Illumina TruSight Oncology 500 DNA/RNA App on Biomek NGeniuS System

Accelerating Answers

BECKMAN COULTER Life Sciences

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App Template Description

App Template: Illumina TruSight Oncology 500 DNA/RNA Automation Kit

Description:

The Illumina* TruSight* Oncology 500 DNA/RNA Automation Kit App Template allows for the creation of Illumina TruSight Oncology 500 DNA and RNA libraries compatible with Illumina sequencing platforms. The App Template allows the user to produce between four and 24 libraries in a single batch with any combination of DNA and RNA samples required. The user has the option of specifying if the batch contains high-quality or low-quality RNA samples and will adjust the RNA fragmentation program accordingly. Refer to the Illumina TruSight Oncology 500 Reference Guide for details concerning RNA input mass and sample quality. DNA samples are pre-sheared on a Covaris* instrument prior to loading onto the Biomek NGeniuS Next Generation Library Prep system. Refer to the Illumina TruSight Oncology 500 Reference Guide for details concerning DNA input mass, sample quality, and shearing parameters. Supplementary HRD enrichment workflow is not supported by the App Template. 80% ethanol wash volumes have been reduced to 50 µL from 200 µL to reduce tip consumption. First and Second Hybridization times have been limited to the minimum time listed in the manual protocol (8 hours for First Hybridization and 1.5 hours for Second Hybridization) to reduce application run time and cannot be changed. The App Template was designed using the Illumina TruSight Oncology 500 Reference Guide (Document# 1000000067621 v10). The App Template utilizes the TruSight Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples) (Illumina Part Number 20045508).

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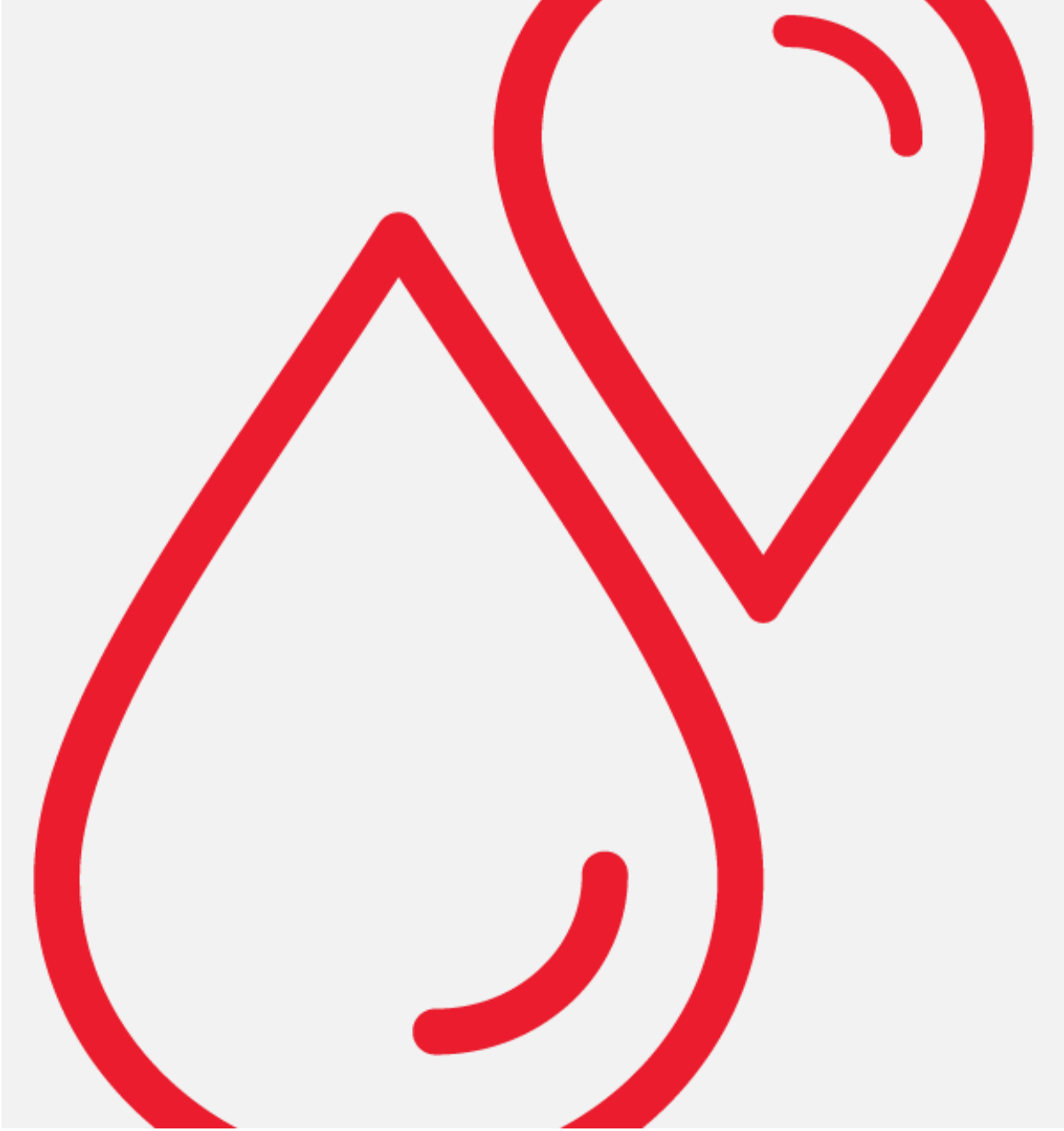
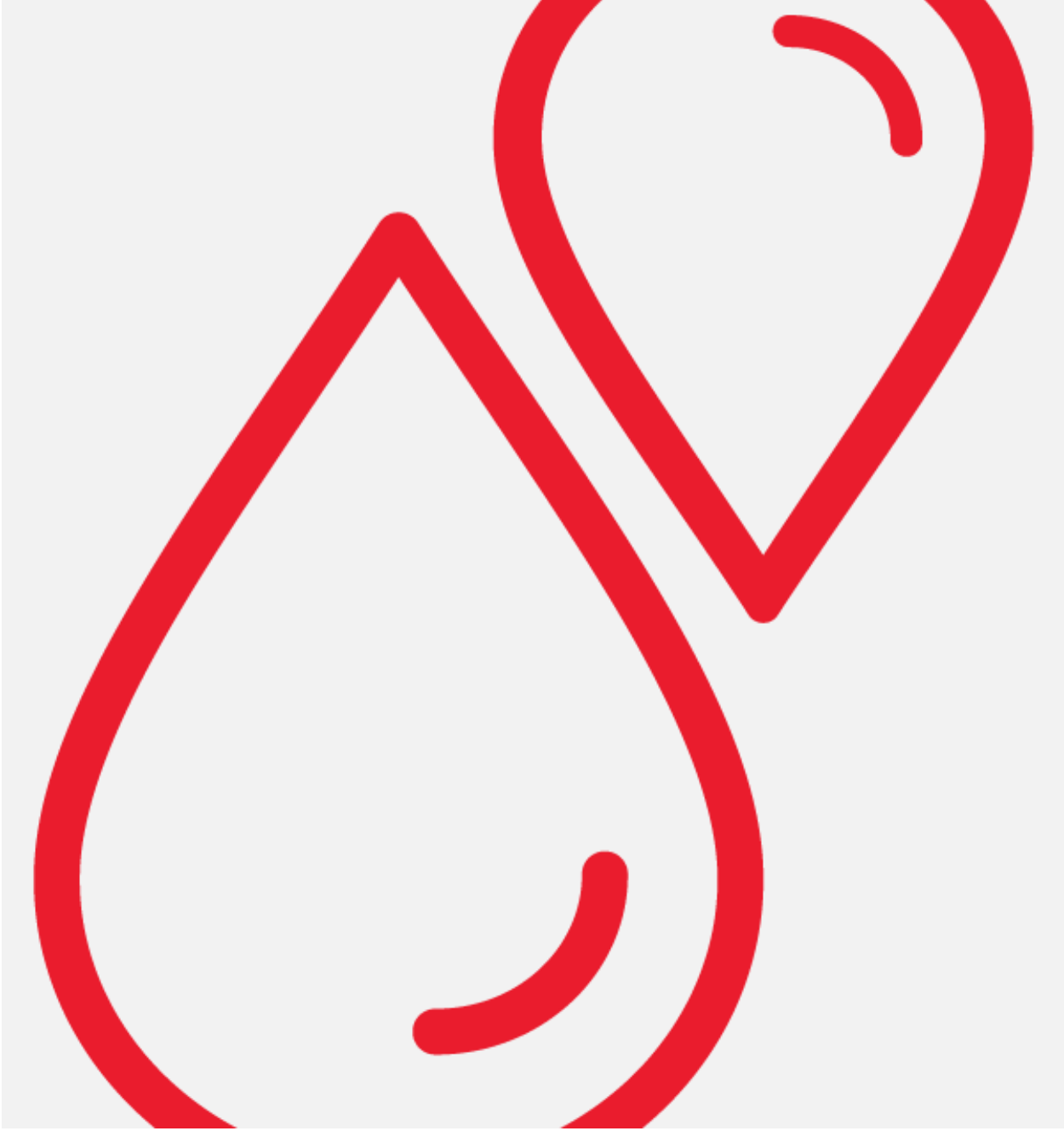
Scoping



Scoping

Scoping Parameter	
Author	Beckman Coulter Life Sciences with Illumina Support
Kit	Illumina TruSight Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples) (Illumina Part Number 20045508).
Supported Features	• This method will support processing 4-24 RNA and DNA samples into Illumina TruSight Oncology 500 libraries. Any combination of RNA and DNA samples (totaling 24 wells) is allowed.) • Method will complete library preparation in less than 3 days. • Method will accommodate 4 batches of 16 libraries per batch.
Excluded Features	HRD workflow
Kit Part Numbers	Illumina TruSight Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples) (Illumina Part Number 20045508).

App Details



Illumina TruSight Oncology 500 DNA/RNA Kit

Illumina TruSight Oncology 500 DNA/RNA kit is designed to prepare and enrich libraries prepared from either DNA Only inputs or a combination of DNA and RNA inputs. Notable features include:

DNA biomarkers include the following:

- Single nucleotide variants (SNVs)
- Insertions
- Deletions
- Gene amplifications
- Multinucleotide variants (MNVs)

RNA biomarkers include the following:

- Fusion
- Splice Events

Illumina TruSight Oncology 500 DNA/RNA kit also detects immunotherapy biomarkers for tumor mutational burden (TMB) and microsatellite instability (MSI) in DNA samples.

Figure 3 TruSight Oncology 500 Kit DNA and RNA Workflow (Part 1)

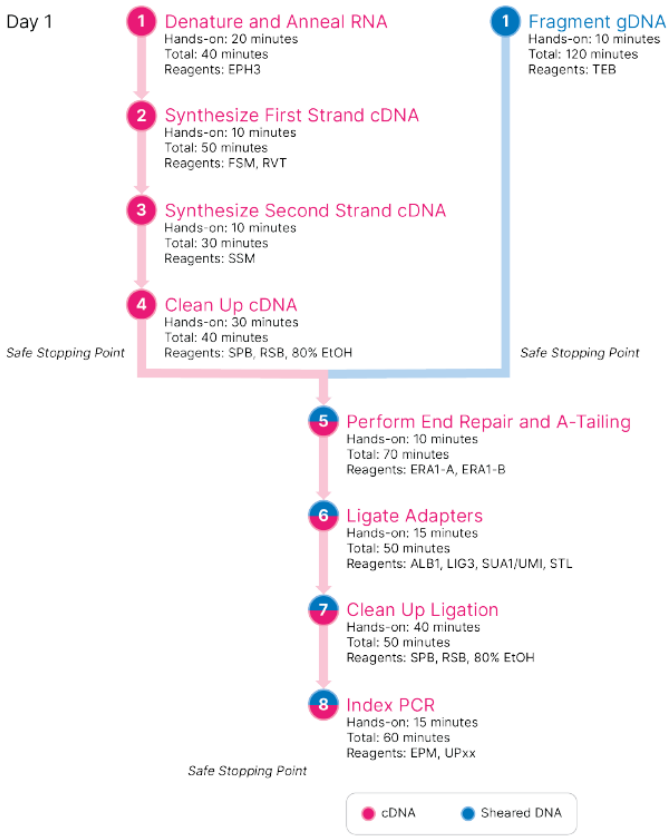
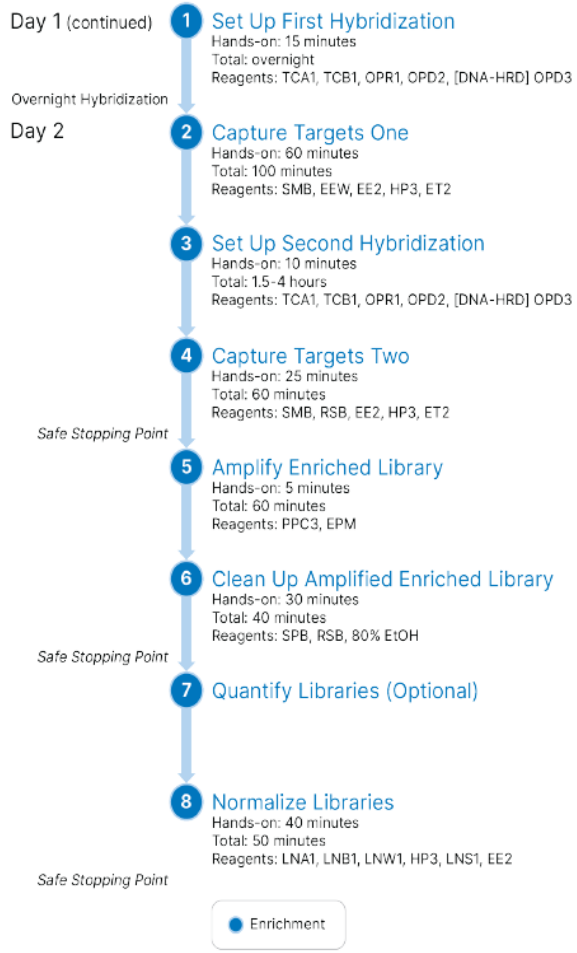


Figure 4 TruSight Oncology 500 Kit DNA and RNA Workflow (Part 2)



Above: Manual Illumina TruSight Oncology 500 DNA/RNA workflow (from the TruSight Oncology 500 Reference Guide)

Sample Input Recommendations

The following recommendations have been reproduced from the Illumina TruSight Oncology 500 Reference Guide (Document # 1000000067621 v10). The Illumina TruSight Oncology 500 DNA/RNA Automation Kit App Template utilizes the same input recommendations.

DNA/RNA Input Recommendations

- The TruSight Oncology 500 assay is optimized to prepare libraries from gDNA that are fragmented to 90–250 bp.
- Use a minimum of 40 ng of DNA/RNA input with the TruSight Oncology 500 Kit assay. Inputs lower than 40 ng can decrease library yield and quality. Quantify the input nucleic acids before beginning the protocol. To obtain sufficient nucleic acid material, isolate nucleic acid from a minimum of 2 mm^3 of FFPE tissue.
    - Use a nucleic acid isolation method that produces high recovery yields, minimizes sample consumption, and preserves sample integrity. The QIAGEN AllPrep DNA/RNA FFPE Kit provides a high yield of nucleic acids, however other extraction chemistries may be used as well.
    - Use a fluorometric quantification method that uses DNA/RNA binding dyes such as AccuClear™ (DNA) or QuantiFluor® (RNA).

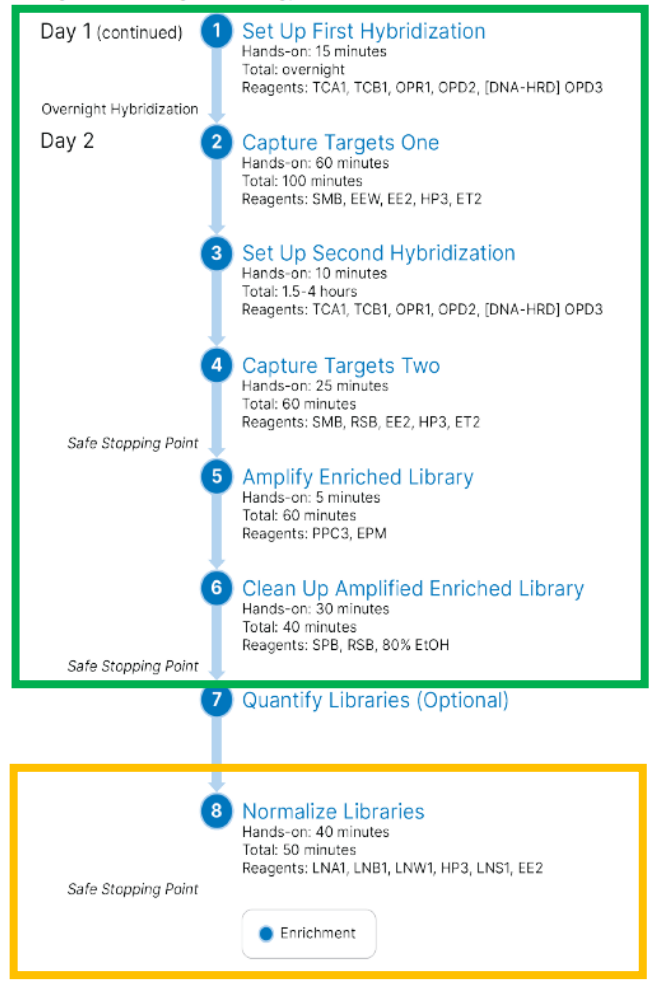
Assess Sample Quality

- DNA samples can be assessed using the Illumina FFPE QC Kit via qPCR.
- Use DNA samples that result in a delta Cq value ≤ 5. Samples with a delta Cq > 5 might result in decreased assay performance.
- RNA samples can be assessed using Advanced Analytical Technologies Fragment Analyzer™ (Standard Sensitivity RNA Analysis Kit) or Agilent Technologies 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit).
- Use RNA samples that result in a DV200 value of ≥ 20%. Using samples with a DV200 value < 20% might result in decreased assay performance.

Sections Automated

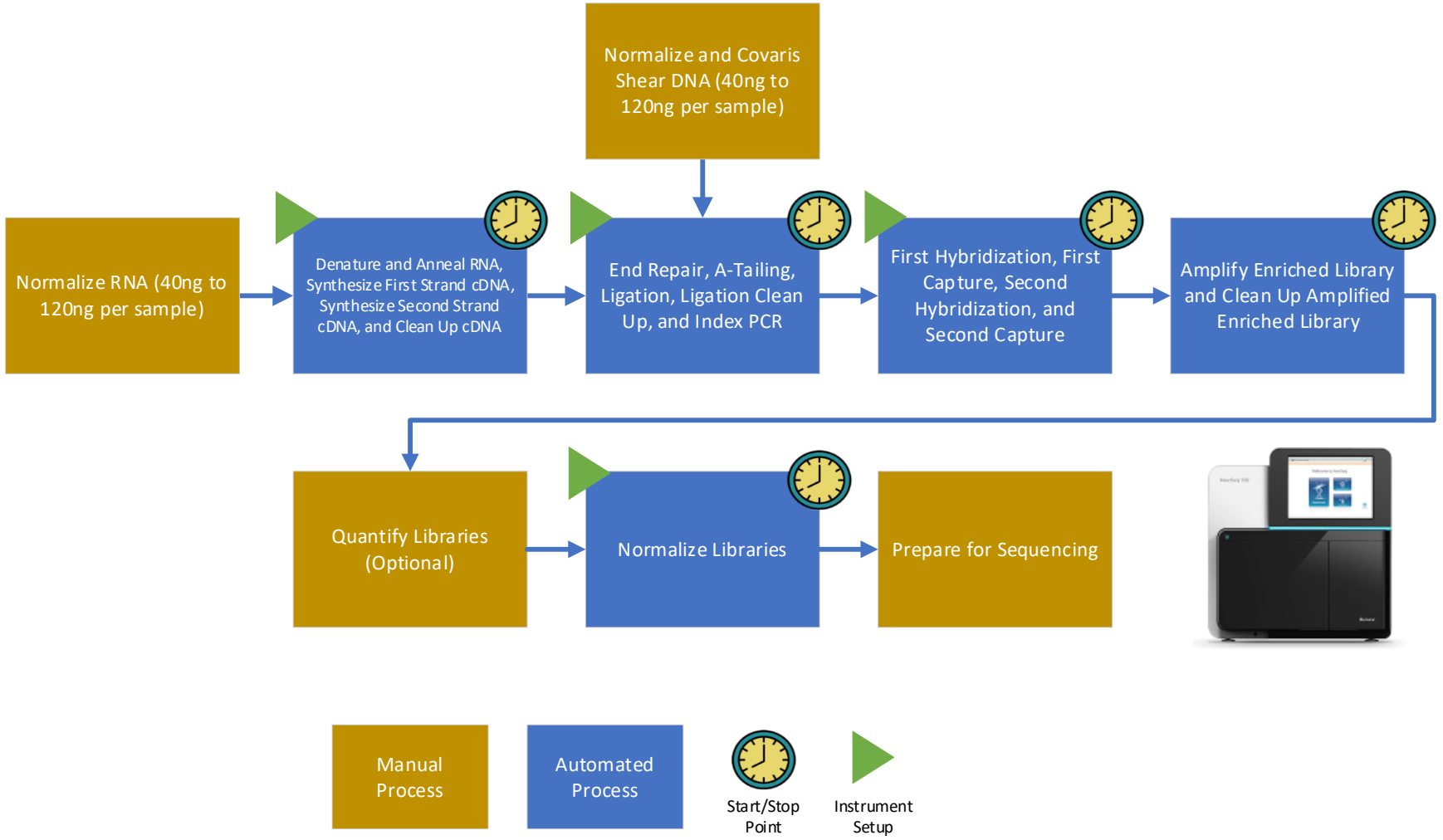
Figure 3 TruSight Oncology 500 Kit DNA and RNA Workflow (Part 1)

Figure 4 TruSight Oncology 500 Kit DNA and RNA Workflow (Part 2)

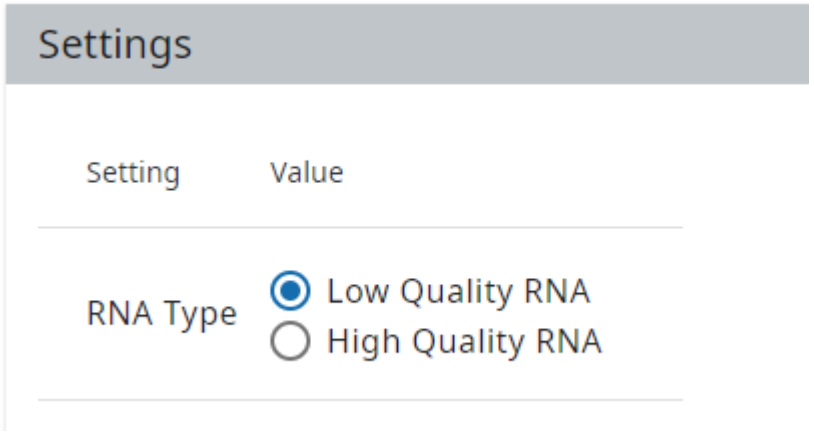


The entire workflow has been automated. The app template breaks the workflow into 4 different sections (3 if running a DNA Only batch), utilizing the established safe stopping points. The safe stopping point after Capture Targets 2 is also supported, but the app template is capable of running through the end of Clean Up Amplified Enriched Library without intervention.

Sections Automated



Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: App Settings



This setting changes the fragmentation time and temperature used for fragmenting RNA samples. For FFPE derived or fragmented RNA, select Low Quality RNA. For cell-line derived or intact RNA, select High Quality RNA.

Note: this setting is ignored for DNA Only batches

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Requested Volumes By Batch Size

Reagent	Reagent Vial on the Biomek NGeniuS System	4 DNA / 4 RNA Batch Run Requested Volume (µl)	8 DNA / 8 RNA Batch Run Requested Volume (µl)	12 DNA / 12 RNA Batch Run Requested Volume (µl)
EPH3	Kit Vial (0.5 ml tube)	65.7	101.4	137.1
FSM	Kit Vial (0.5 ml tube)	81.3	119	156.9
RVT	Kit Vial (0.5 ml tube)	39.3	43.4	47.4
SSM	Kit Vial (2.0 ml tube)	135	240	345
cDNA RSB	1.5 mL conical Sarstedt Reformat Tube	274.6	545.1	767.7
cDNA 80% EtOH	Bulk Reservoir	3400	3800	4200
cDNA SPB	Bulk Reservoir	1340	1680	2020

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Requested Volumes By Batch Size

Reagent	Reagent Vial on the Biomek NGeniuS System	4 DNA / 4 RNA Batch Run Requested Volume (µl)	8 DNA / 8 RNA Batch Run Requested Volume (µl)	12 DNA / 12 RNA Batch Run Requested Volume (µl)
ERA1-A	Kit Vial (0.5 ml tube)	55.3	83.6	112
ALB1	Kit Vial (2.0 ml tube)	544	1048	1552
LIG3	Kit Vial (0.5 ml tube)	72	114	156
UMI1	Kit Vial (0.5 ml tube)	67	109	151
SUA1	Kit Vial (0.5 ml tube)	67	109	151
EPM	Kit Vial (2.0 ml tube)	198	366	534
Index (UP01-16)	Kit Vial (0.5 ml tube)	12.1 (each)	12.1 (each)	12.1 (each)
ERA1-B	Kit Vial (0.5 ml tube)	114	186.4	258.9
STL	Kit Vial (2.0 ml tube)	82	124	166
RSB	1.5 mL conical Sarstedt Reformat Tube	335.4	570.8	806.2
80% EtOH	Bulk Reservoir	3800	4600	5400
SPB	Bulk Reservoir	1840	2680	3520

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Requested Volumes By Batch Size

Reagent	Reagent Vial on the Biomek NGeniuS System	4 DNA / 4 RNA Batch Run Requested Volume (µl)	8 DNA / 8 RNA Batch Run Requested Volume (µl)	12 DNA / 12 RNA Batch Run Requested Volume (µl)
EPM	Kit Vial (2.0 ml tube)	198	366	534
OPD2	Kit Vial (0.5 ml tube)	57	99	141
OPR1	Kit Vial (0.5 ml tube)	57	99	141
TCA1	Kit Vial (2.0 ml tube)	218	386	554
HP3	Kit Vial (0.5 ml tube)	97.7	115.5	133.3
ET2	Kit Vial (0.5 ml tube)	134	218	302
PPC3	Kit Vial (0.5 ml tube)	62	104	146
TCB1	Kit Vial (2.0 ml tube)	312	564	816
EE2	Kit Vial (2.0 ml tube)	389.9	722.9	1055.9
EPCR SPB	5.0 mL Sarstedt Reformat Tube	1292	2174	3056
SMB1*	5.0 mL Sarstedt Reformat Tube	1630	2890	4150
SMB2*	5.0 mL Sarstedt Reformat Tube	1630	2890	4150
EEW	Bulk Reservoir	7300	12100	16900
RSB	Bulk Reservoir	3448.8	5397.6	7346.4
80% EtOH	Bulk Reservoir	3800	4600	5400

*SMB1 and SMB2 are both taken from the SMB reagent tubes supplied from the kit.

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Requested Volumes By Batch Size

Reagent	Reagent Vial on the Biomek NGeniuS System	4 DNA / 4 RNA Batch Run Requested Volume (µl)	8 DNA / 8 R1026NA Batch Run Requested Volume (µl)	12 DNA / 12 RNA Batch Run Requested Volume (µl)
HP3	Kit Vial (0.5 ml tube)	96.7	110.3	123.9
EE2	Kit Vial (2.0 ml tube)	381.2	641.4	901.7
LNB1	Kit Vial (5.0 ml tube)	348.4	415.6	482.8
LNA1	Kit Vial (5.0 ml tube)	665.4	1035	1404.6
LNS1	Kit Vial (5.0 ml tube)	522	774	1026
LNW1	Kit Vial (5.0 ml tube)	1166	1922	2678

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Reagents Required But Not Supplied

Reagent	Supplier	Part Number
Ethyl Alcohol, pure (500 ml)	Sigma Aldrich	E7023
Ultrapure water, nuclease-free	General Lab Supplier	N/A

See the Illumina TruSight Oncology 500 Reference Guide (Document # 1000000067621 v10) Consumables and Equipment list for additional reagents and equipment needed for operations performed off the Biomek NGeniuS System

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Batches Per Kit

Batch Size	4 RNA / 4 DNA	8 RNA / 8 DNA	12 RNA / 12 DNA	8 DNA	16 DNA	24 DNA
Batches per kit	5	4	2	6	3	2
Samples	20 RNA and 20 DNA	32 RNA and 32 DNA	24 RNA and 24 DNA	48 DNA	48 DNA	48 DNA
Largest batch with leftover volume	0	0	0	16 DNA	16 DNA	4 DNA
Total samples from kit	40	64	48	64	64	52 DNA

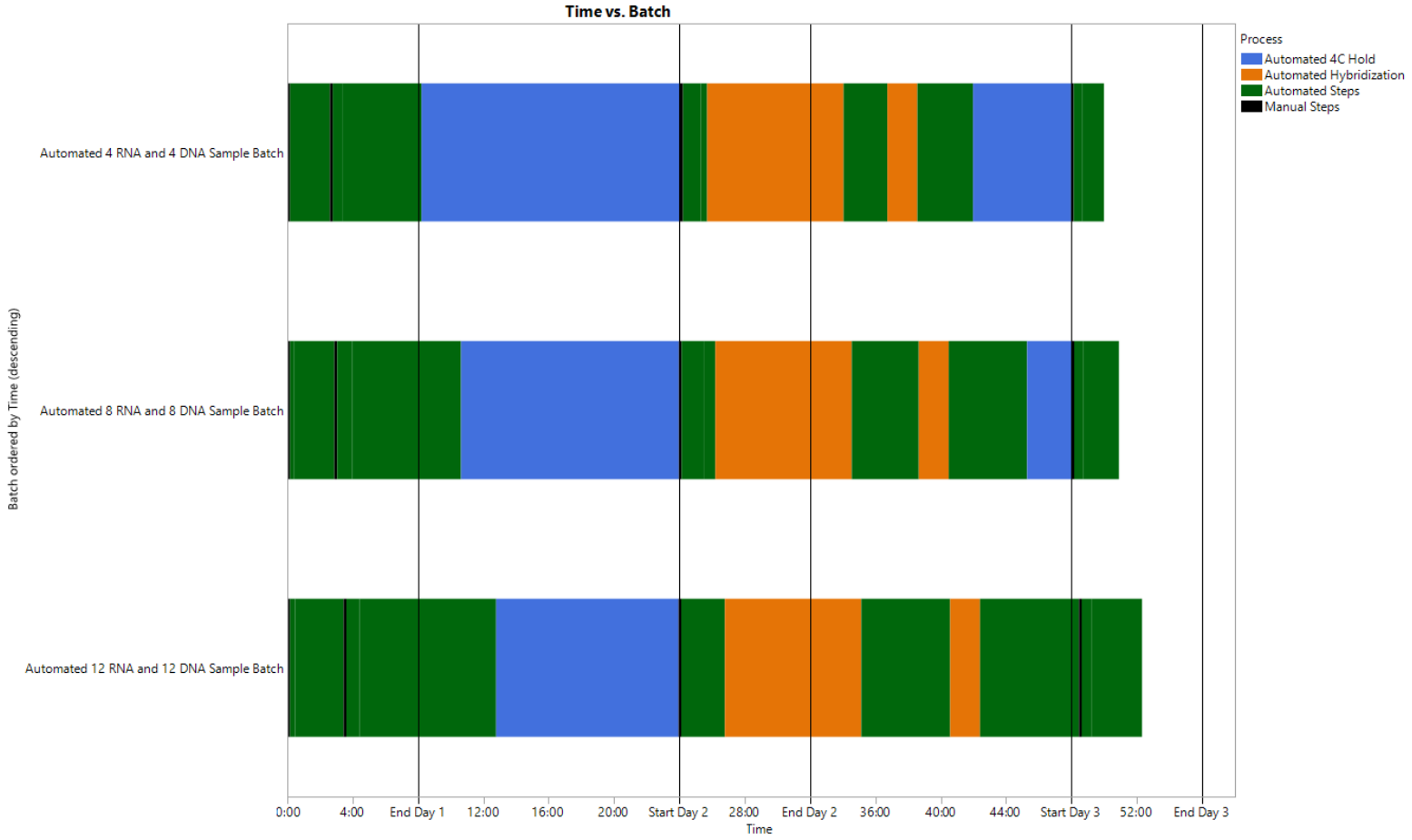
- Illumina TruSight Oncology 500 DNA/RNA Automation Kit (Illumina Part Number 20045508) is a 32DNA + 32 RNA Library Prep kit.
- The ***Batch size*** can be run ***Batches per kit*** times, leaving enough reagent volume to do one additional batch with ***Largest batch with leftover volume*** samples.
- Run combinations calculated based on **published** reagent vial volumes.
- Due to the ability to run any combination of DNA and RNA samples (with a minimum total batch size of 4 and a maximum batch size of 24), many other types of batches are possible than are presented here. Generally, smaller batch sizes will exhaust a kit faster than larger batch sizes.

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Estimated Time of Completion

Customer Portal Section	Operation	4 DNA / 4 RNA Batch Run Time (hr:min)	8 DNA / 8 RNA Batch Run Time (hr:min)	12 DNA / 12 RNA Batch Run Time (hr:min)
1	Reagent Aliquoting- Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA	0:13	0:16	0:19
1	Sample Processing- Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA	2:14	2:27	2:58
3	Reagent Aliquoting- End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR	0:38	0:55	0:48
3	Sample Processing- End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR	4:48	6:40	8:22
5 and 6	Reagent Aliquoting- First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library	1:06	1:21	1:37
5 and 6	Sample Processing- First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library	16:40	19:48	22:44
8	Reagent Aliquoting- Normalize Libraries	0:30	0:34	0:38
8	Sample Processing- Normalize Libraries	1:21	2:10	3:04
All	Total Instrument Run Time	27:30	36:11	42:10

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Estimated Time of Completion

If running RNA samples, the most efficient way to operate the App Template is to perform the first section (Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA) and then start the third section (End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR) and allow it to run overnight so that the fifth and sixth sections (First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library) can be started on the morning of Day 2. This in turn should allow the operator to complete the batch by the end of Day 3. If running only DNA samples the Biomek NGeniuS system should be able to complete the batch by the end of Day 2 by allowing overnight runs.



Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Consumables

		Batch Size		
Consumable	**Part number**	**4 DNA / 4 RNA Batch Run**	**8 DNA / 8 RNA Batch Run**	**12 DNA / 12 RNA Batch Run**
RVs	C62705	20	20	20
Bulk Reservoirs	C62707	4	4	4
Lids	C62706	17	17	17
Millitips (boxes)	C59585	258 (3)	462 (5)	658 (7)
Microtips (boxes)	C62712	760 (2)	1472 (4)	2192 (6)
Seal plate	C70665	2	2	2
Sarstedt Inc 5 mL SCTUBE 15.3X92FCBSG/PK1000 (Sarstedt Part Number 62.611)	Fisher Scientific (P/N NC9236303)	3	3	3
Sarstedt Inc 1.5 mL SC MTUBE CAP PCR/PK1000 (Sarstedt Part Number 72.692.	Fisher Scientific (P/N NC0308763)	2	2	2
Price Per Sample ($)*	-	$39.28	$28.64	$25.09

* Costs assume using fresh tip boxes. Some clean tips will remain each run, reducing cost of subsequent runs. Costs do not include empty tip boxes for tip disposal. Costs calculated based on prices as of May 2024; subject to change.

Illumina TruSight Oncology 500 DNA/RNA Kit on Biomek NGeniuS System: Price Per Sample

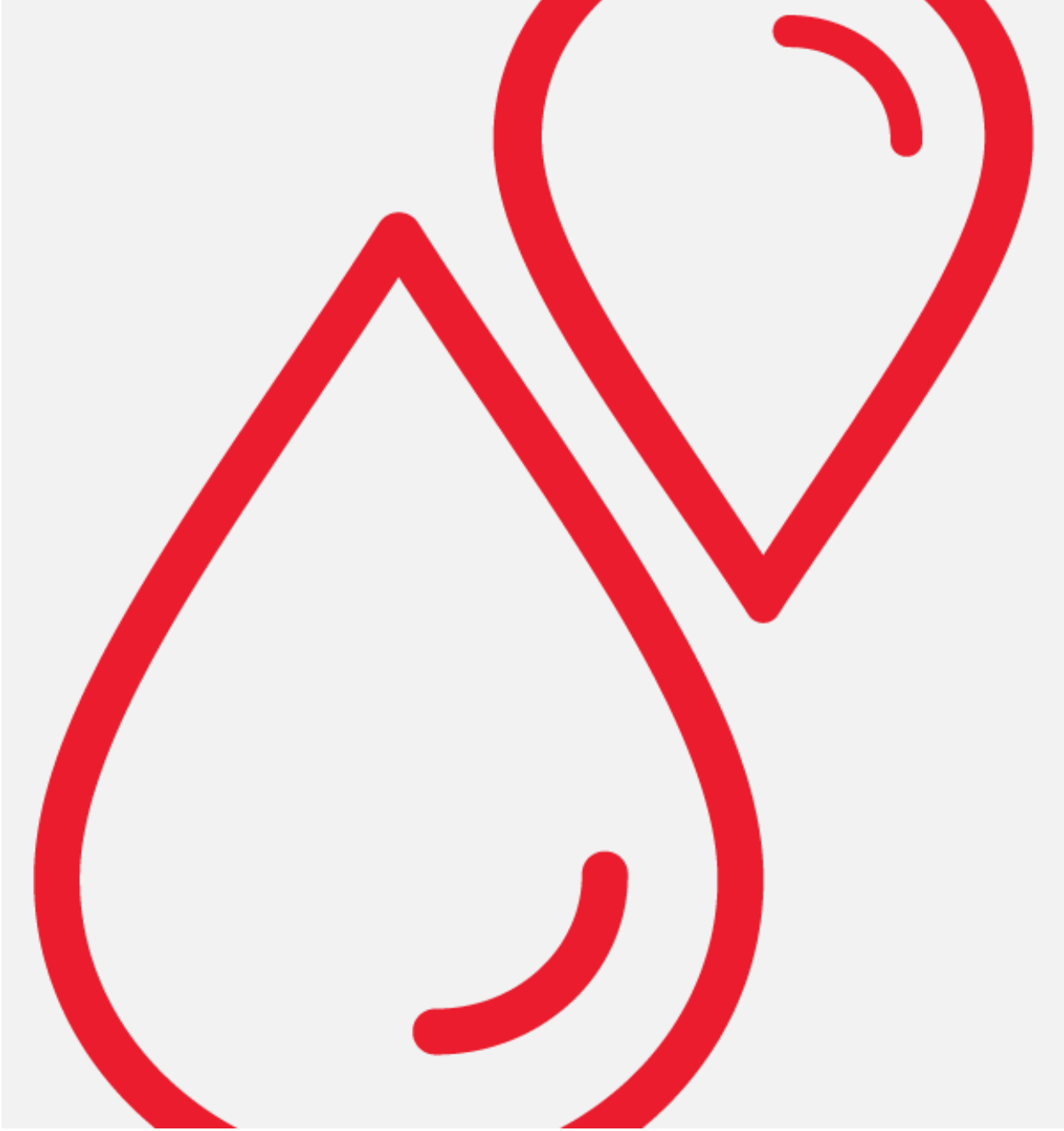
Manual Processing	Per Sample* for 8 RNA / 8 DNA batch
Library Prep Kit	$375.65
Plastic Consumables	$47.77
Total Consumables	$420.42

Biomek NGeniuS System Processing	Per Sample* for 8 RNA / 8 DNA batch
Library Prep Kit	$375.65
Plastic Consumables	$28.64
Total Consumables	$404.29

Per Sample	
Biomek NGeniuS System	$404.29
Manual	$420.42
Difference	-$16.13

* Prices calculations as of May 2024; subject to change; for reference only

Demonstration Data (App Template Version 1.0.1)



Demonstration Acceptance Criteria

Number	Metric Source	Metric
1	Illumina TruSight Oncology 500 Reference Guide (1000000067621 v10)	Pre-sequencing: Pre-library normalization yield should be 3ng/µl or higher as determined by Qubit.
2	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Sequencing: Sequencing run percent pass filter reads should be 80% or higher
3	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	RNA Sequencing: Median CV Gene 500X Coverage should be less than or equal to 93.
4	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	RNA Sequencing: Total On Target Reads should be greater than or equal to 9000000.
5	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	RNA Sequencing: Median Insert Size should be greater than or equal to 80.
6	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Contamination Score should be less than 1457
7	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Median Insert Size should be greater than or equal to 70bp
8	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Median Exon Coverage (Count) should be greater than or equal to 150
9	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Percent Exon Coverage 50% should be greater than or equal to 90
10	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Usable MSI Sites (Count) should be greater than or equal to 40
11	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Gene_Scaled_MAD (Count) should be less than 0.134
12	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	DNA Sequencing: Median Bin Count CNV Target (Count) should be greater than or equal to 1
13	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	DNA Sequencing: 5% Variant Allele Frequency (VAF) for small variants
14	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	DNA Sequencing: 2.2X fold-change for Copy Number Variants (CNV)
15	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	DNA Sequencing: Analytical sensitivity greater than 96% for all variant types at 5% VAF or higher

On Instrument Demonstration Runs

Experiment	Library Number	Input Mass (ng)	Samples	App Settings	Instrument	Sequencing	Analysis Workflow
1	2 RNA / 2 DNA	40	1 SeraCare RNA, 1 negative control, 2 SeraCare DNA	Low Quality RNA	BEC Instrument 1	2x101 bp sequencing run using NextSeq500 High Output Kit	DRAGEN TSO500 Solid Eval App | Version: 2.5.1
2	8 RNA / 8 DNA	40	8 SeraCare RNA, 1 negative control, 1 SeraCare DNA, 2 Horizon HD798, 2 Horizon HD799, and 2 Horizon HD803	Low Quality RNA	BEC Instrument 2	2x101 bp sequencing run using NextSeq500 High Output Kit	DRAGEN TSO500 Solid Eval App | Version: 2.5.1
3	12 RNA / 12 DNA	80	12 SeraCare RNA, 1 negative control, 5 SeraCare DNA, 2 Horizon HD798, 2 Horizon HD799, and 2 Horizon HD803	High Quality RNA	BEC Instrument 1	Two 2x101 bp sequencing run using NextSeq500 High Output Kit	DRAGEN TSO500 Solid Eval App | Version: 2.5.1

Control Samples Used

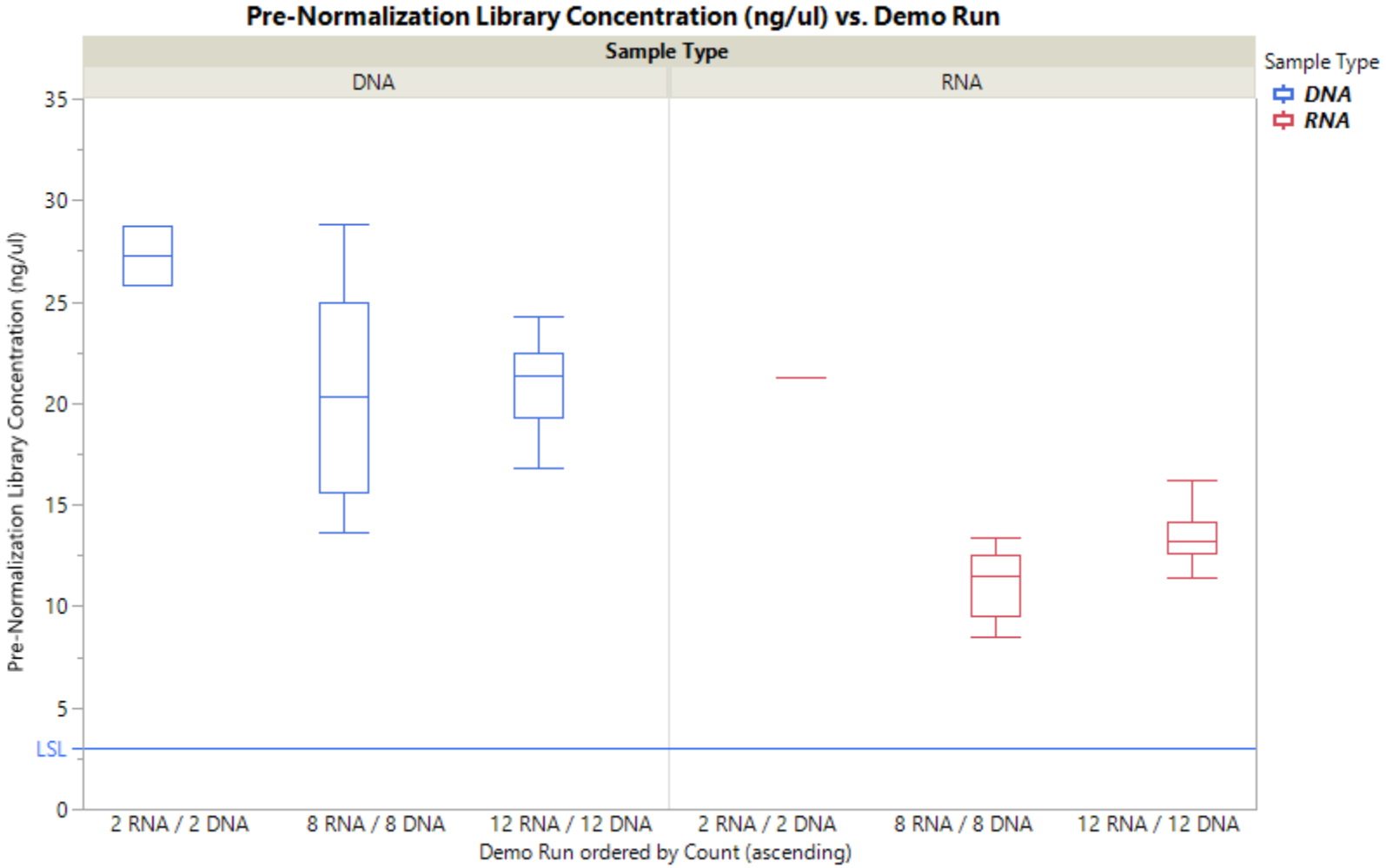
Vendor	Part Number	Control Sample
LGC Clinical Diagnostic	0710-0496	Seraseq® FFPE Tumor Fusion RNA v4 Reference Material
LGC Clinical Diagnostic	0710-0097	Seraseq® Tri-Level Tumor Mutation DNA Mix v2 HC
Horizon Discovery	HD798	Quantitative Multiplex Reference Standard (mild)
Horizon Discovery	HD799	Quantitative Multiplex Reference Standard (moderate)
Horizon Discovery	HD803	Quantitative Multiplex Reference Standard (severe)

Off Instrument Demonstration Runs

Experiment	Library Number	App Settings
1	11 DNA Only	Low Quality RNA
2	15 RNA Only	Low Quality RNA

Two virtual demonstration runs were performed on a virtual machine running instrument software version 1.58.0 to prove that the app template is capable of supporting both DNA only and RNA only batches in addition to the combined DNA/RNA batches used during On Instrument Demonstration Runs. Both runs completed successfully.

TSO500 DNA/RNA Demonstration Data: Pre-Normalization Library Concentration



Sequencing Runs (5/8/24, 5/10/24, and 5/15/24)

TSO500 DNA/RNA 12 RNA / 12 DNA Run (5/8/24)

The sequencing run generated 277,262,102 pass filter reads (86.5% of total reads), of which 86% of reads were successfully identified. 89.22% of all bases were Q30 or higher. Total sequencing output was 117.7 Gb. Sequencing results should have 80% of reads pass filter with 80% of bases Q30 or higher.

TSO500 DNA/RNA 12 RNA / 12 DNA Run Part 2 and 2 RNA / 2 DNA Run (5/10/24)

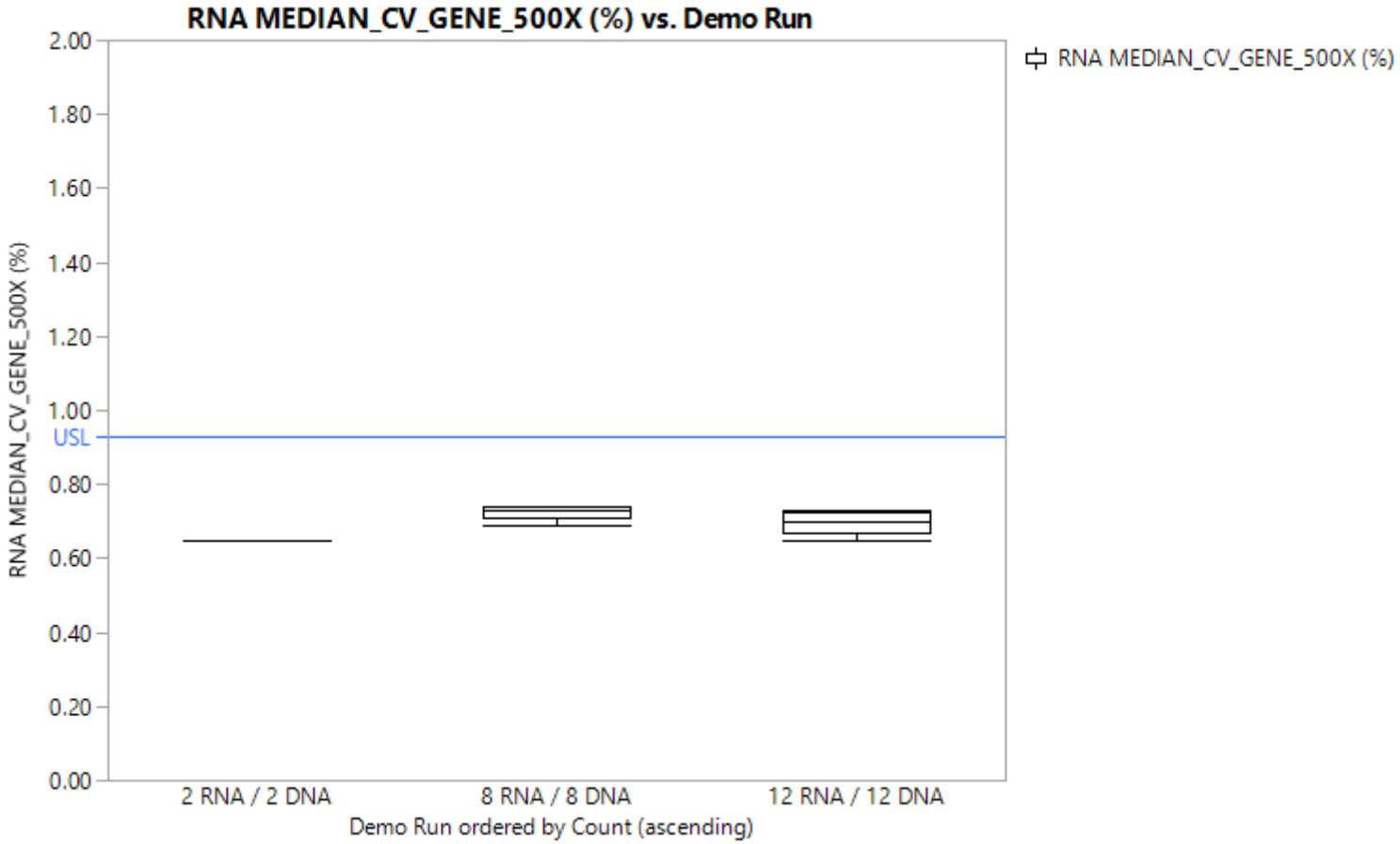
The sequencing run generated 260,009,438 pass filter reads (89.2% of total reads), of which 89.2% of reads were successfully identified. 91.08% of all bases were Q30 or higher. Total sequencing output was 110.73 Gb. Sequencing results should have 80% of reads pass filter with 80% of bases Q30 or higher.

TSO500 DNA/RNA 8 RNA / 8 DNA Run (5/15/24)

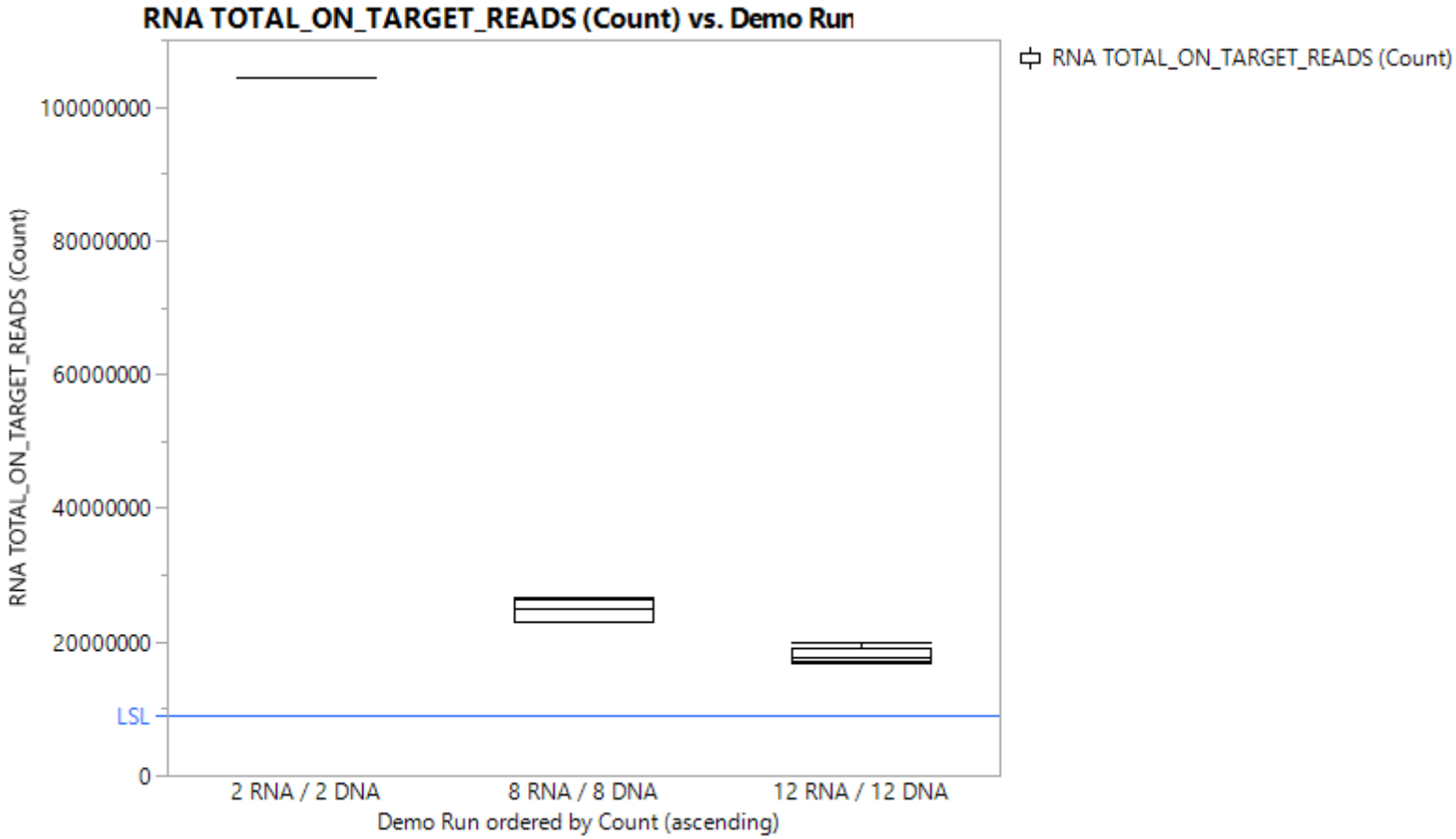
The sequencing run generated 278,011,010 pass filter reads (85.3% of total reads), of which 89.4% of reads were successfully identified. 88.36% of all bases were Q30 or higher. Total sequencing output was 117.75 Gb. Sequencing results should have 80% of reads pass filter with 80% of bases Q30 or higher.

Sequencing results were analyzed on BaseSpace using the TSO500 DRAGEN Evaluation App. The results were then entered into JMP and graphed along with Upper and Lower Spec Limits when appropriate.

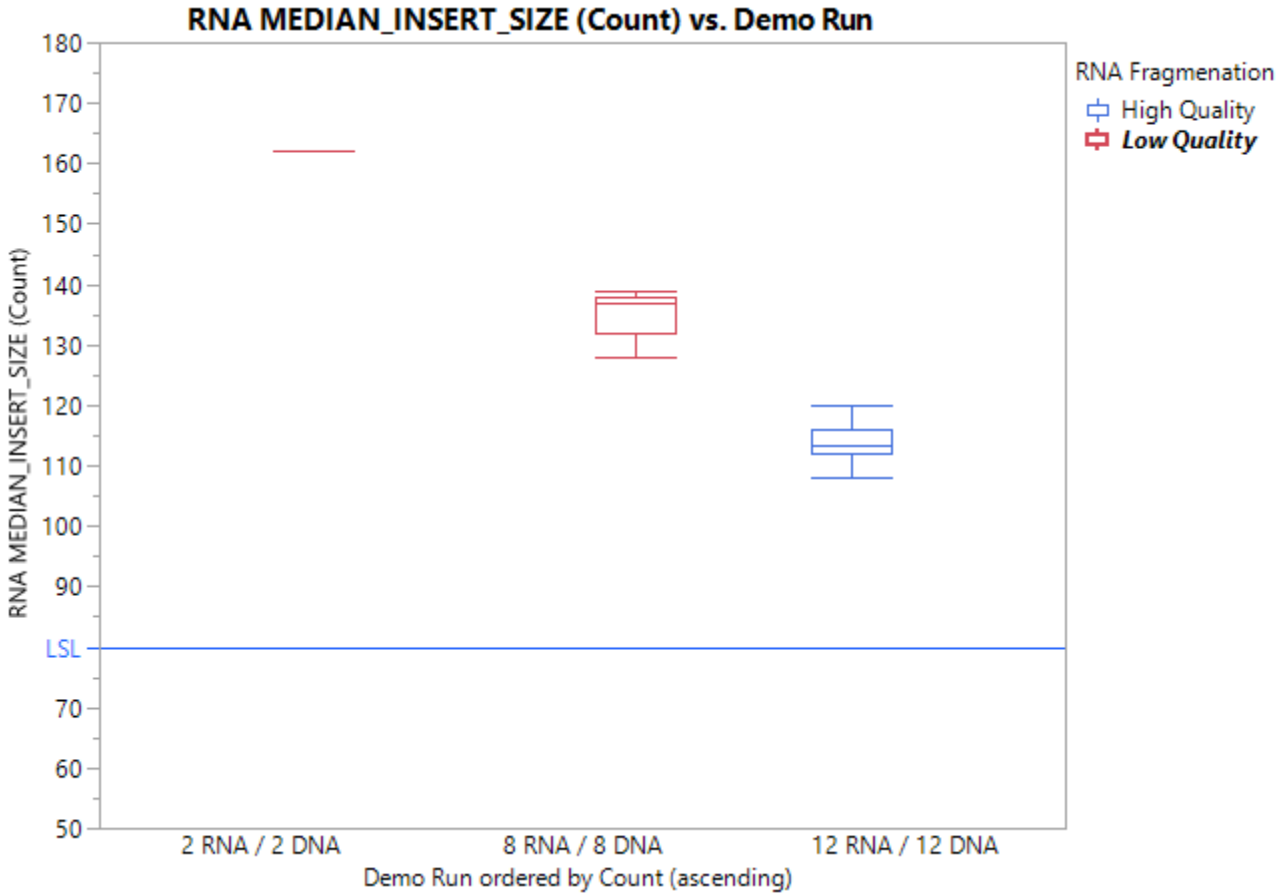
RNA Metrics



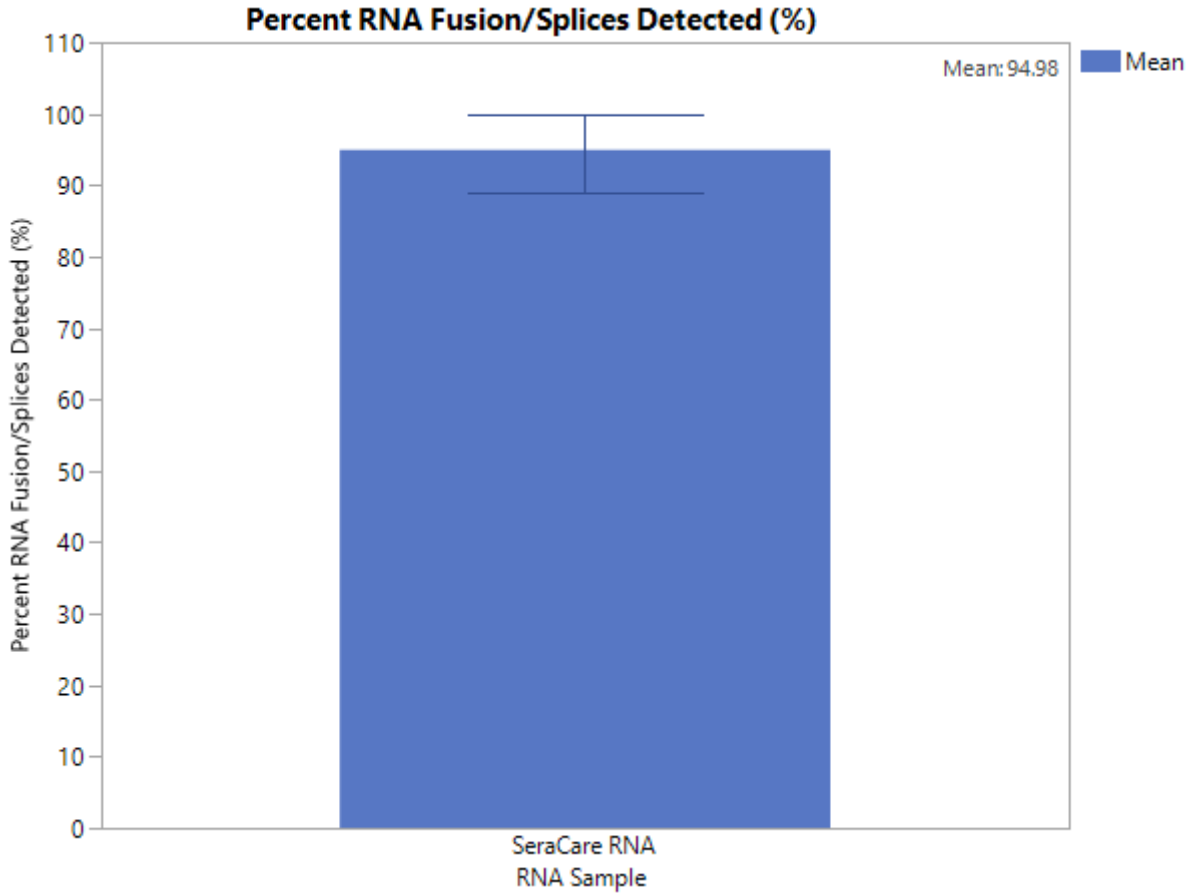
RNA Metrics



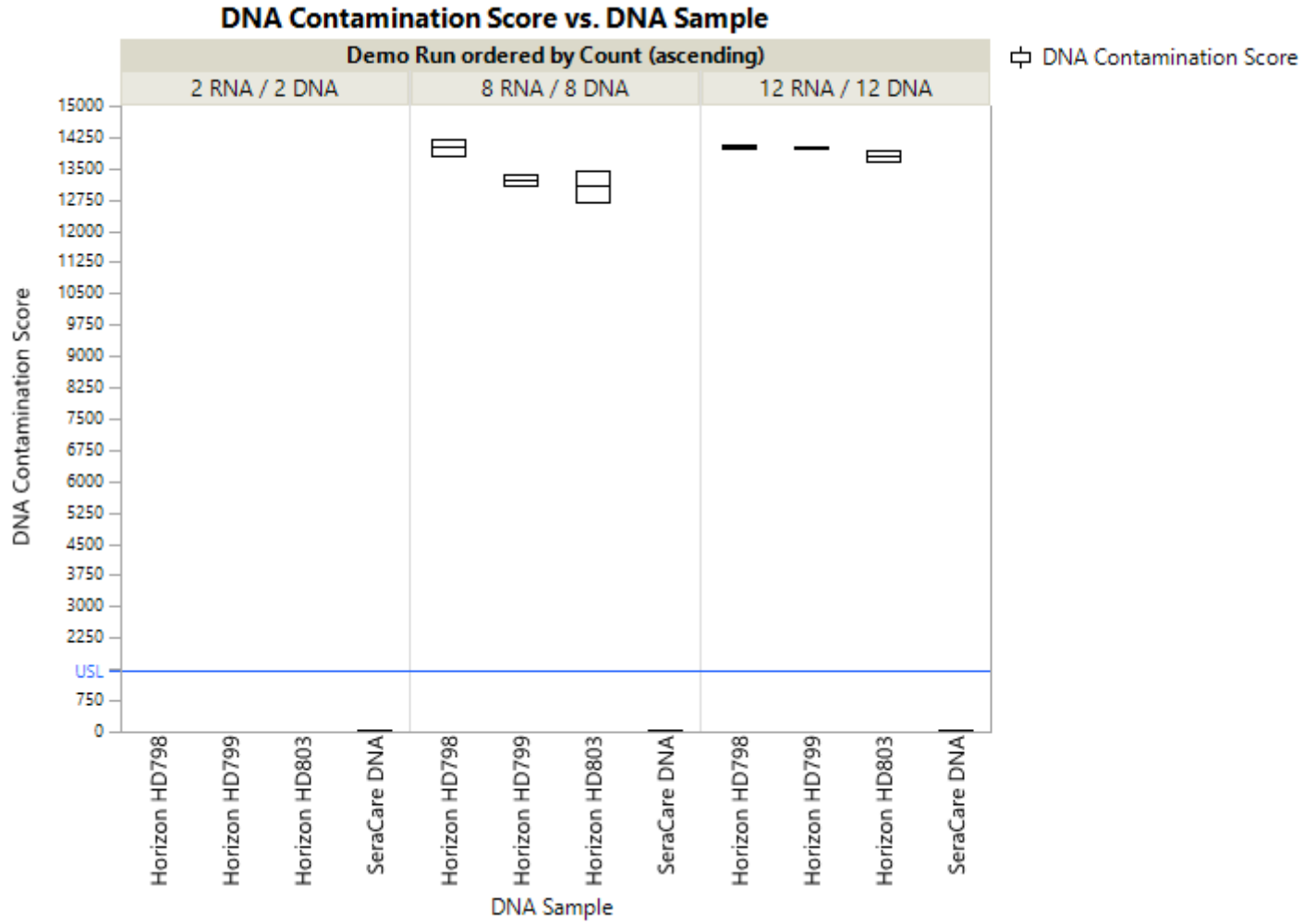
RNA Metrics



RNA Metrics

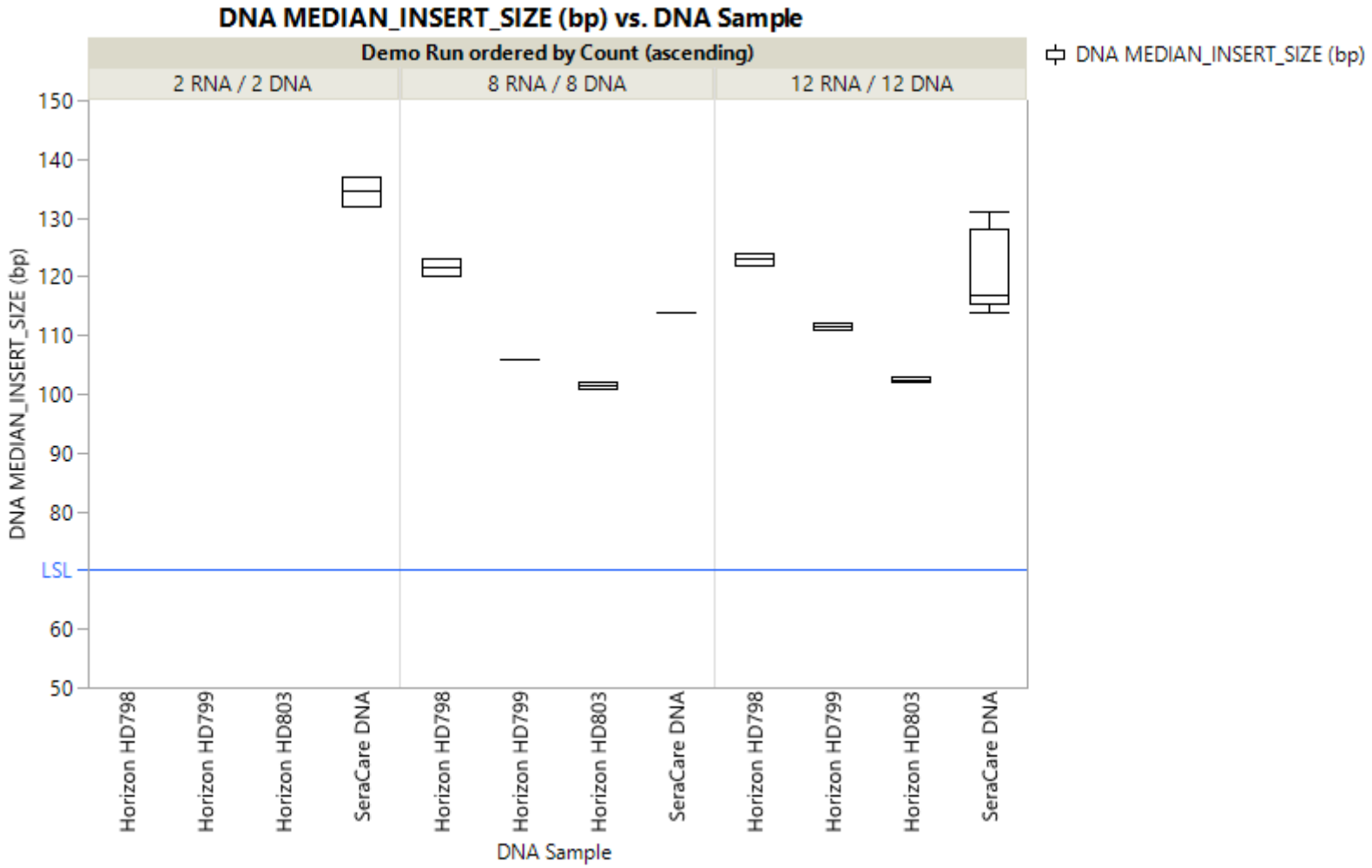


DNA Metrics



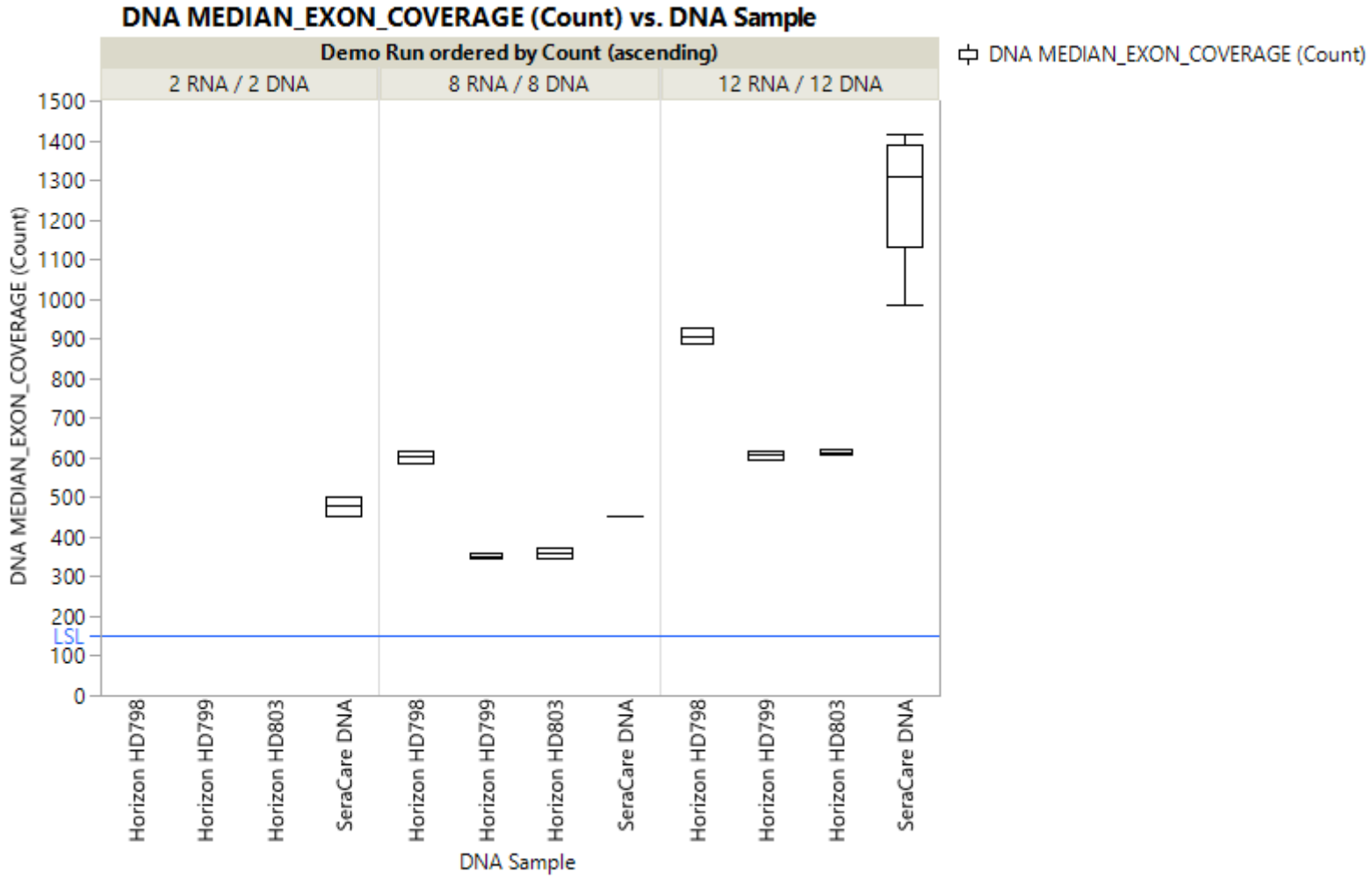
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



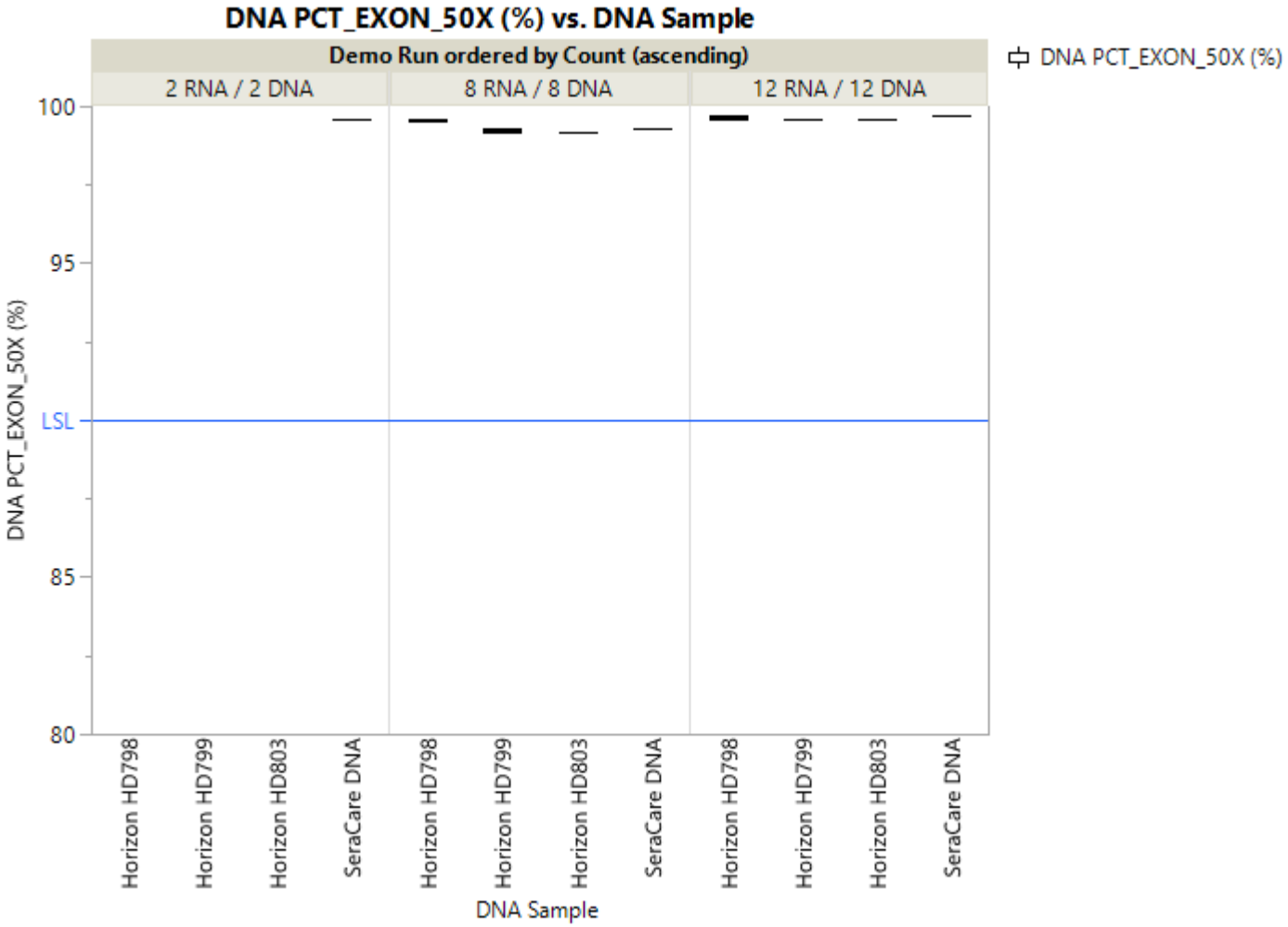
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



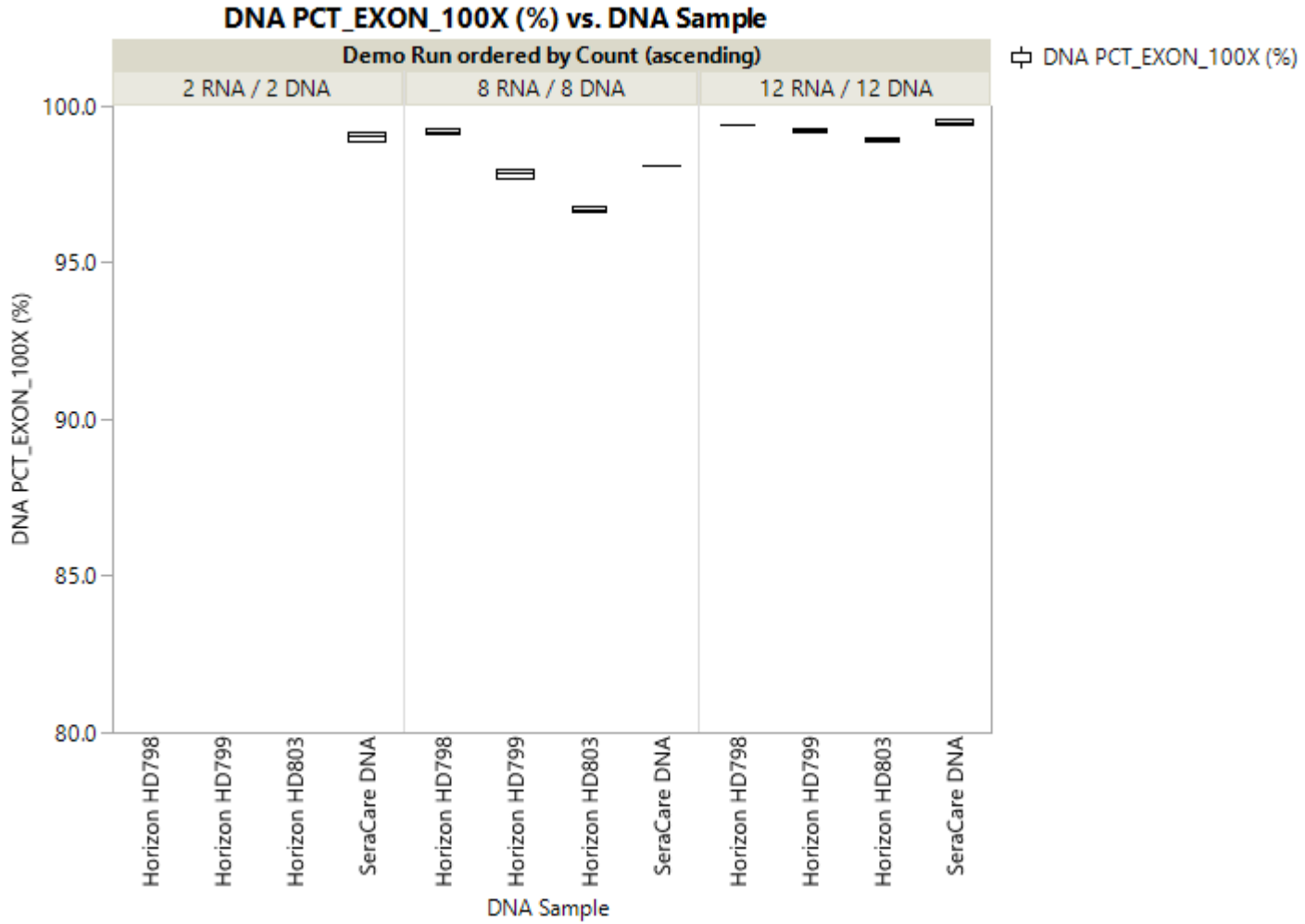
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



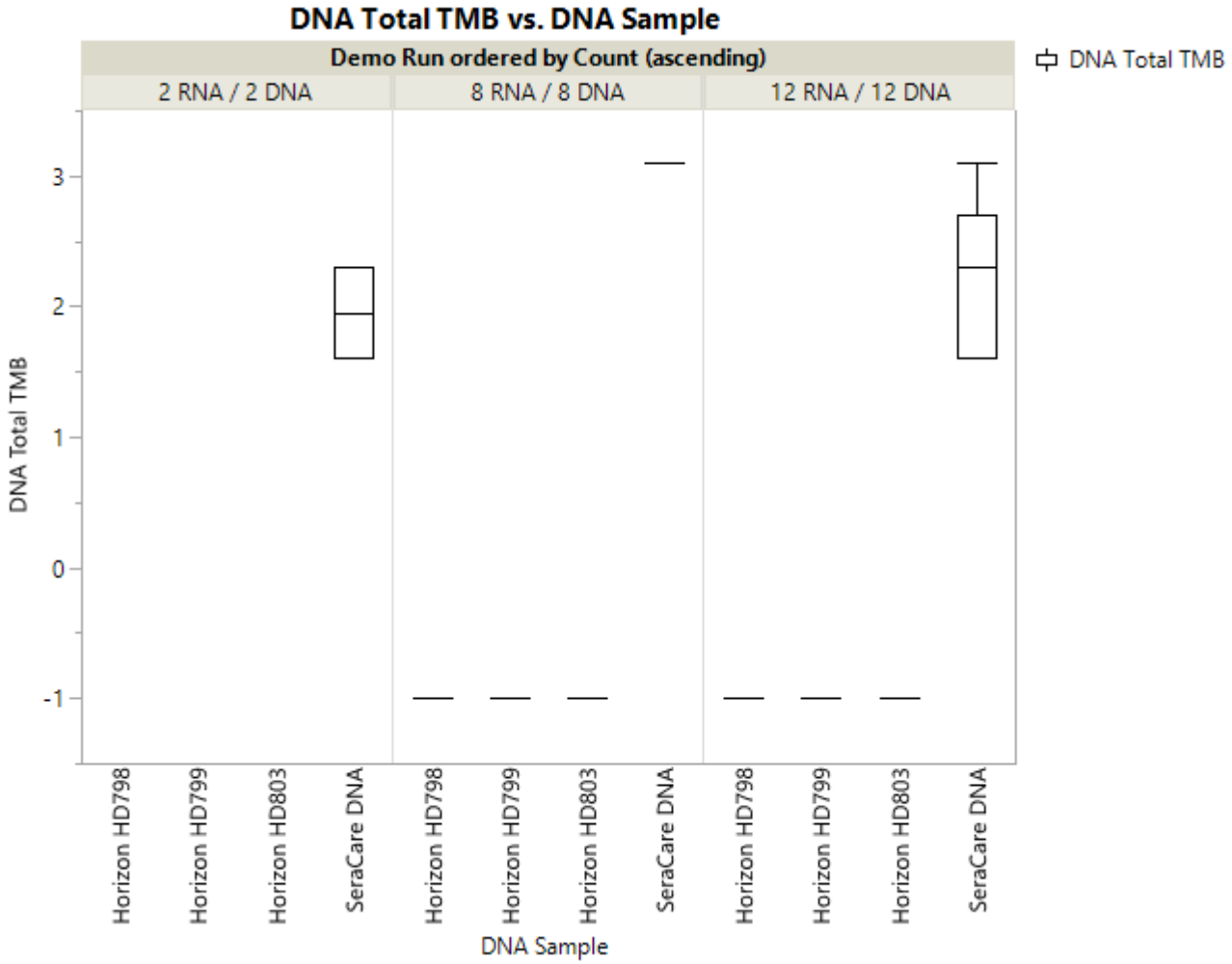
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



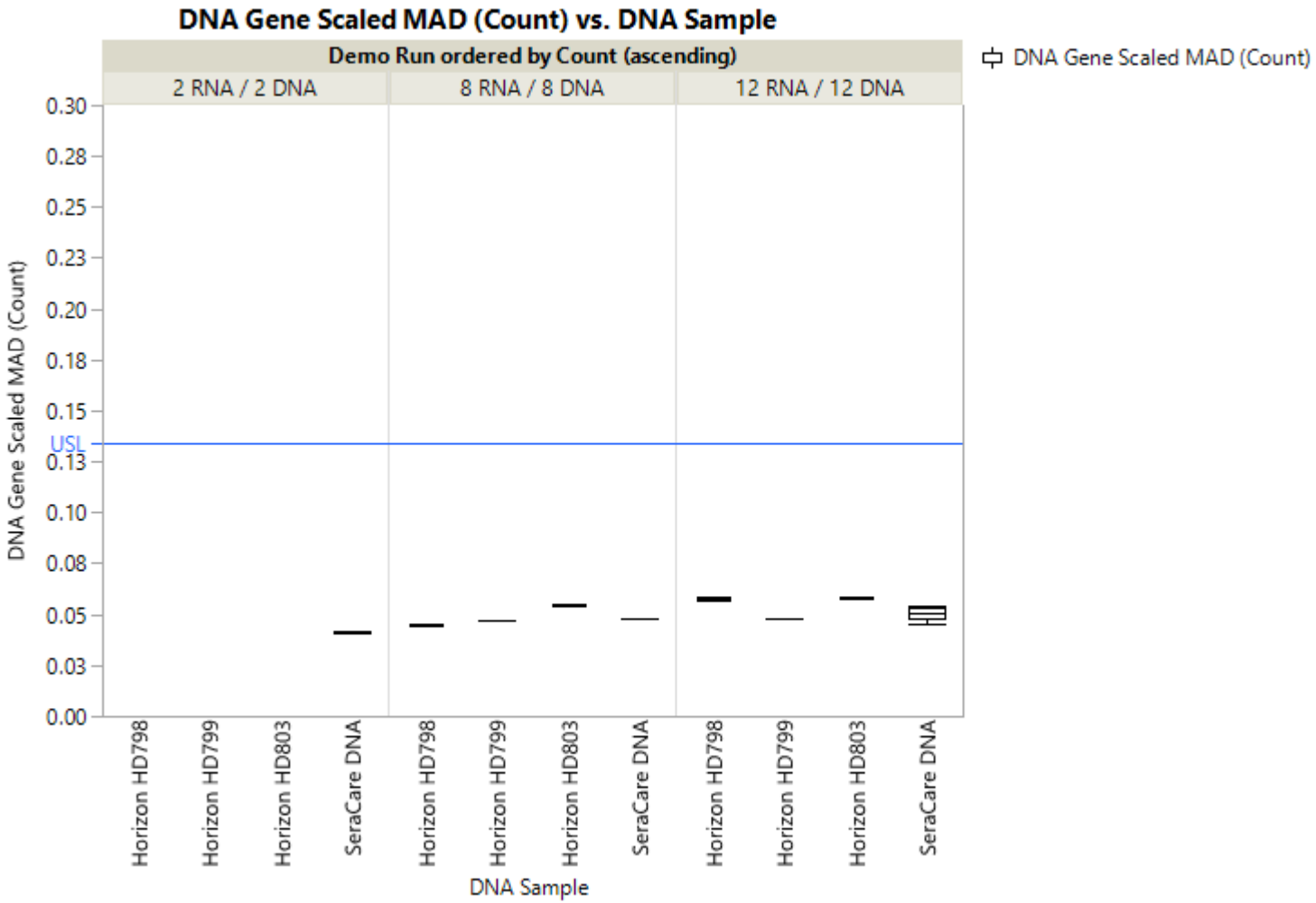
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



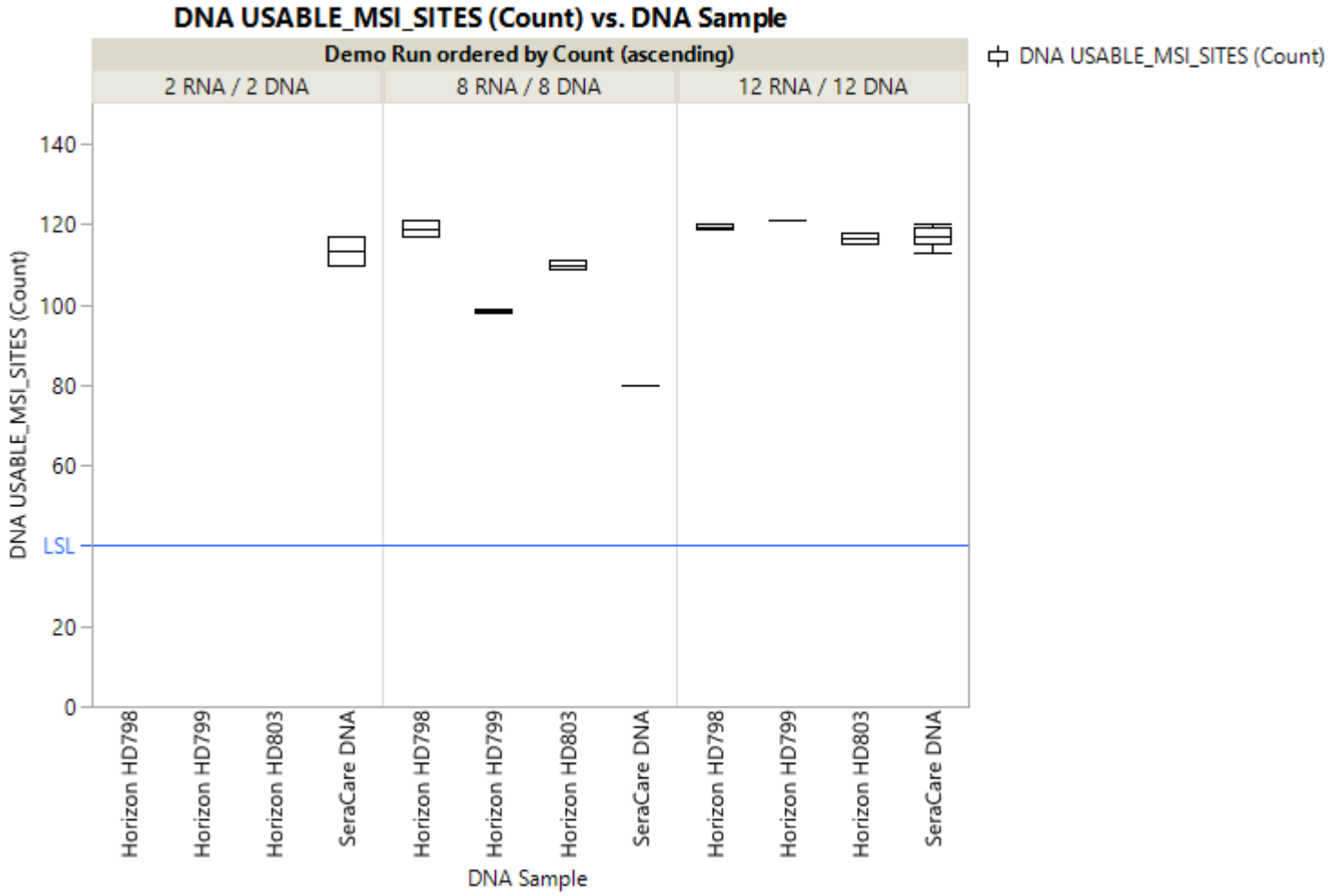
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



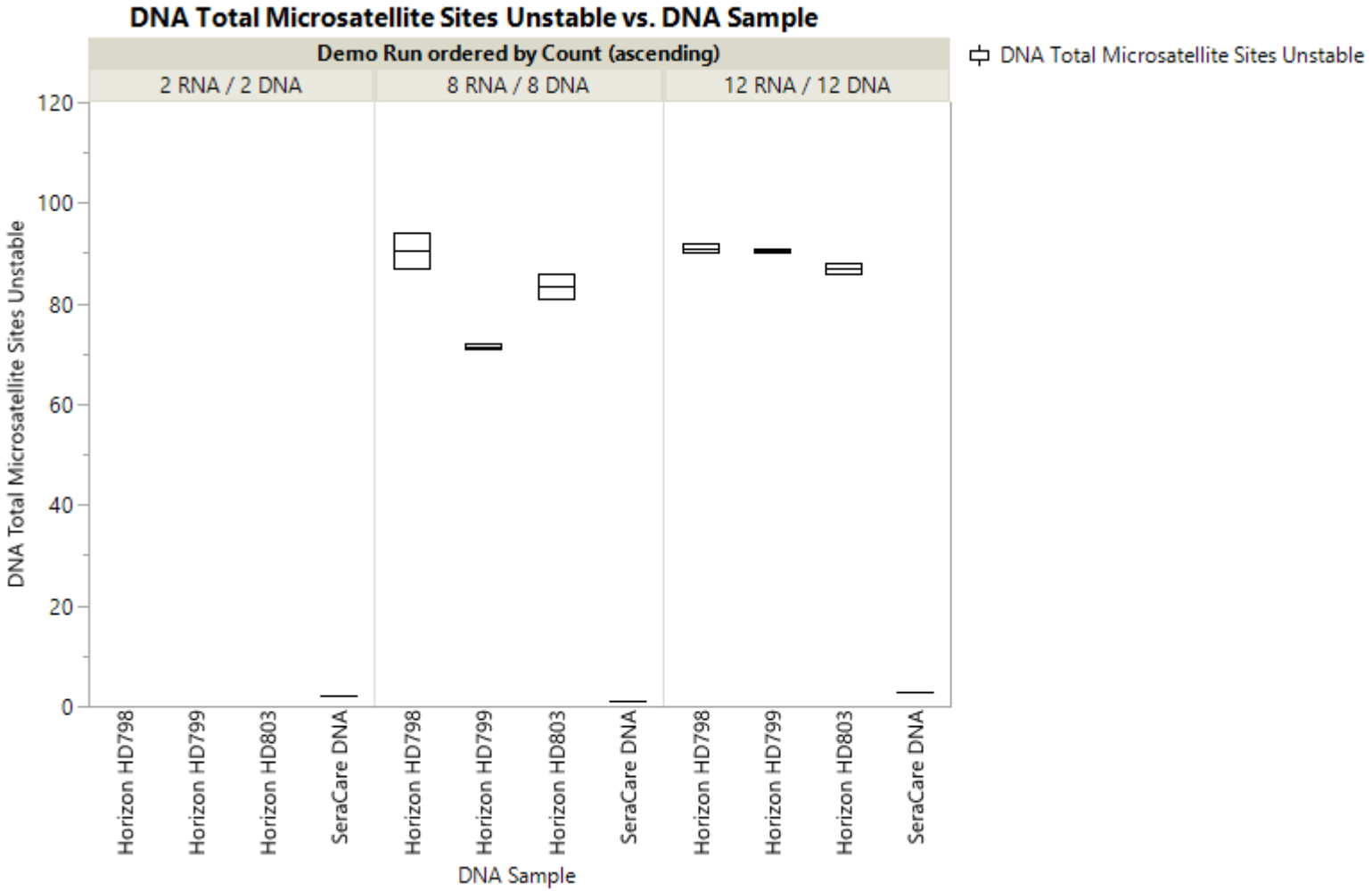
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



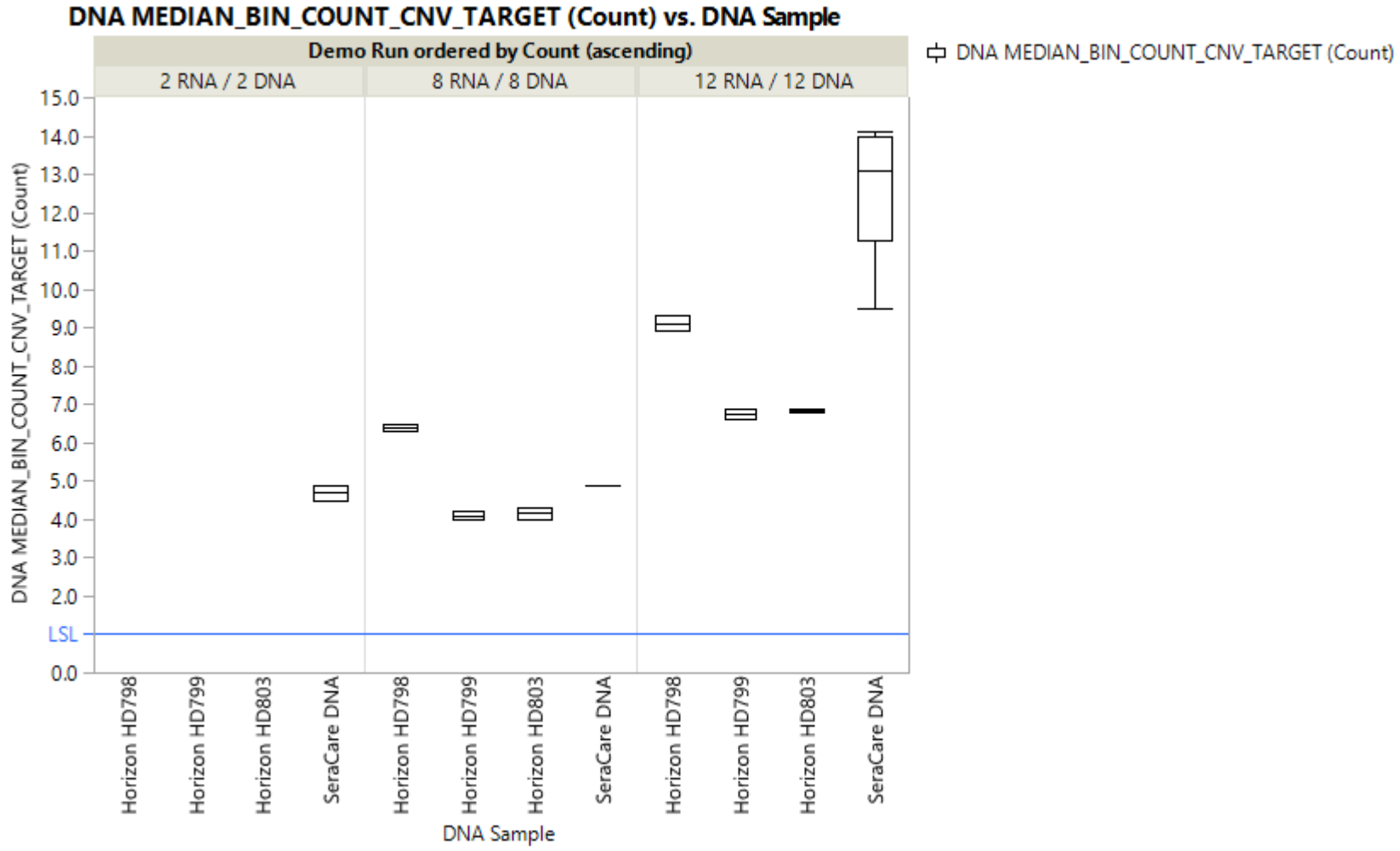
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



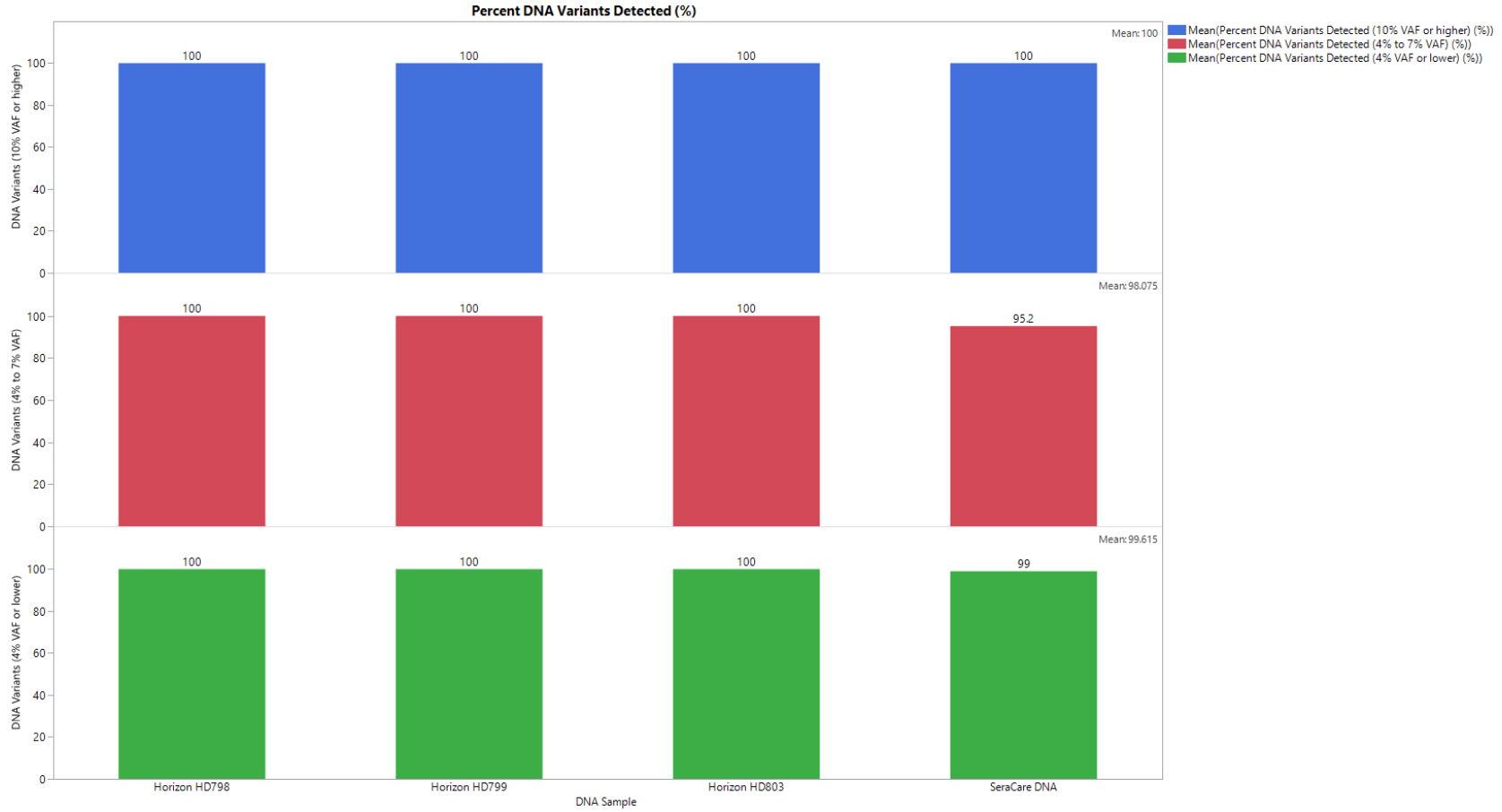
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics



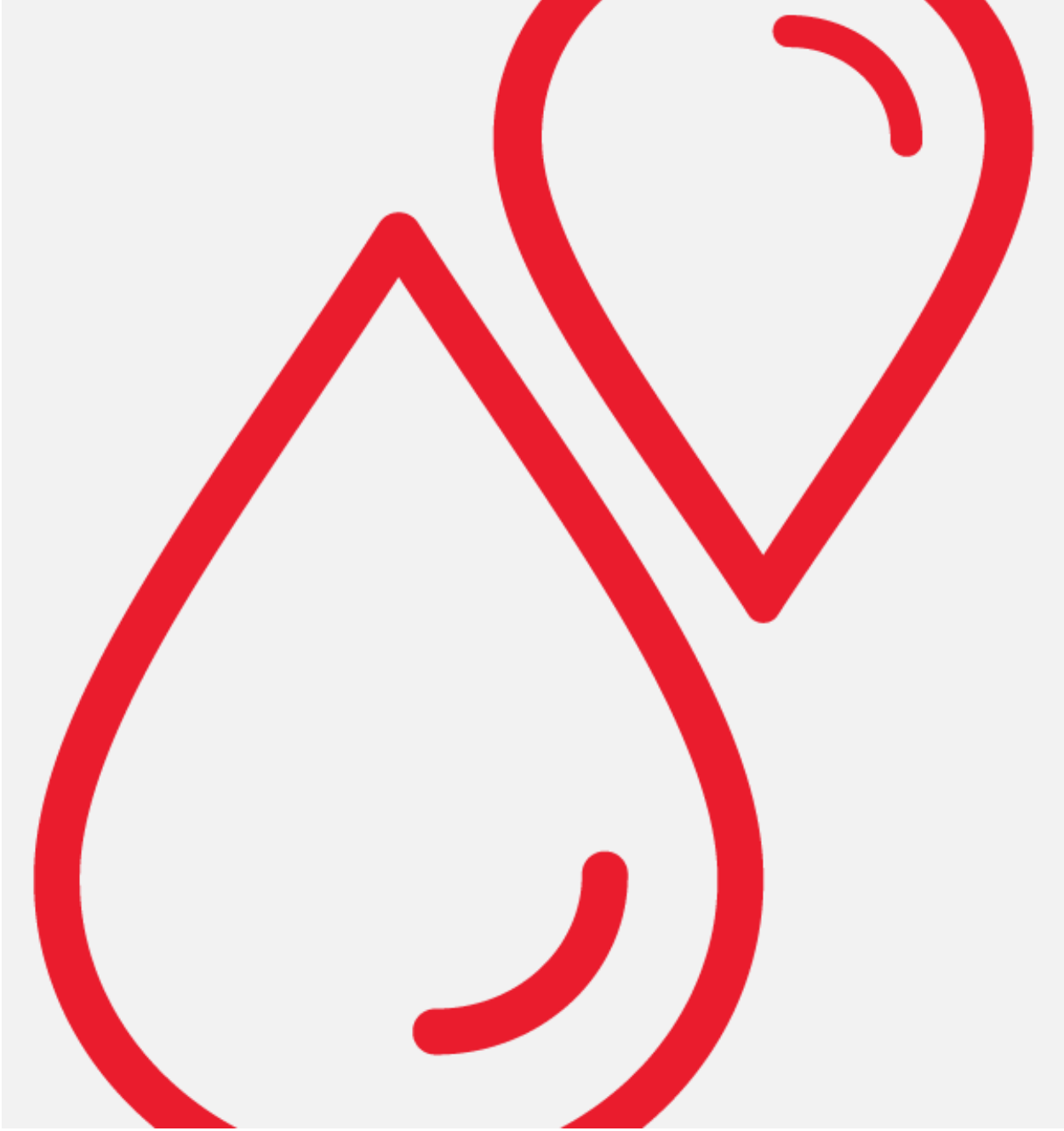
No Horizon samples were included in the 2 RNA / 2 DNA demonstration run

DNA Metrics: SeraCare Small Variants



The *NPM1* insertion element c.863_864insTCTG (7% frequency) was identified in 4 of 10 NGeniuS SeraCare replicates.

Demonstration Summary



Demonstration Summary

Number	Metric	Results
1	Pre-sequencing: Pre-library normalization yield should be 3ng/µl or higher as determined by Qubit.	Passed (44 of 44)*
2	Sequencing: Sequencing run percent pass filter reads should be 80% or higher	Passed (86.5%, 89.2%, and 85.7%)
3	RNA Sequencing: Median CV Gene 500X Coverage should be less than or equal to 93.	Passed (21 of 21 RNA Libraries)
4	RNA Sequencing: Total On Target Reads should be greater than or equal to 9000000.	Passed (21 of 21 RNA Libraries)
5	RNA Sequencing: Median Insert Size should be greater than or equal to 80.	Passed (21 of 21 RNA Libraries)
6	DNA Sequencing: Contamination Score should be less than 1457	Passed (20 of 20 DNA Libraries)†
7	DNA Sequencing: Median Insert Size should be greater than or equal to 70bp	Passed (20 of 20 DNA Libraries)
8	DNA Sequencing: Median Exon Coverage (Count) should be greater than or equal to 150	Passed (20 of 20 DNA Libraries)
9	DNA Sequencing: Percent Exon Coverage 50% should be greater than or equal to 90	Passed (20 of 20 DNA Libraries)
10	DNA Sequencing: Usable MSI Sites (Count) should be greater than or equal to 40	Passed (20 of 20 DNA Libraries)
11	DNA Sequencing: Gene_Scaled_MAD (Count) should be less than 0.134	Passed (20 of 20 DNA Libraries)
12	DNA Sequencing: Median Bin Count CNV Target (Count) should be greater than or equal to 1	Passed (20 of 20 DNA Libraries)
13	DNA Sequencing: 5% Variant Allele Frequency (VAF) for small variants	Passed. Variants as low as 4% frequency detected.
14	DNA Sequencing: 2.2X fold-change for Copy Number Variants (CNV)	Passed (20 of 20 DNA Libraries)
15	DNA Sequencing: Analytical sensitivity greater than 96% for all variant types at 5% VAF or higher	Passed (99% of all 10% and 7% variants identified)

Runs contain a total of 44 libraries (21 RNA, 20 DNA, and 3 negative controls). Negative control libraries were negative for all metrics.

General Automation Considerations

- Please read and understand Biomek NGeniuS System IFU, C43212
- Please read and understand the Illumina TruSight Oncology 500 DNA RNA Automation Kit App Template Setup Guide for Biomek NGeniuS System
- Reagents left over from aliquoting can be saved and used in subsequent runs
- Do not use other sized kits as their reagents might come in an unexpected tube size
  - Chance for misread OCR token on vial
  - Chance for failed chemistry
  - Chance for damaged instrument
- Avoid bubbles in reagent tubes to ensure accurate liquid level sensing and aliquoting
  - EEW, SMB, LNB are bubbly
- The Work Aid requests more volume than what is consumed
  - Dead volume is needed in source tubes to ensure enough is available due to tolerance stack-ups
- Dead volume will be left behind in some storage wells
  - The nature of automation, tolerance stack-ups, and environment necessitates some overage
- Make sure bulk reagents wet the entire length of reservoir
  - Ensures accurate liquid volume sensing
- Start with sample volumes >= 2 mL
  - Biomek NGeniuS System will calculate needed sample volume based on provided sample concentration
  - System *will* accept smaller input volumes, but more variation is observed

Change Log

Illumina TruSight Oncology 500 DNA/RNA Automation Kit App Template version 1.0.2

- Corrected part number for 5ml reformat tubes. No impact on pipetting.

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