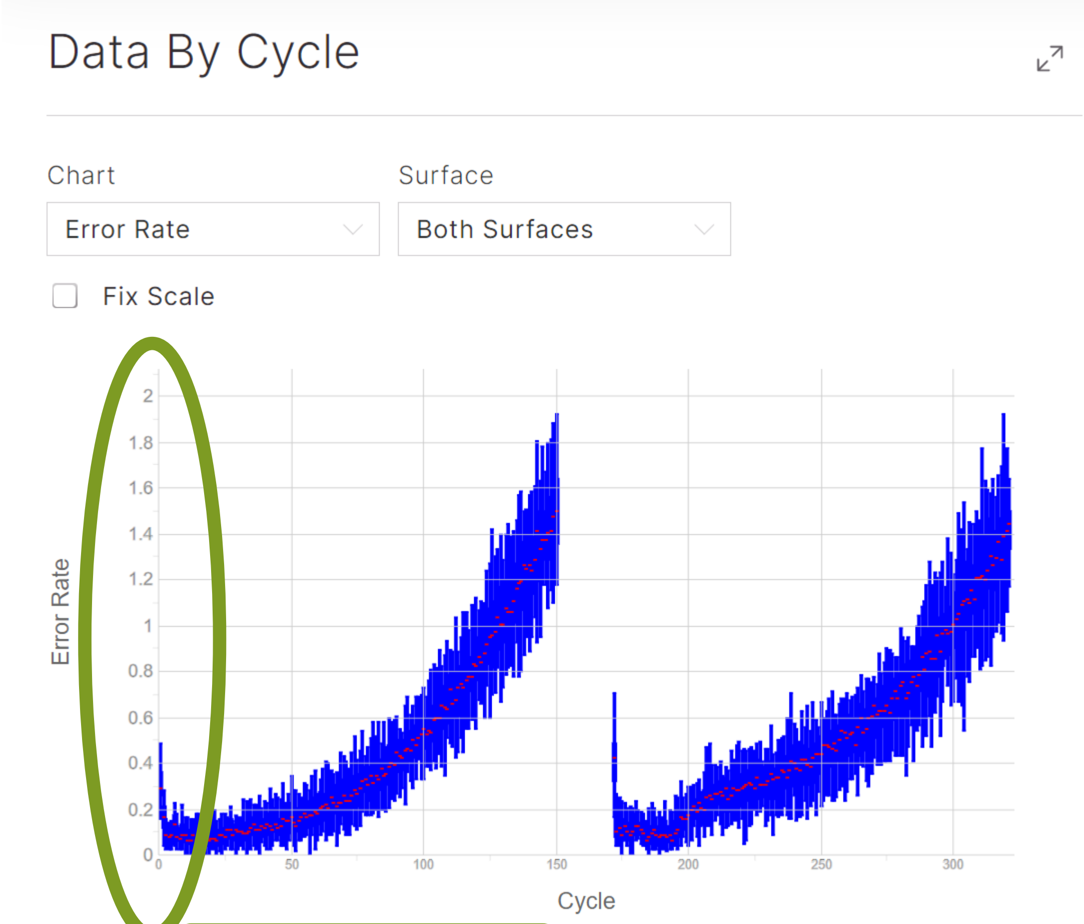
Surface: Both Surfaces

☐ Fix Scale ☐ Accum

2x301bp

Error Rate

Error Rate Only Calculated if PhiX is Spiked In



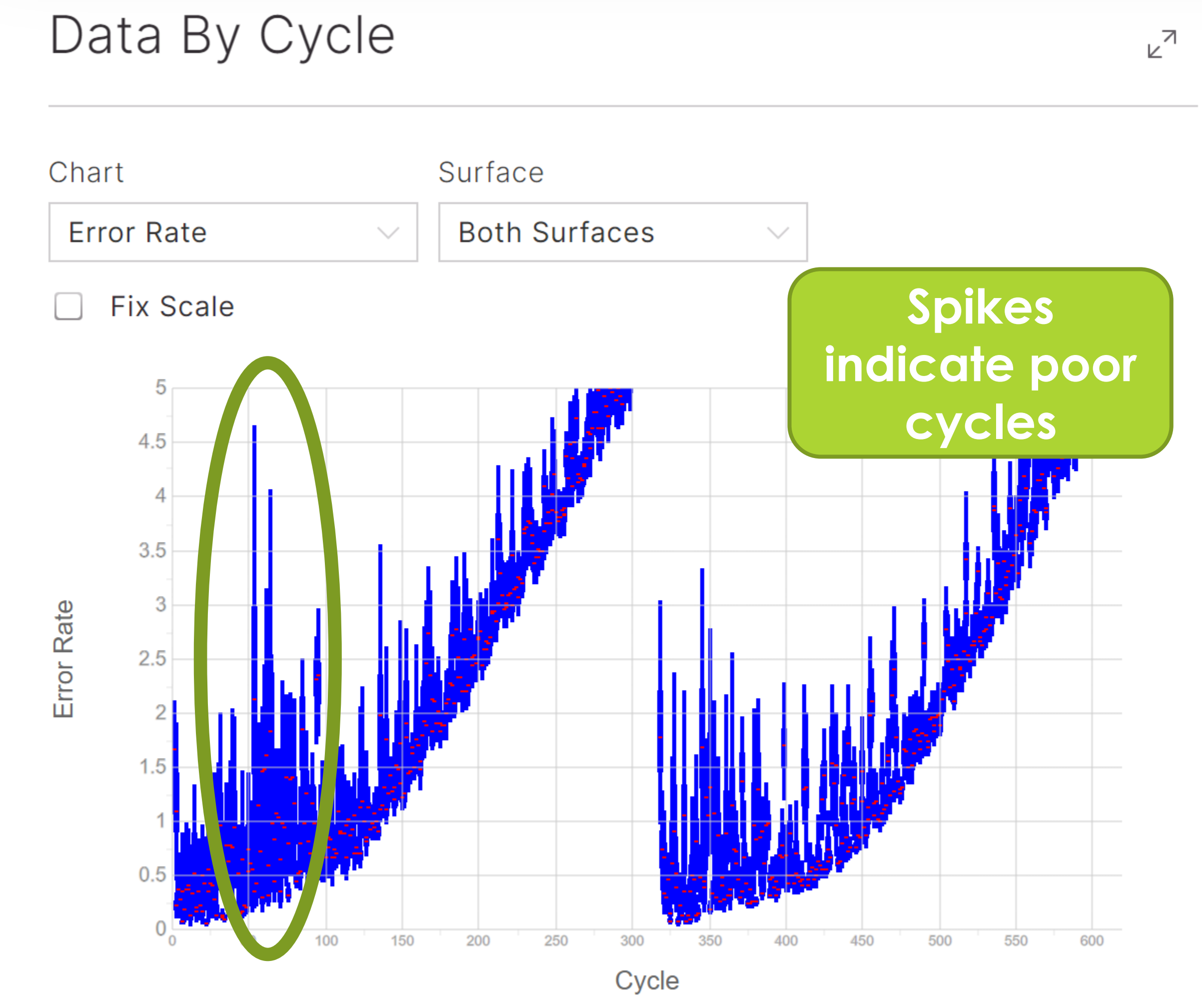
Data By Cycle

Chart: Error Rate

Surface: Both Surfaces

☐ Fix Scale

Watch Scale



Data By Cycle

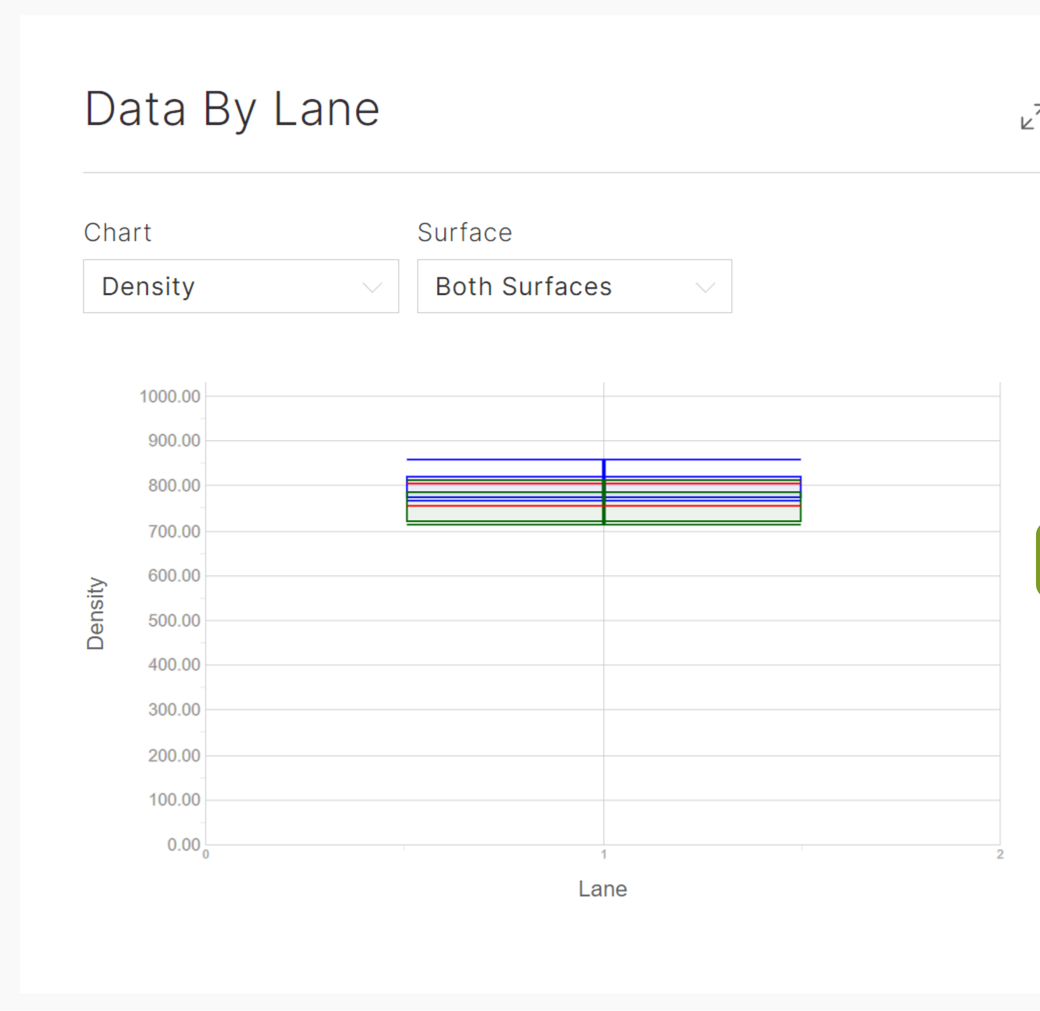
Chart: Error Rate

Surface: Both Surfaces

☐ Fix Scale

Spikes indicate poor cycles

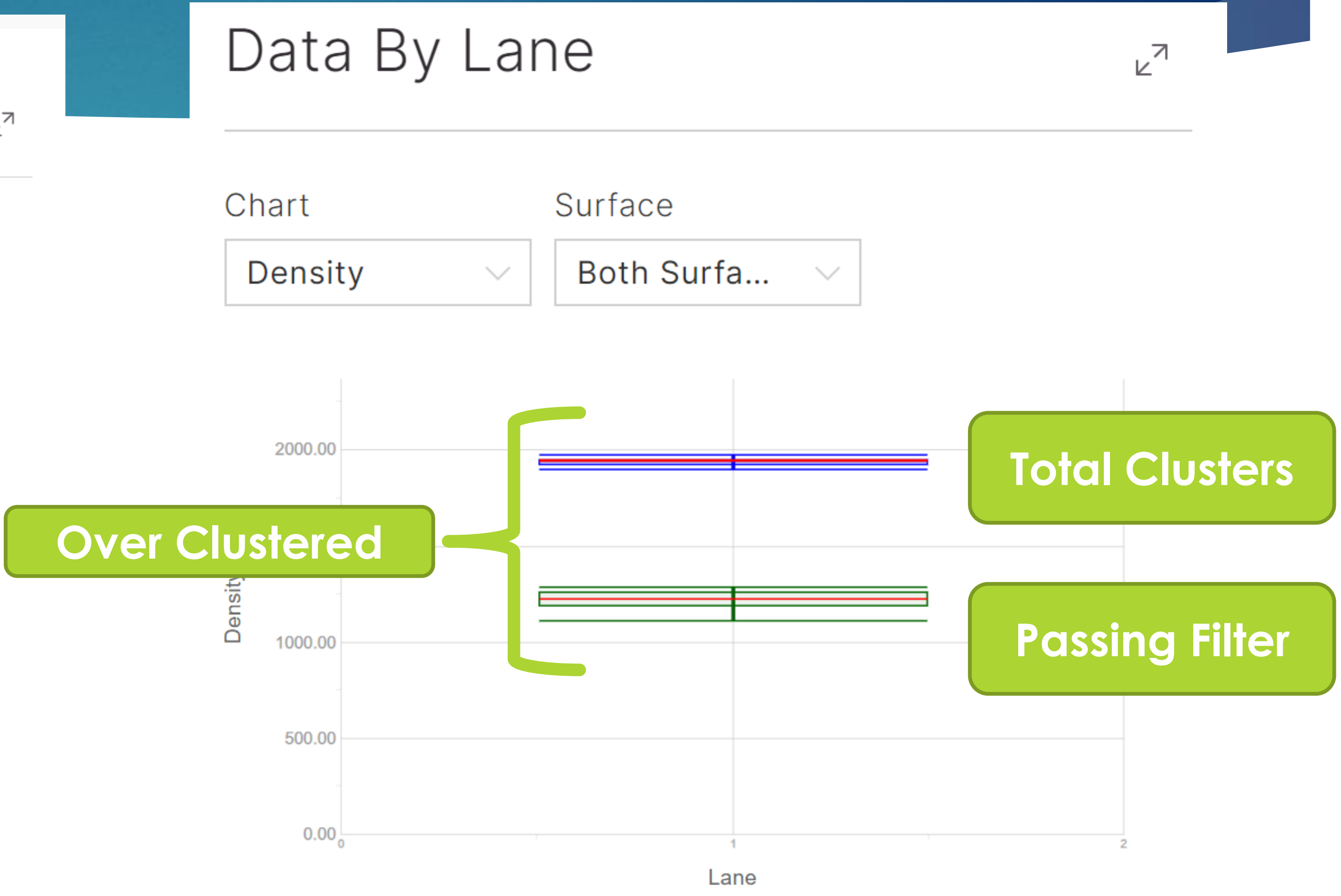
Cluster Density



Data By Lane

Chart: Density

Surface: Both Surfaces



Data By Lane

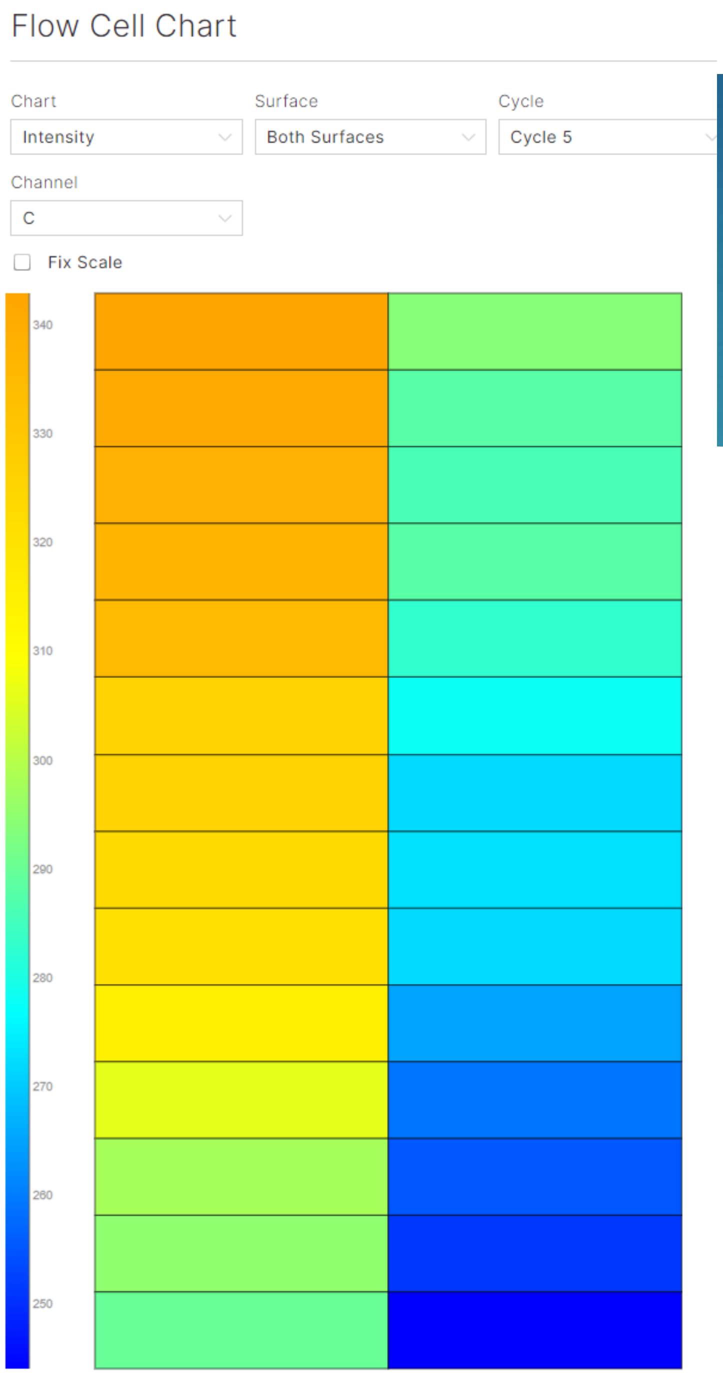
Chart: Density

Surface: Both Surfa...

Over Clustered

Total Clusters

Passing Filter



Flow Cell Chart

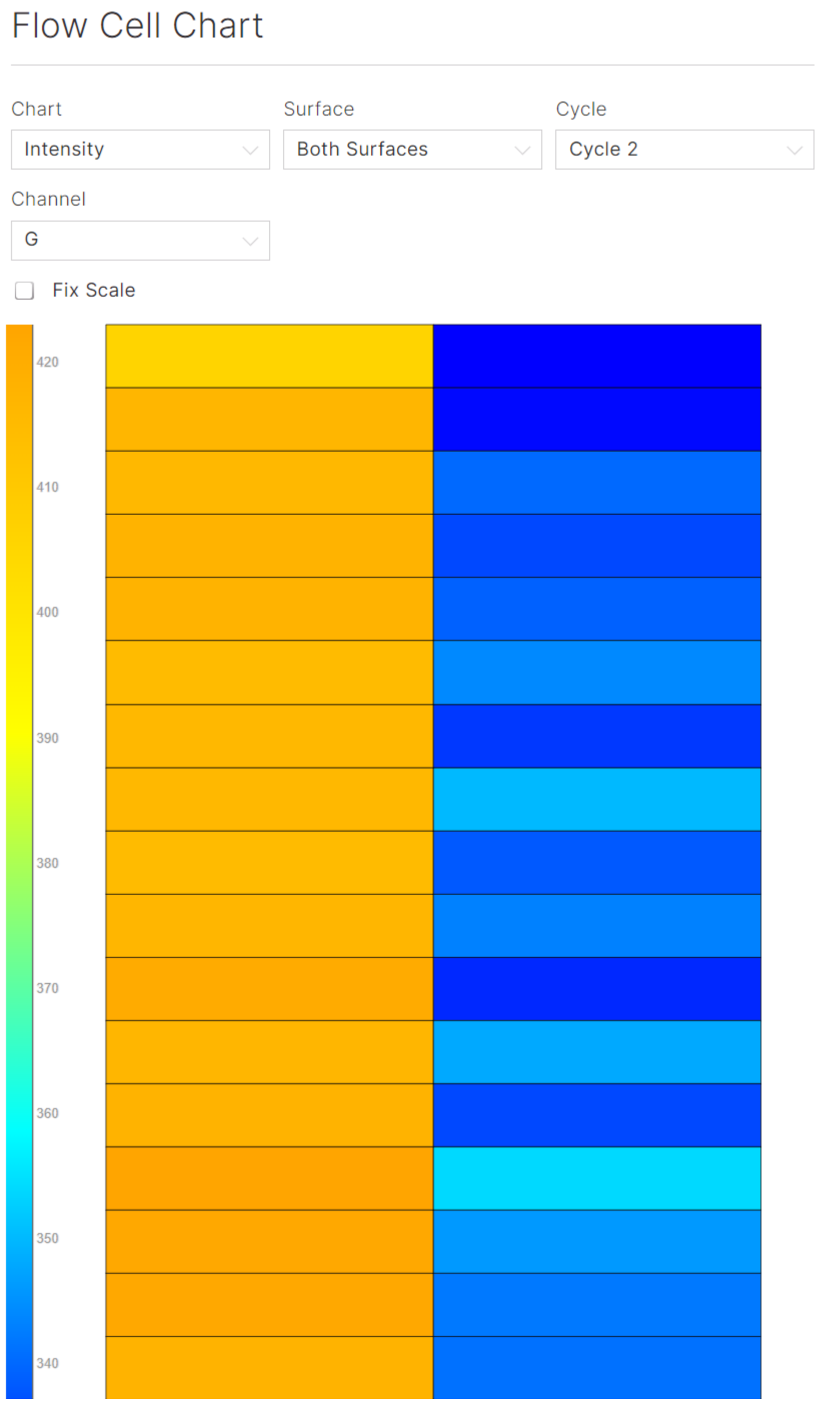
Chart: Intensity

Surface: Both Surfaces

Cycle: Cycle 5

Channel: C

☐ Fix Scale



Flow Cell Chart

Chart: Intensity

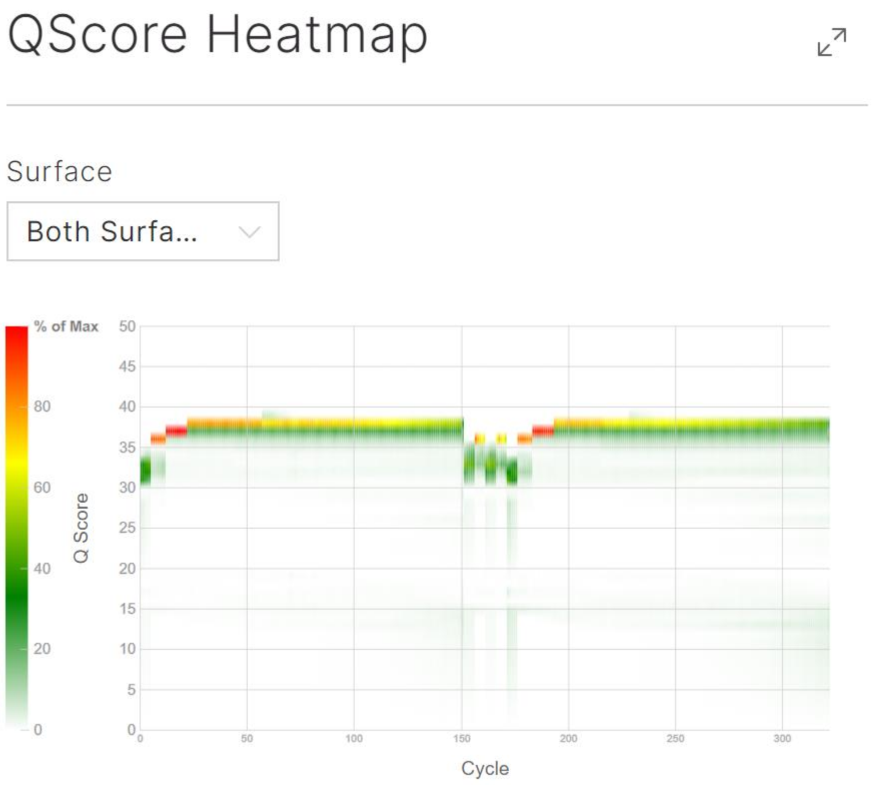
Surface: Both Surfaces

Cycle: Cycle 2

Channel: G

☐ Fix Scale

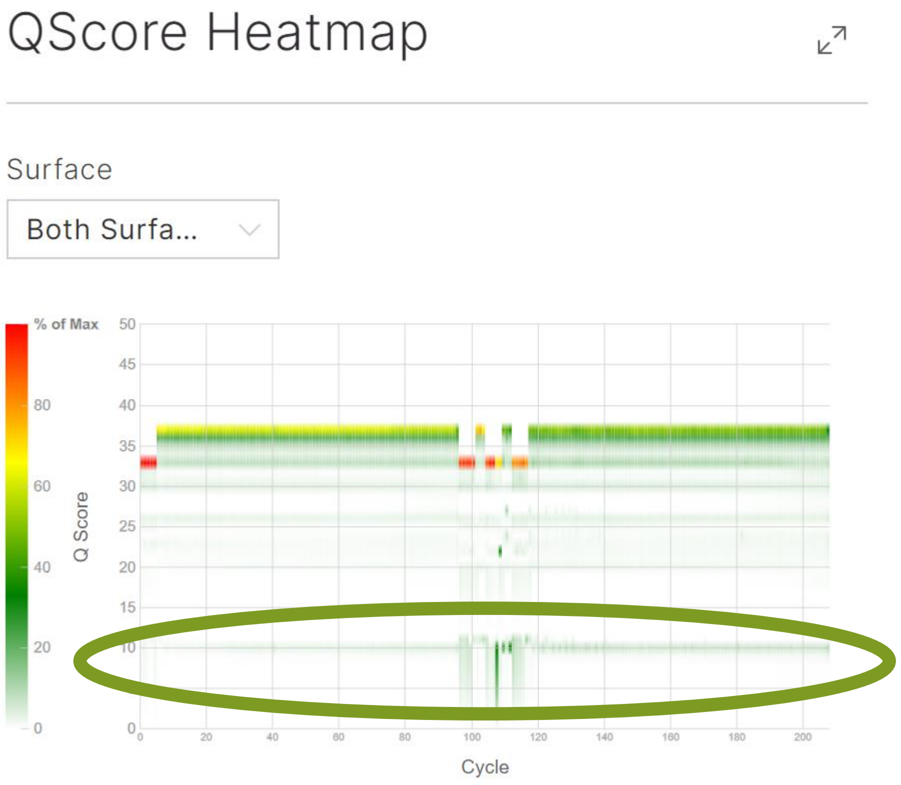
Quality Score Heatmap



QScore Heatmap

Surface

Both Surfa...

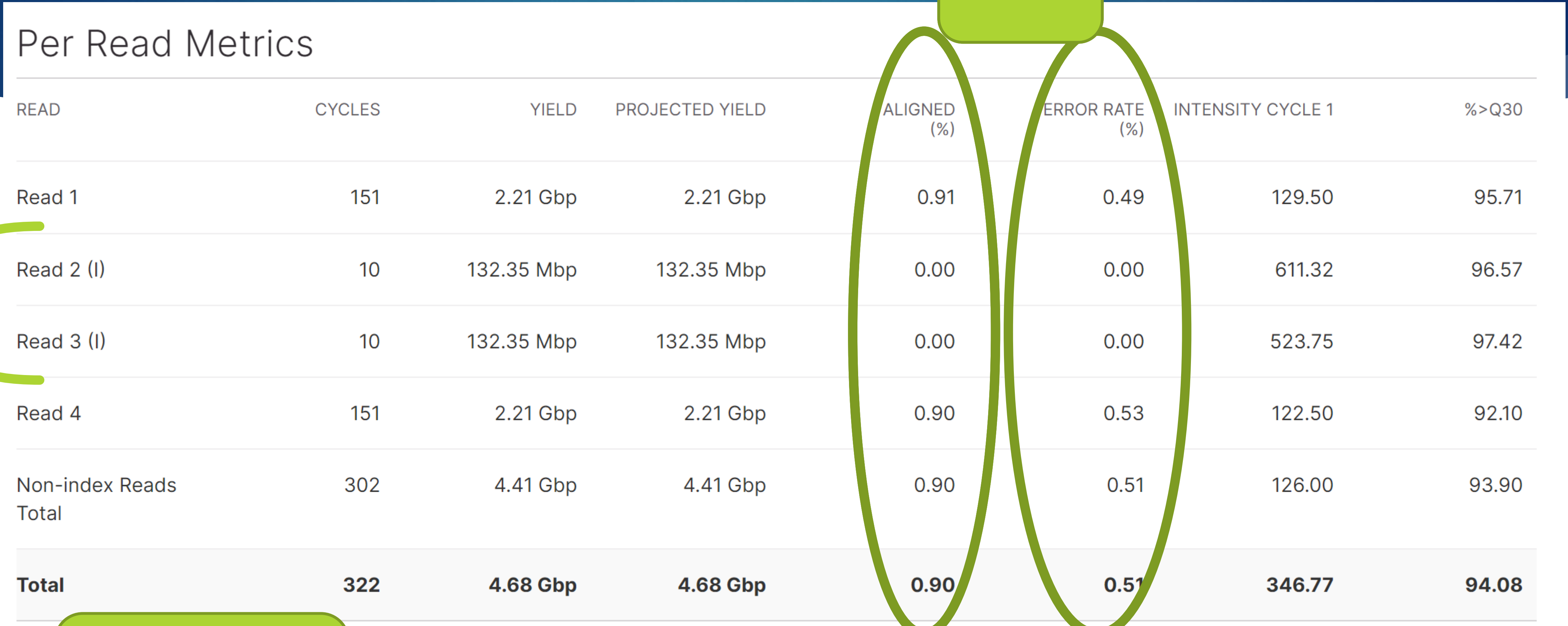


QScore Heatmap

Surface

Both Surfa...

Lower Q Scores

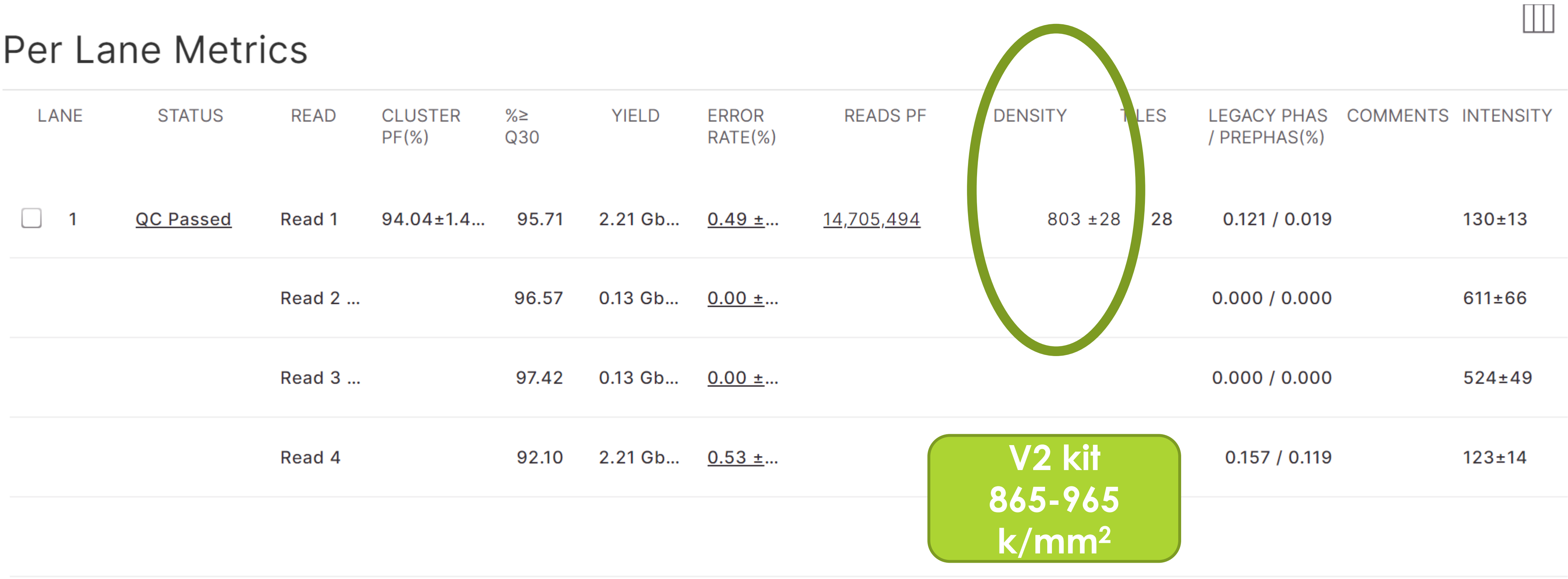


Per Read Metrics

READ	CYCLES	YIELD	PROJECTED YIELD	ALIGNED (%)	ERROR RATE (%)	INTENSITY CYCLE 1	%>Q30
Read 1	151	2.21 Gbp	2.21 Gbp	0.91	0.49	129.50	95.71
Read 2 (I)	10	132.35 Mbp	132.35 Mbp	0.00	0.00	611.32	96.57
Read 3 (I)	10	132.35 Mbp	132.35 Mbp	0.00	0.00	523.75	97.42
Read 4	151	2.21 Gbp	2.21 Gbp	0.90	0.53	122.50	92.10
Non-index Reads Total	302	4.41 Gbp	4.41 Gbp	0.90	0.51	126.00	93.90
Total	**322**	**4.68 Gbp**	**4.68 Gbp**	**0.90**	**0.51**	**346.77**	**94.08**

PhiX

Note: Illumina calls Index Reads, R2 & R3

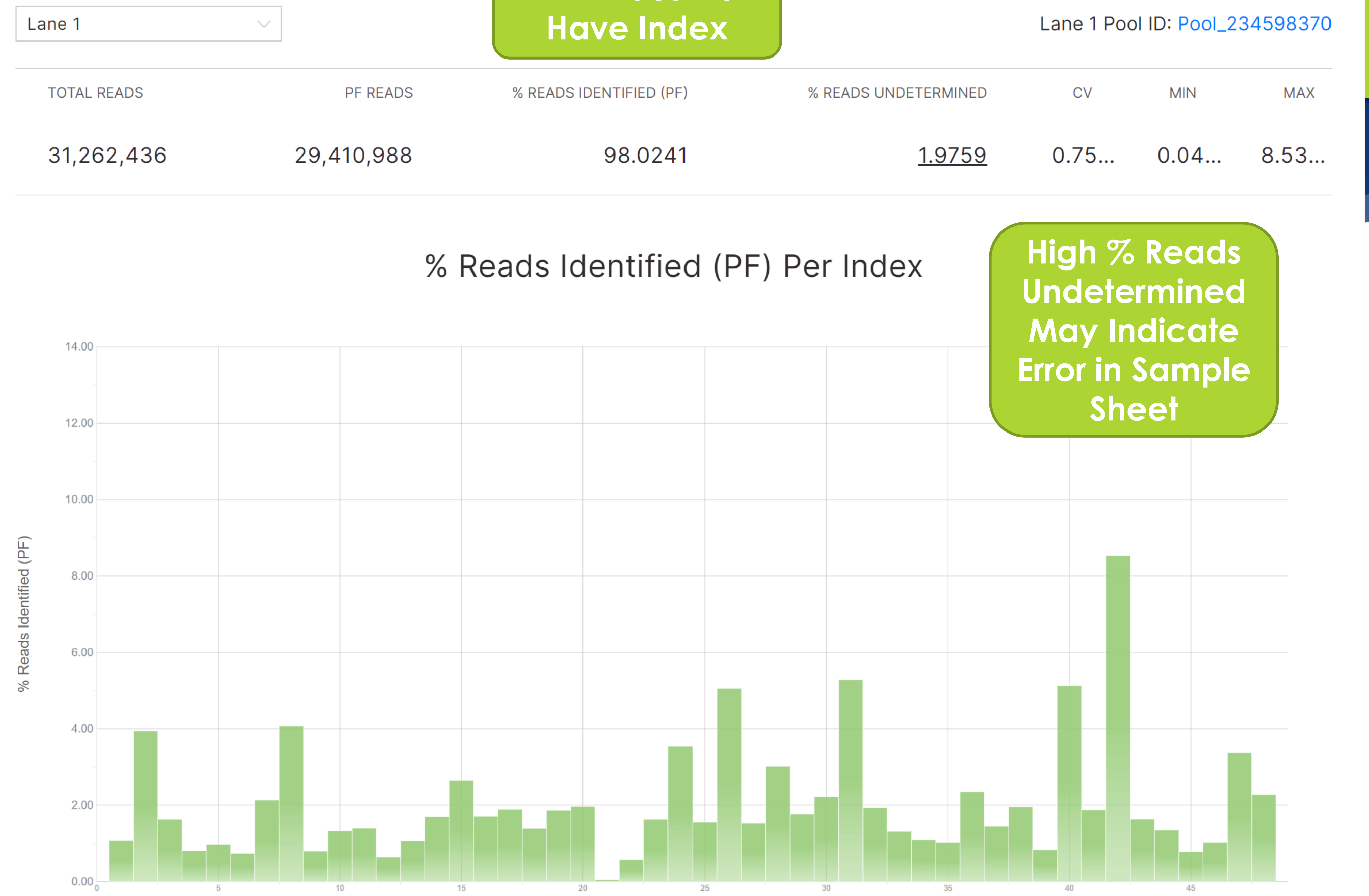


Per Lane Metrics

LANE	STATUS	READ	CLUSTER PF(%)	%≥ Q30	YIELD	ERROR RATE(%)	READS PF	DENSITY	TILES	LEGACY PHAS / PREPHAS(%)	COMMENTS	INTENSITY
☐ 1	QC Passed	Read 1	94.04±1.4...	95.71	2.21 Gb...	0.49 ±...	14,705,494	803 ±28	28	0.121 / 0.019		130±13
		Read 2 ...		96.57	0.13 Gb...	0.00 ±...				0.000 / 0.000		611±66
		Read 3 ...		97.42	0.13 Gb...	0.00 ±...				0.000 / 0.000		524±49
		Read 4		92.10	2.21 Gb...	0.53 ±...				0.157 / 0.119		123±14

V2 kit
865-965
k/mm^2

PhiX Does Not Have Index



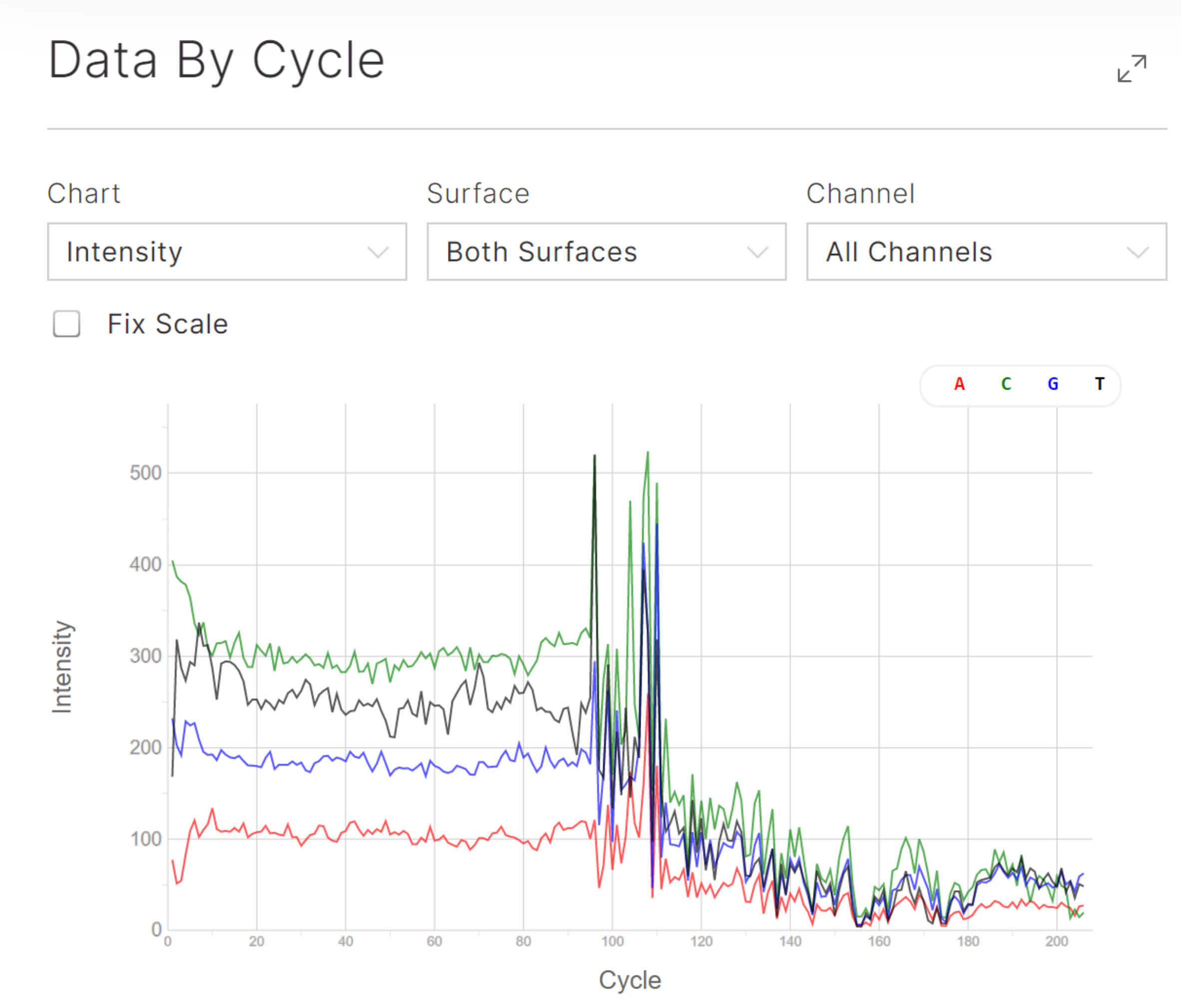
Lane 1 Pool ID: Pool_234598370

TOTAL READS	PF READS	% READS IDENTIFIED (PF)	% READS UNDETERMINED	CV	MIN	MAX
31,262,436	29,410,988	98.0241	1.9759	0.75...	0.04...	8.53...

% Reads Identified (PF) Per Index

High % Reads Undetermined May Indicate Error in Sample Sheet

Failed Sequencing Run



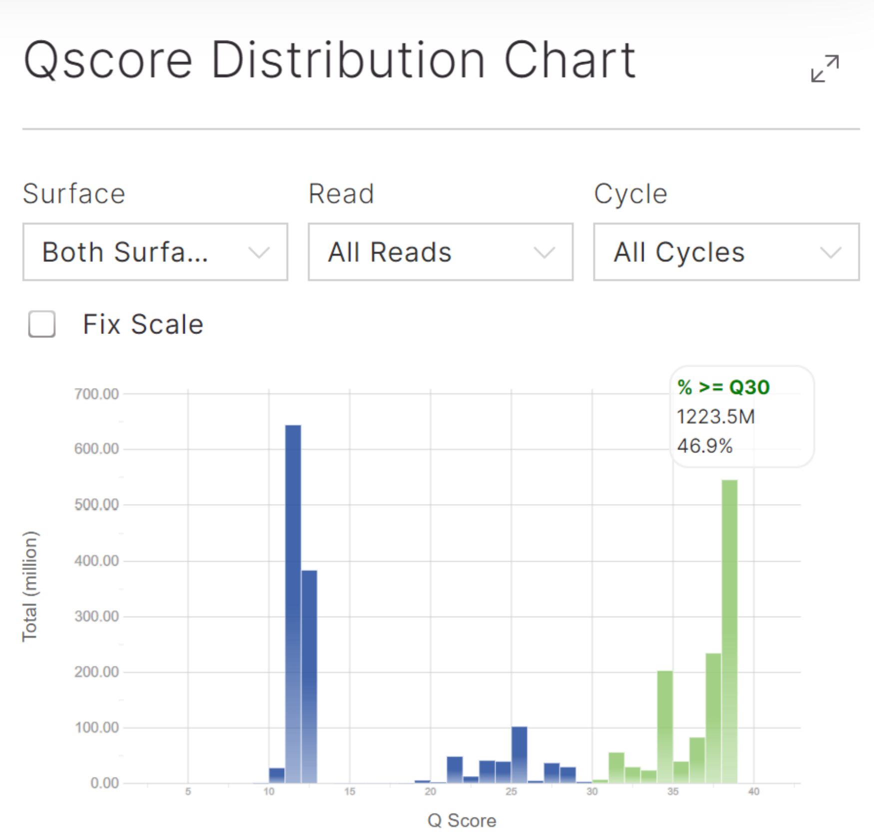
Data By Cycle

Chart: Intensity

Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale



Qscore Distribution Chart

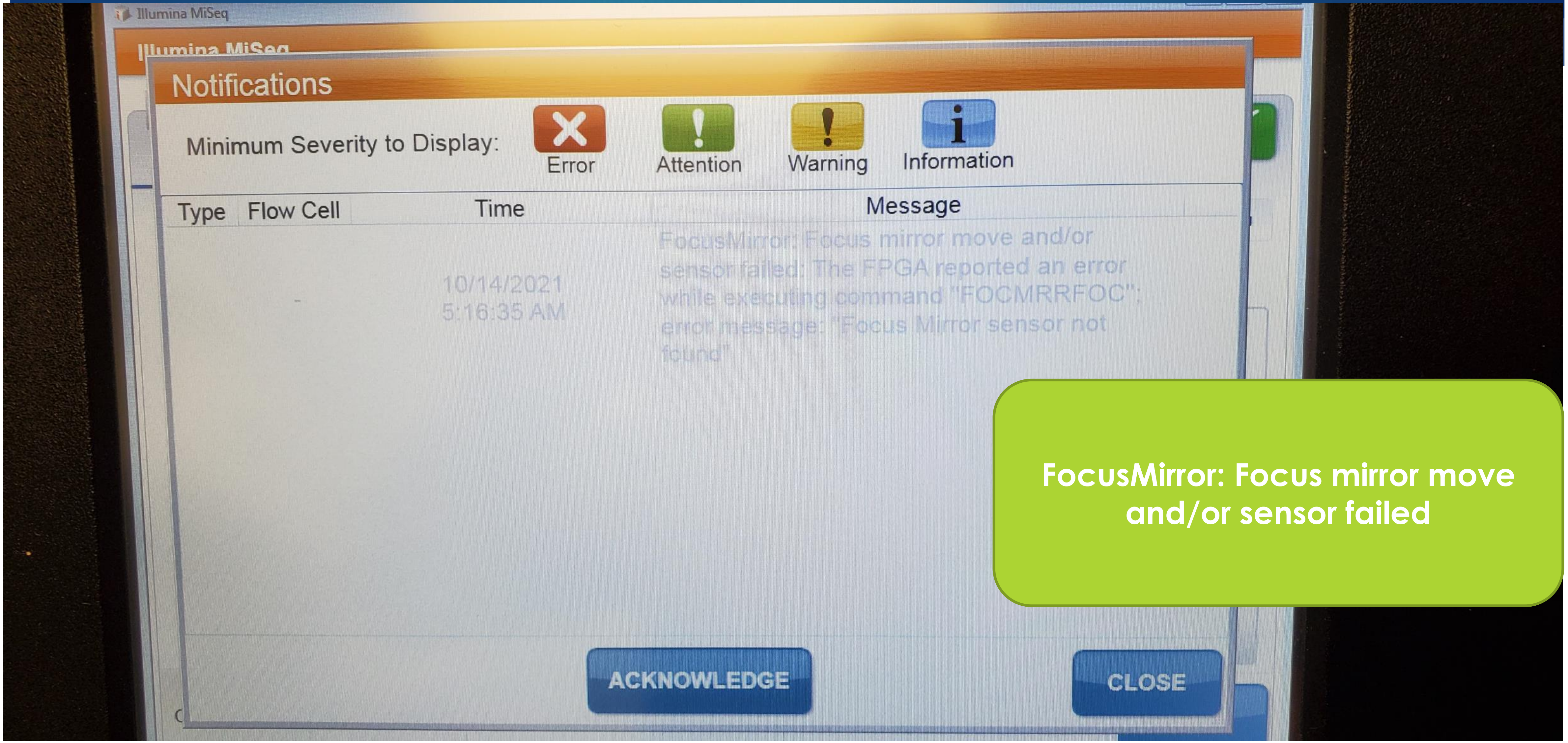
Surface: Both Surfa...

Read: All Reads

Cycle: All Cycles

☐ Fix Scale

Illumina MiSeq Failed Run

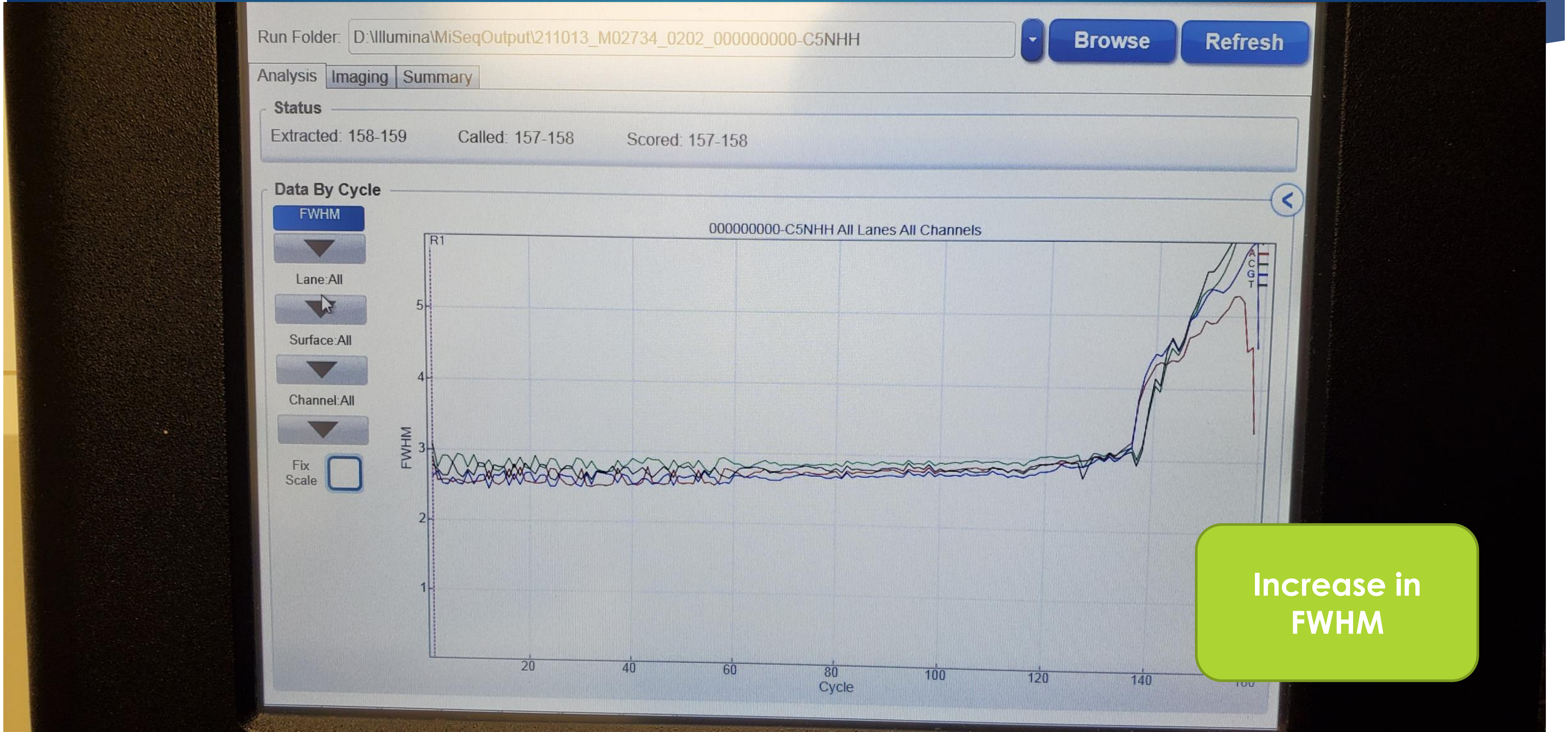


Notifications

Minimum Severity to Display:

Type	Flow Cell	Time	Message
	-	10/14/2021 5:16:35 AM	FocusMirror: Focus mirror move and/or sensor failed: The FPGA reported an error while executing command "FOCMRRFOC"; error message: "Focus Mirror sensor not found"

FocusMirror: Focus mirror move and/or sensor failed

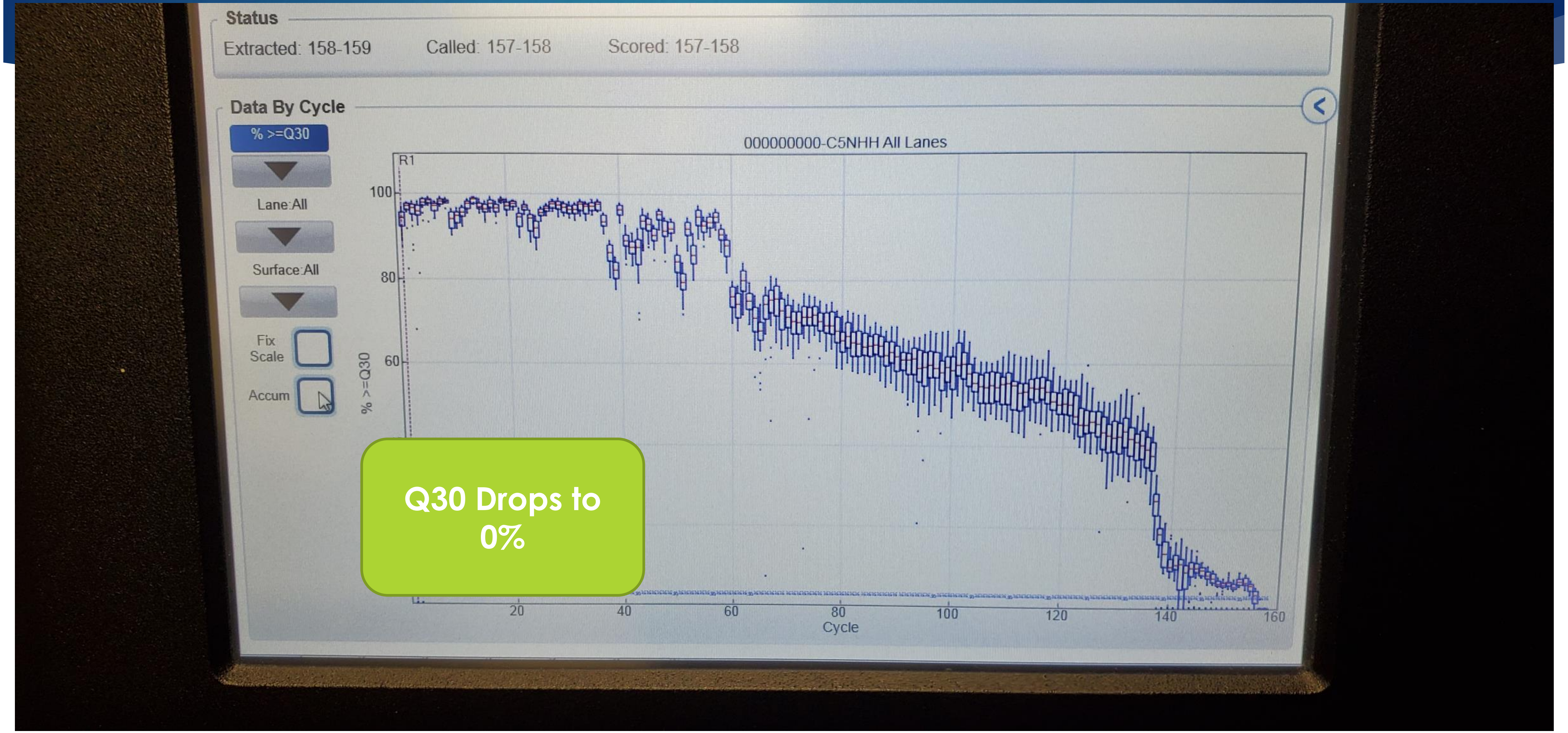


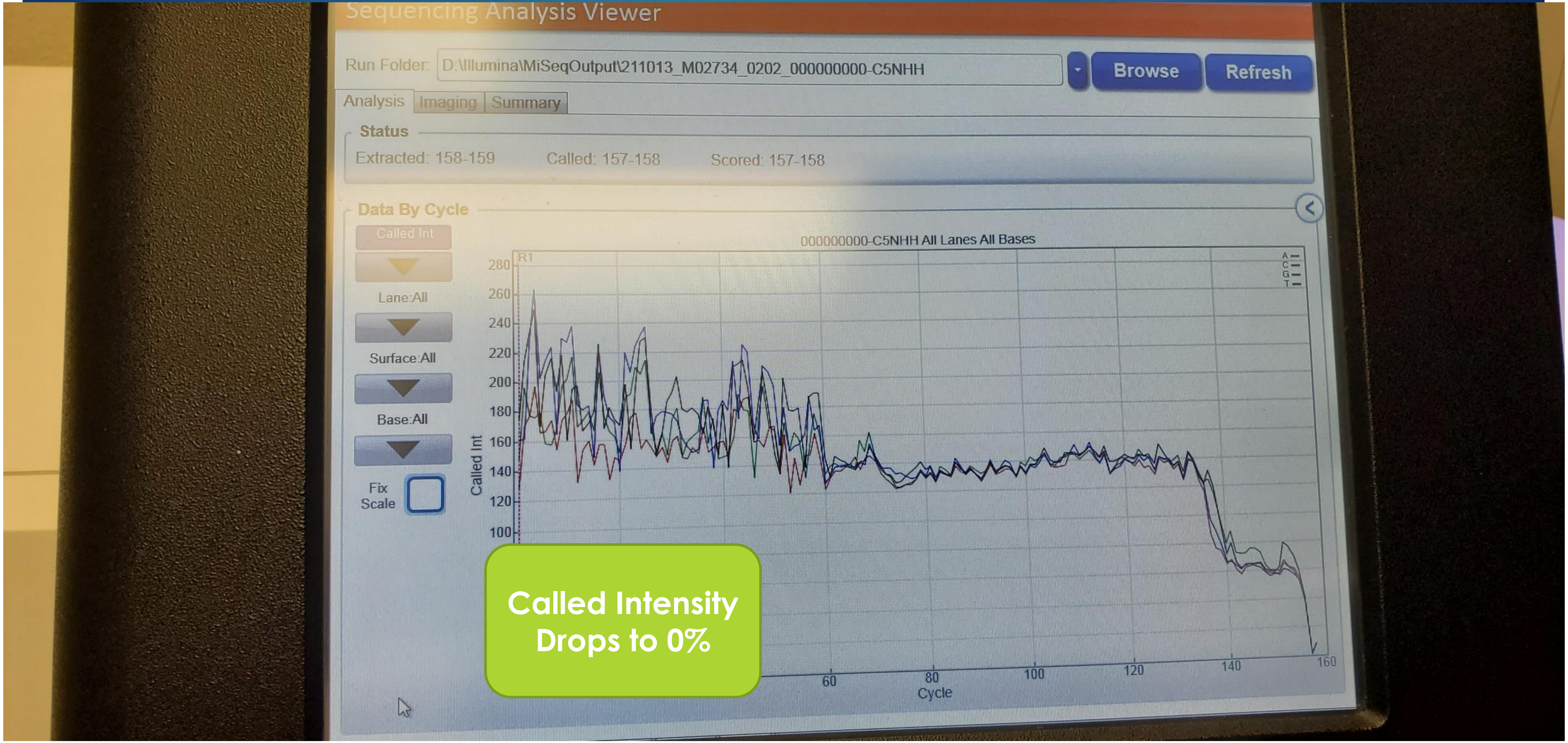
Run Folder: D:\Illumina\MiSeqOutput\211013_M02734_0202_000000000-C5NHH

Status

Extracted: 158-159 Called: 157-158 Scored: 157-158

Increase in FWHM





Run Folder: D:\Illumina\MiSeqOutput\211013_M02734_0202_000000000-C5NHH

Status

Extracted: 158-159 Called: 157-158 Scored: 157-158

Called Intensity Drops to 0%

Cycle 1=Clear Clusters

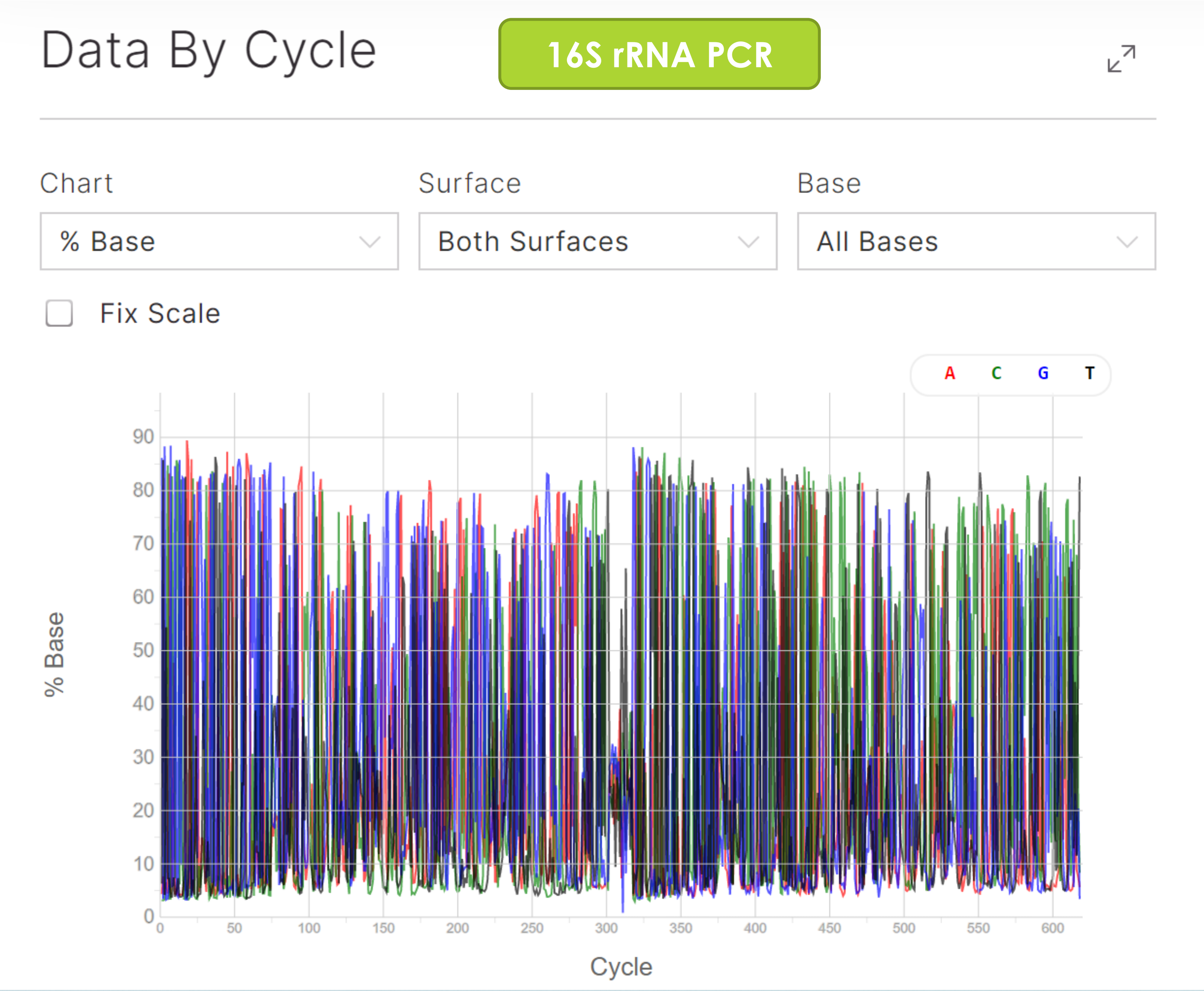
Cycle 150= Clusters Out of Focus

Outcome

Broken Part Needed Replacement

16S rRNA

16S rRNA PCR



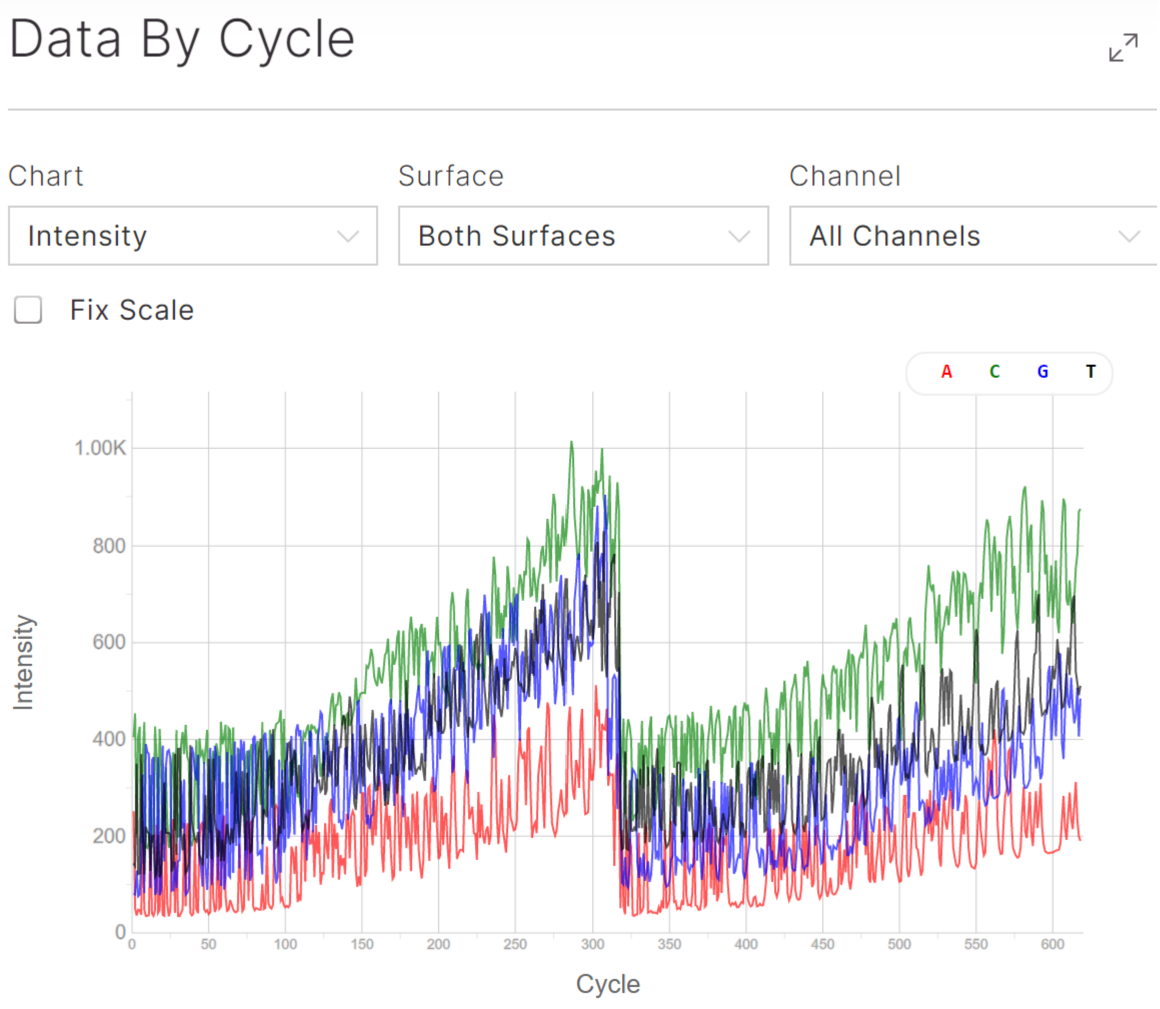
Data By Cycle

Chart: % Base

Surface: Both Surfaces

Base: All Bases

☐ Fix Scale



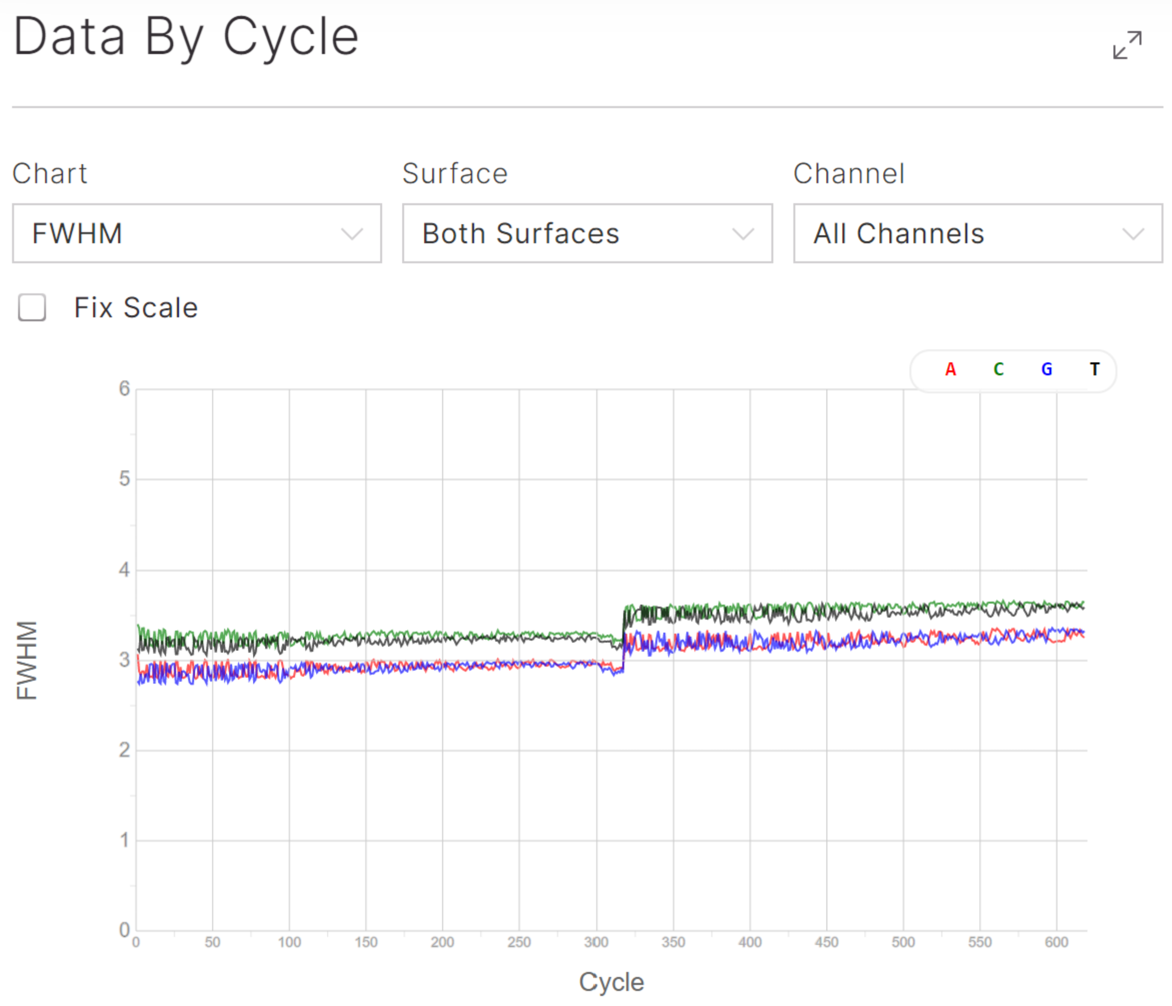
Data By Cycle

Chart: Intensity

Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale



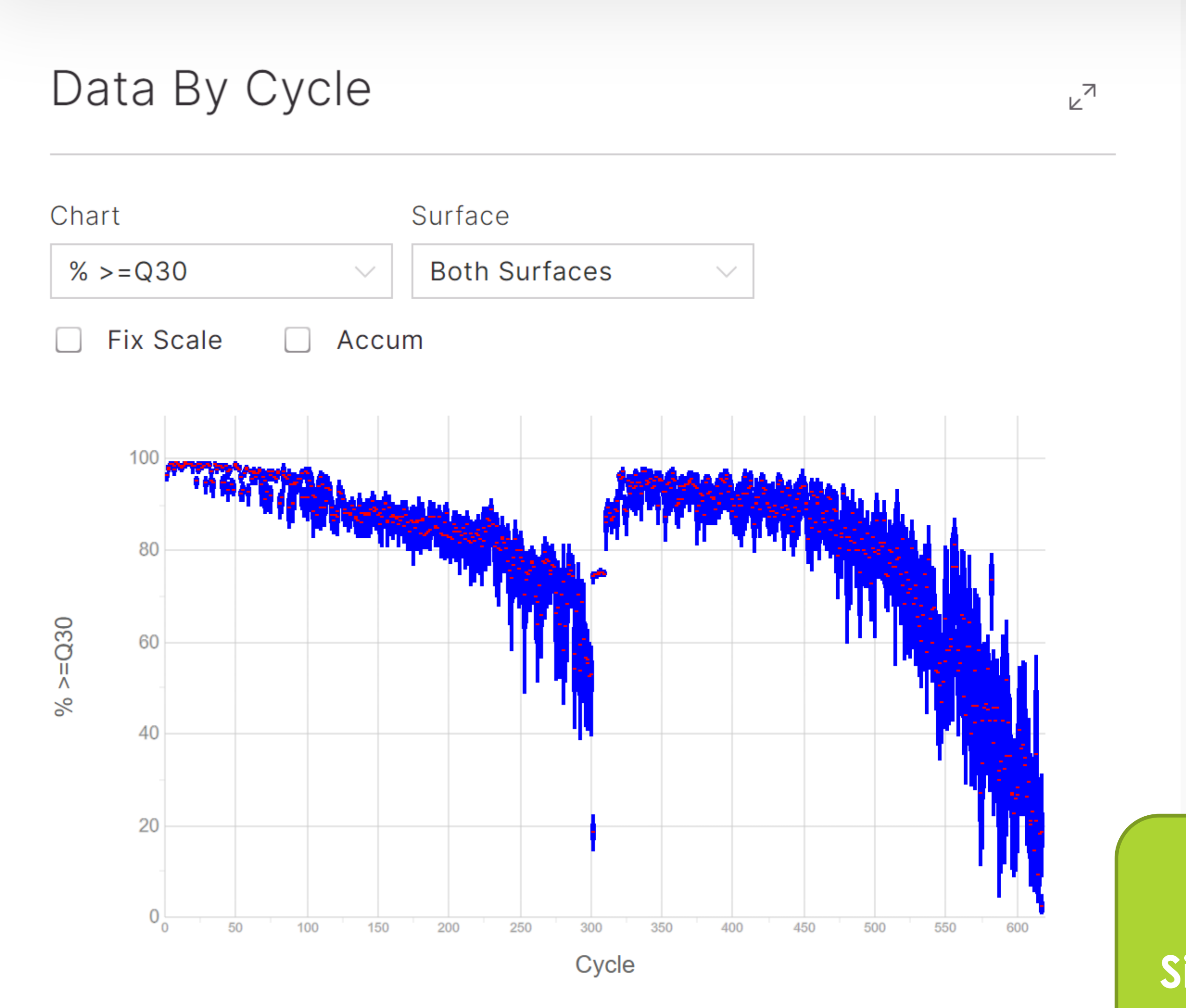
Data By Cycle

Chart: FWHM

Surface: Both Surfaces

Channel: All Channels

☐ Fix Scale

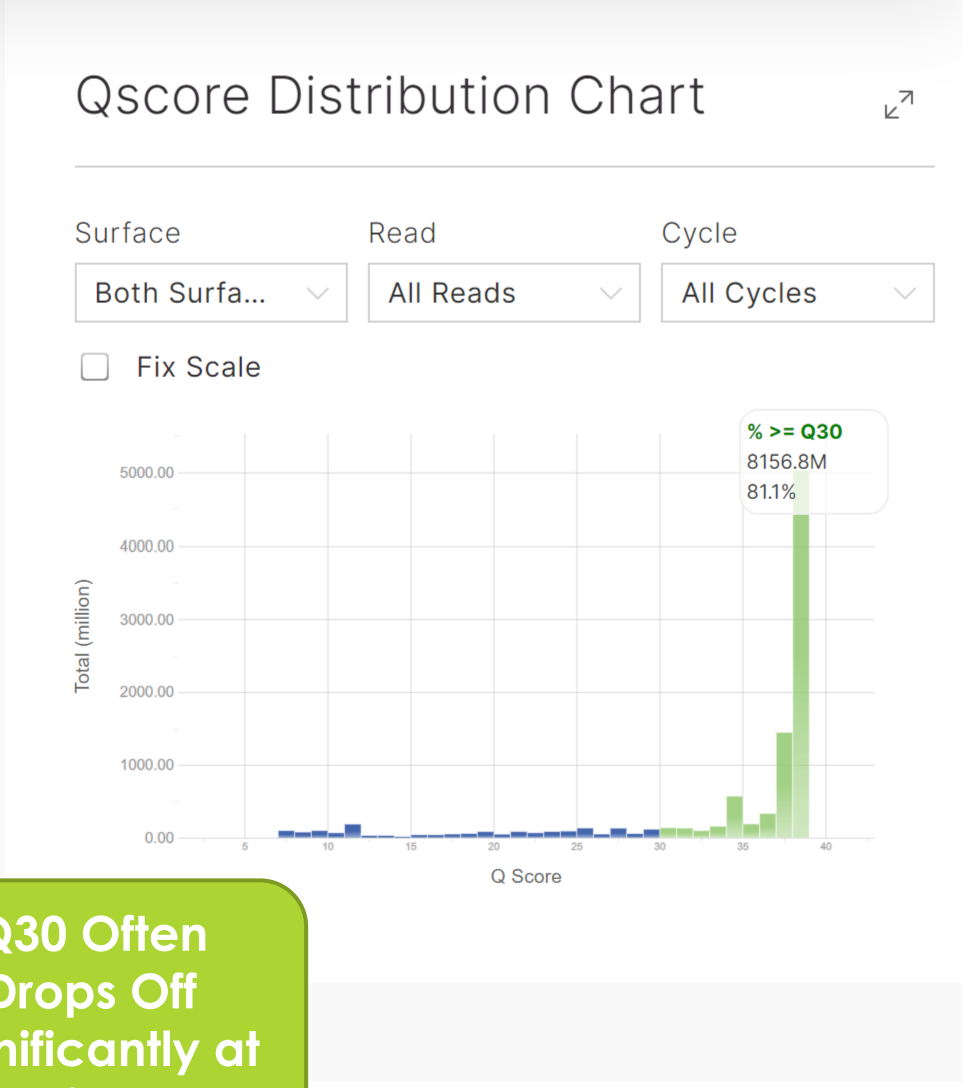


Data By Cycle

Chart: % >=Q30

Surface: Both Surfaces

☐ Fix Scale ☐ Accum



Qscore Distribution Chart

Surface: Both Surfa...

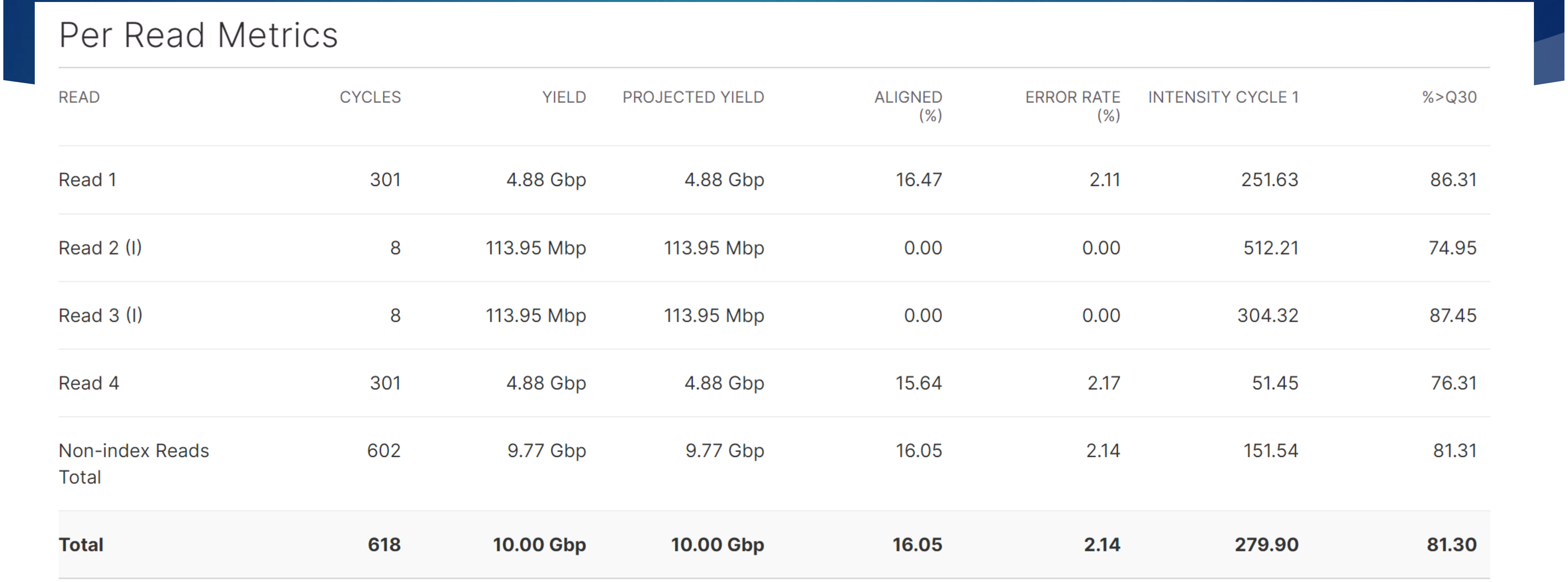
Read: All Reads

Cycle: All Cycles

☐ Fix Scale

% >= Q30
8156.8M
81.1%

Q30 Often Drops Off Significantly at end of Read 2



Per Read Metrics

READ	CYCLES	YIELD	PROJECTED YIELD	ALIGNED (%)	ERROR RATE (%)	INTENSITY CYCLE 1	%>Q30
Read 1	301	4.88 Gbp	4.88 Gbp	16.47	2.11	251.63	86.31
Read 2 (I)	8	113.95 Mbp	113.95 Mbp	0.00	0.00	512.21	74.95
Read 3 (I)	8	113.95 Mbp	113.95 Mbp	0.00	0.00	304.32	87.45
Read 4	301	4.88 Gbp	4.88 Gbp	15.64	2.17	51.45	76.31
Non-index Reads Total	602	9.77 Gbp	9.77 Gbp	16.05	2.14	151.54	81.31
Total	**618**	**10.00 Gbp**	**10.00 Gbp**	**16.05**	**2.14**	**279.90**	**81.30**

Requires ~15% PhiX to Create Sequence Diversity

Agenda Update

- No sessions next week due to Eid al-Fitr (May 1- May 8)
- Next session: Tuesday May 10, 2022
- Week 12 : Genomic epidemiology and Nextstrain pipeline (Domman)

Questions?