Method Biography for Illumina TruSight™ Oncology 500 DNA Only on Biomek NGeniuS System

Presented by Zach Smith | 1/26/2024

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Scoping

Illumina TruSight Oncology 500 DNA Only Kit

Kit Highlights:

The kit is optimized to provide high sensitivity and specificity for low-frequency somatic variants across 523 genes.

DNA biomarkers include the following:

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

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

Figure 1 TruSight Oncology 500 Kit DNA Only Workflow (Part 1)

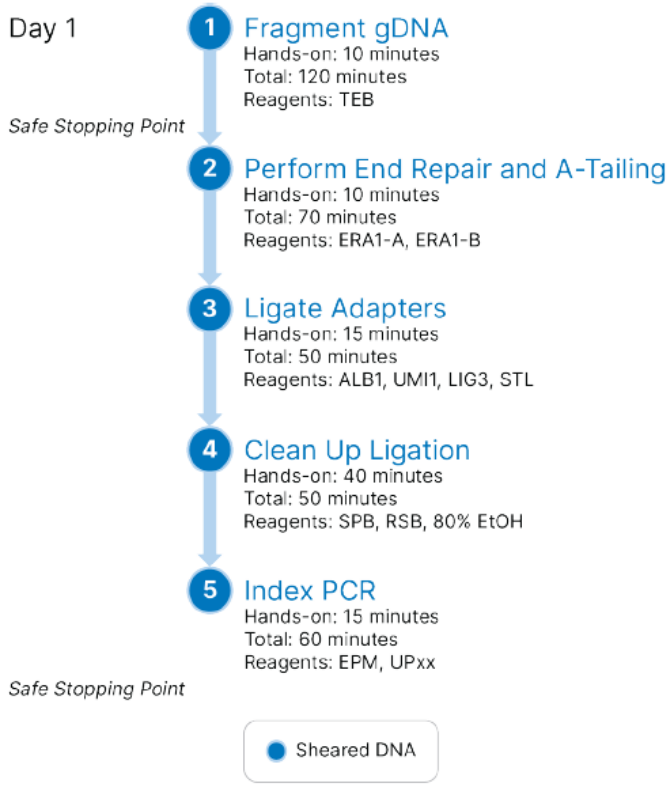
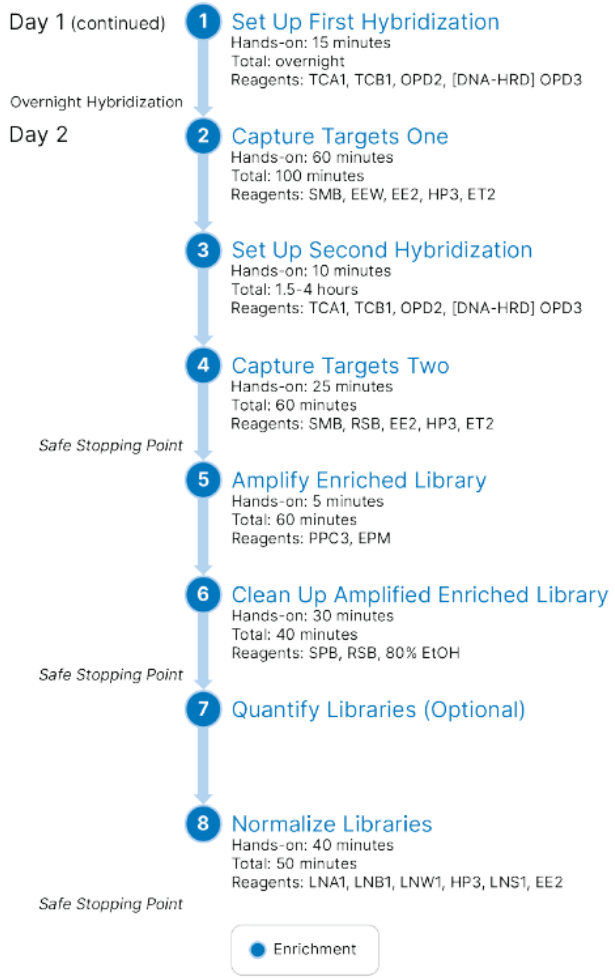


Figure 2 TruSight Oncology 500 Kit DNA Only Workflow (Part 2)



Above: Illumina TSO 500 DNA Only Workflow (adapted from TruSight Oncology 500 Reference Guide

App Template Description

The Illumina* TruSight* Oncology 500 DNA Automation Kit App Template allows for the creation of Illumina TruSight Oncology 500 DNA Only libraries compatible with Illumina sequencing platforms. The App Template allows the user to produce between 4 to 16 libraries in a single batch utilizing pre-sheared DNA samples. Refer to the Illumina TruSight Oncology 500 Reference Guide for details concerning DNA input mass, sample quality, and shearing parameters. Combined DNA/RNA workflow is not supported by the App Template. 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) 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 Automation Kit (16 indexes, 64 Samples) (Illumina Part Number 20045504).

Document ID 2023-GBL-EN-101637-v1

TSO500 DNA Only Workflow on Biomek NGeniuS System

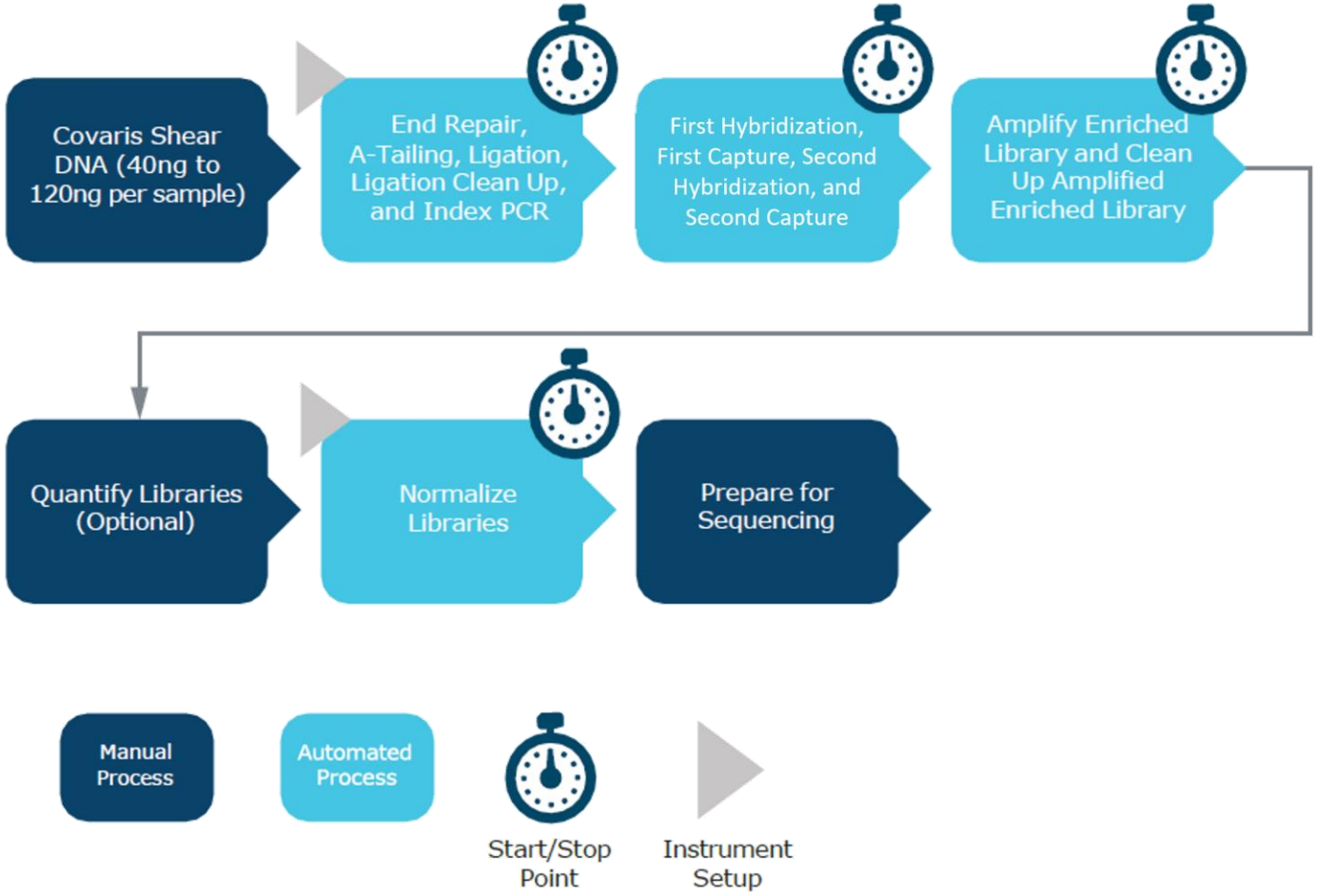
Features of the TSO500 DNA Only App

- Process any number of samples from 4 to 16, including odd numbers of samples.
- Can process a 16-sample batch with samples on sequencer by the end of Day 2.
- Built for the TruSight Oncology 500 DNA Automation Kit (16 indexes, 64 Samples) (Illumina Part Number 20045504).
- Can process up to four 16-sample batches from a single kit.

Not Included in the TSO500 DNA Only App

- Support for processing RNA and DNA samples in the same batch.
- Support for the supplemental HRD workflow.

TSO500 DNA Only Workflow on Biomek NGeniuS System



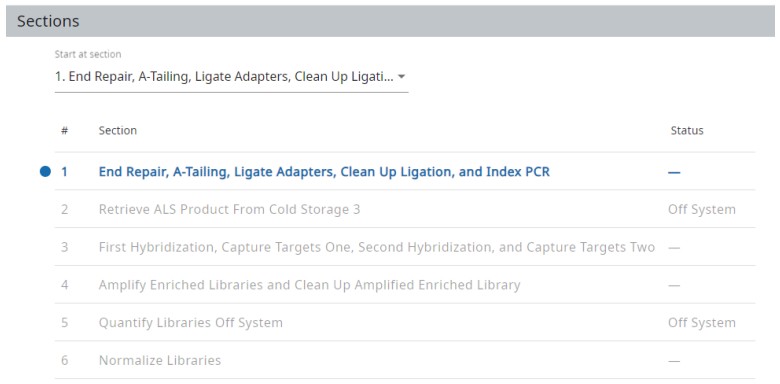
Expectation (From Reference Guide and Other Materials)

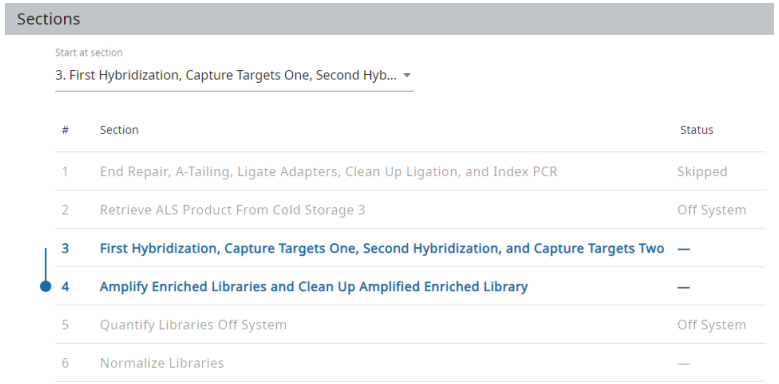
Metric Location	Metric Source	Results Metrics
Pre-Sequencing	Illumina TruSight Oncology 500 Reference Guide (1000000067621 v10)	Pre-library normalization yield should be 3ng/ul or higher as determined by Qubit.
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Sequencing run percent pass filter reads should be 80% or higher
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Post Sequencing: Contamination Score should be less than 1457
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Median Insert Size should be greater than or equal to 70bp
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Median Exon Coverage (Count) should be greater than or equal to 150X
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Percent Exon Coverage 50% should be greater than or equal to 90%
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Usable MSI Sites (Count) should be greater than or equal to 40
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Gene_Scaled_MAD (Count) should be less than 0.134
Post Sequencing	TSO500 DRAGEN Evaluation App MetricsOutput.tsv	Median Bin Count CNV Target (Count) should be greater than or equal to 1
Post Sequencing	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	5% Variant Allele Frequency (VAF) for small variants
Post Sequencing	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	2.2X fold-change for Copy Number Variants (CNV)
Post Sequencing	TruSight Oncology 500 and TruSight Oncology 500 High-Throughput Data Sheet (M-GL-00173 v5.0)	Analytical sensitivity greater than 96% for all variant types at 5% VAF or higher

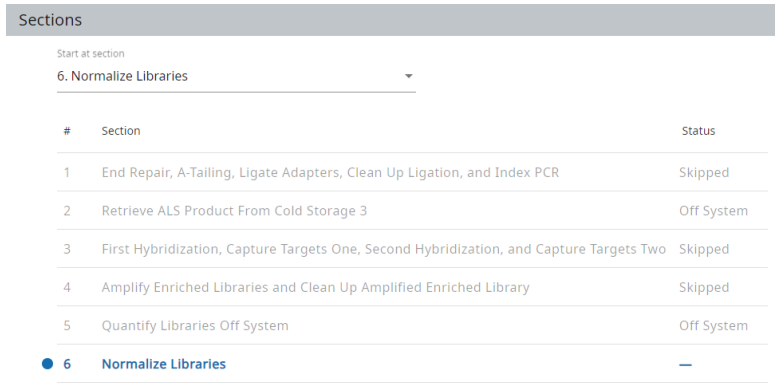
Operating the Illumina TruSight Oncology 500 DNA Only App Template

TSO500 DNA Only Workflow on Biomek NGeniuS System

Batches can be executed with a minimum of three user interactions

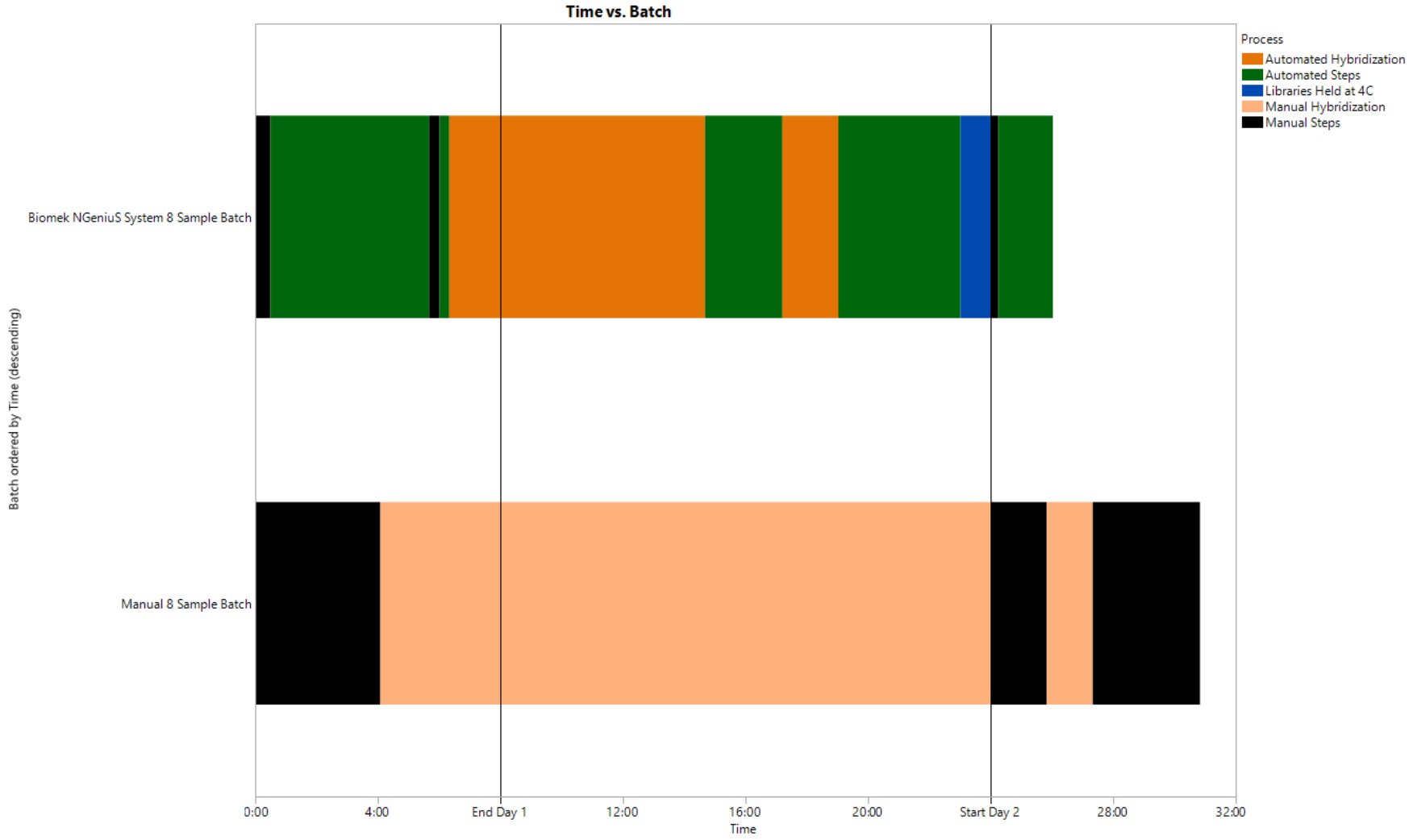




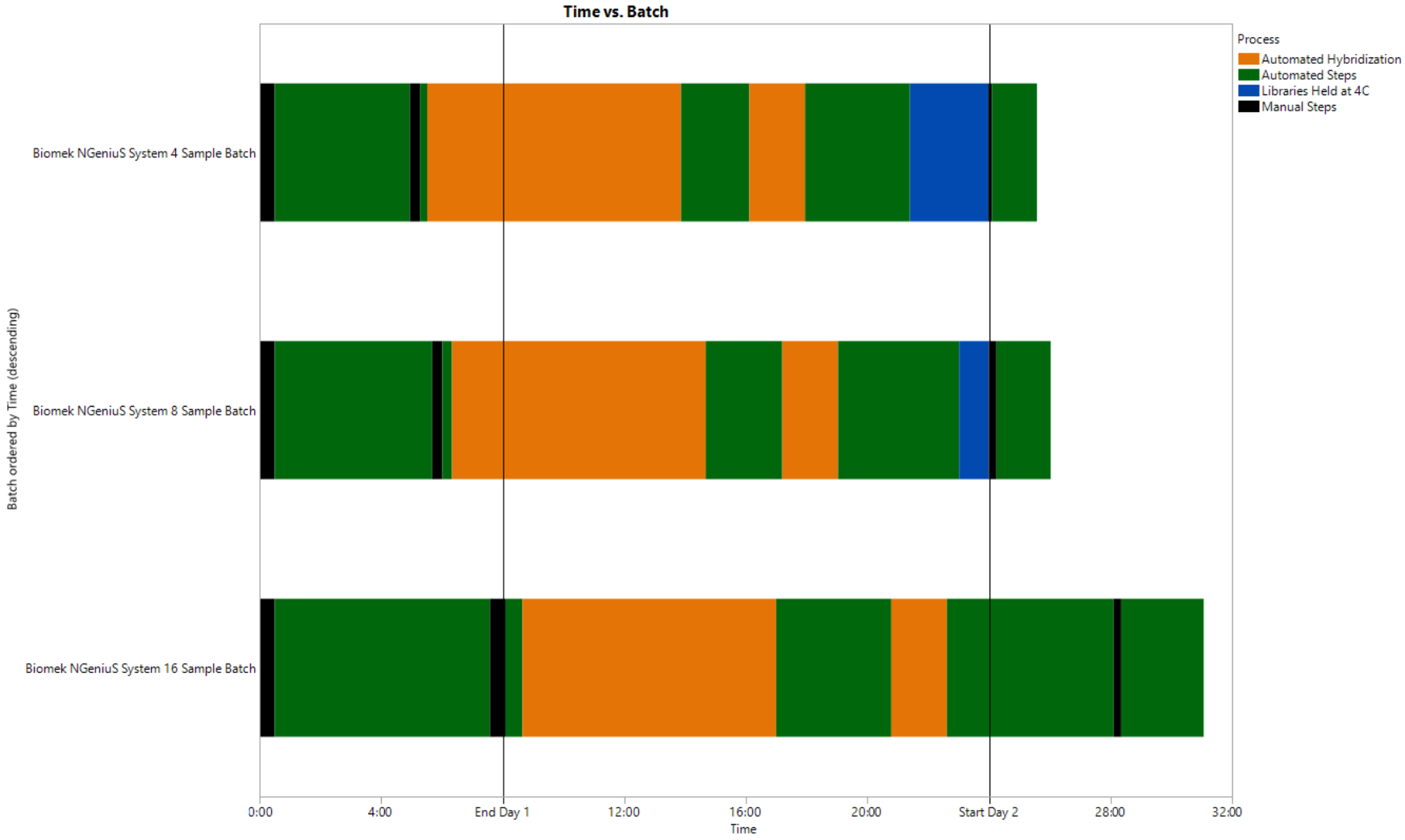


There are two off-system sections that require a user interaction. In the first one, the user is shown in the App Template Instructions for Use how to retrieve the ALS product that isn't being used for the first hybridization. The second off-system section is for the quantification of the libraries prior to bead-based library normalization.

TSO500 DNA Only on Biomek NGeniuS System Run Time and Hands-On Time



TSO500 DNA Only on Biomek NGeniuS System Run Time and Hands-On Time



TSO500 DNA Only on Biomek NGeniuS System Consumables Required By Batch Size

Part Number	Name	Number Required by App Template (tip boxes, reaction vessels, etc.) for 4 Samples	Number Required by App Template (tip boxes, reaction vessels, etc.) for 8 Samples	Number Required by App Template (tip boxes, reaction vessels, etc.) for 16 Samples	Quantity of Part Number to Purchase
C62705	Biomek Reaction Vessel, 24 well	19	19	19	1
C62706	Biomek Lid, 24 well	15	15	15	1
C62707	Biomek Bulk Reservoir, 25 mL/section	5	5	5	1
C59585	Case, Tips, 1025µL, Conductive, Filtered, 480 Tips	1	3	6	1
C62712	Case, Tips, 70 µL, Conductive, Filtered, 3840 Tips	2	2	4	1
C70672	Case, Biomek 1025 µL Tip Box, Empty, 5 Racks	1	1	1	1
C70673	Case, Biomek 70 µL Tip Box, Empty, 10 Racks	1	1	1	1
C70665*	Biomek Seal Plate, 24 well	3	3	3	1

* For the updated seal plate information please see Notification letter – Biomek Seal Plate Revision

TSO500 DNA Only on Biomek NGeniuS System Cost per Sample

Manual Processing	Per Sample*
Library Prep Kit	$389.96
Plastic Consumables	$46.34
Total Consumables	$436.30

Biomek NGeniuS Syatem Processing	Per Sample*
Library Prep Kit	$389.96
Plastic Consumables	$32.33
Total Consumables	$422.29

Per Sample	
NGeniuS	$422.29
Manual	$436.30
Difference	-$14.01

* Prices calculated for a 16-sample batch. Prices calculates as of Jan 2024; subject to change; for reference only

Batch runs per kit

Batch Size	4	5	6	7	8	9	10	11	12	13	14	15	16
Number of Batches per Kit	6	6	6	6	6	6	5	5	5	4	4	4	4
Samples	24	30	36	42	48	54	50	55	60	52	56	60	64
Largest Batch with Leftover Volume	16	16	16	11	5	0	7	0	0	9	5	0	0
Total Samples from Kit	**40**	**46**	**52**	**53**	**53**	**54**	**57**	**55**	**60**	**61**	**61**	**60**	**64**

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.

Demonstration Data

TSO500 DNA Only Demonstration Runs

TSO500 DNA Only 16-Sample Demo Run

Nine 40 ng Sera Care DNA shear replicates, two HD798 replicates, two HD799 replicates, two HD803 replicates, and one negative control (water) were used for the run.

TSO500 DNA Only 9-Sample Demo Run

Five 80 ng Sera Care DNA shear replicates, one 80 ng HD798 replicate, one 80 ng HD799 replicate, one 80 ng HD803 replicate, and one negative control (water) were used for the run.

TSO500 DNA Only 4-Sample Demo Run

Three 120 ng Sera Care DNA shear replicates and one negative control (water) were used for the run.

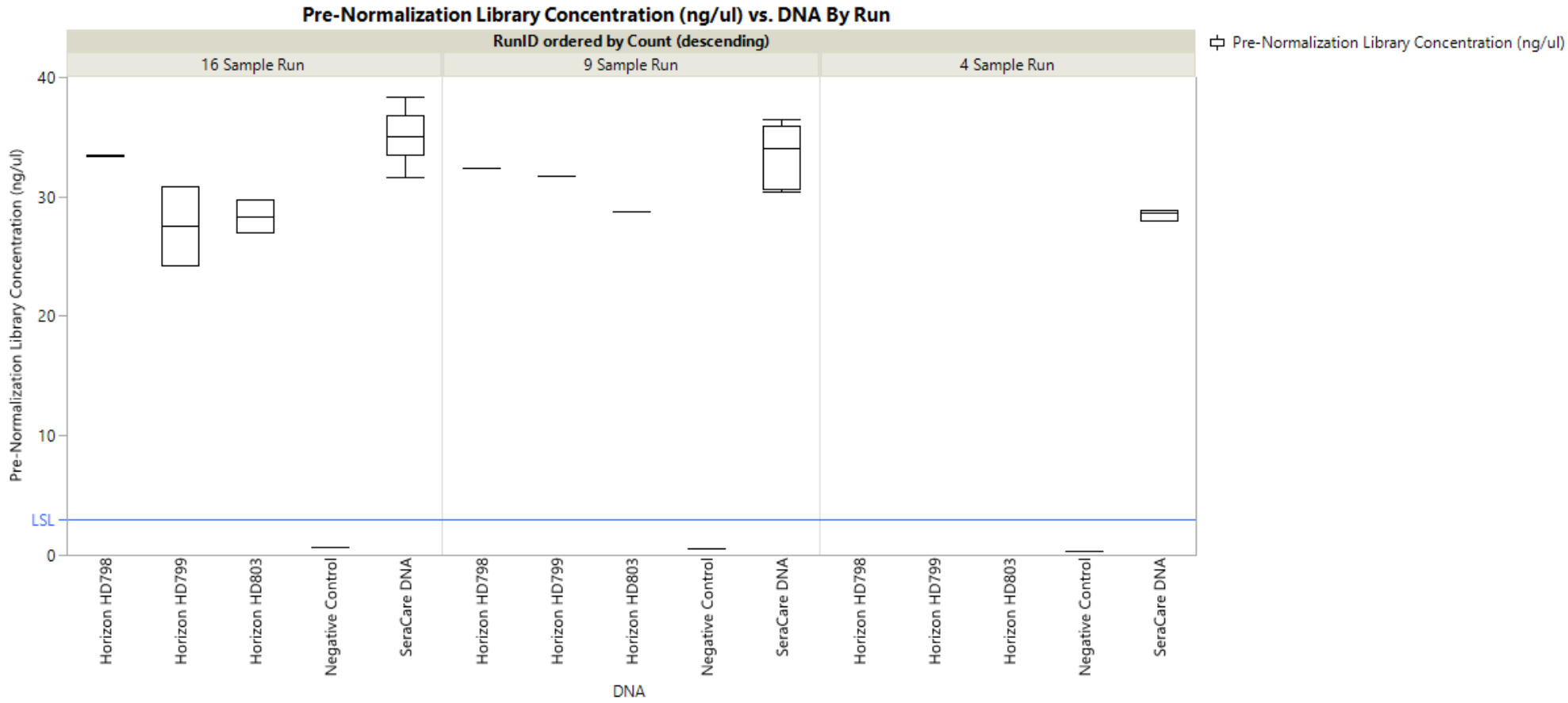
https://www.seracare.com/Seraseq-TriLevel-Tumor-Mutation-DNA-Mix-v2-HC-0710-0097/

https://horizondiscovery.com/en/reference-standards/products/quantitative-multiplex-reference-standard-fcdna-mild?catalognumber=HD798

https://horizondiscovery.com/en/reference-standards/products/quantitative-multiplex-reference-standard-fcdna-moderate?catalognumber=HD799

https://horizondiscovery.com/en/reference-standards/products/quantitative-multiplex-reference-standard-fcdna-severe

TSO500 DNA Only Demonstration Data: Pre-Normalization Library Concentration



Sequencing Runs

TSO500 DNA Only 16-Sample Demo Run

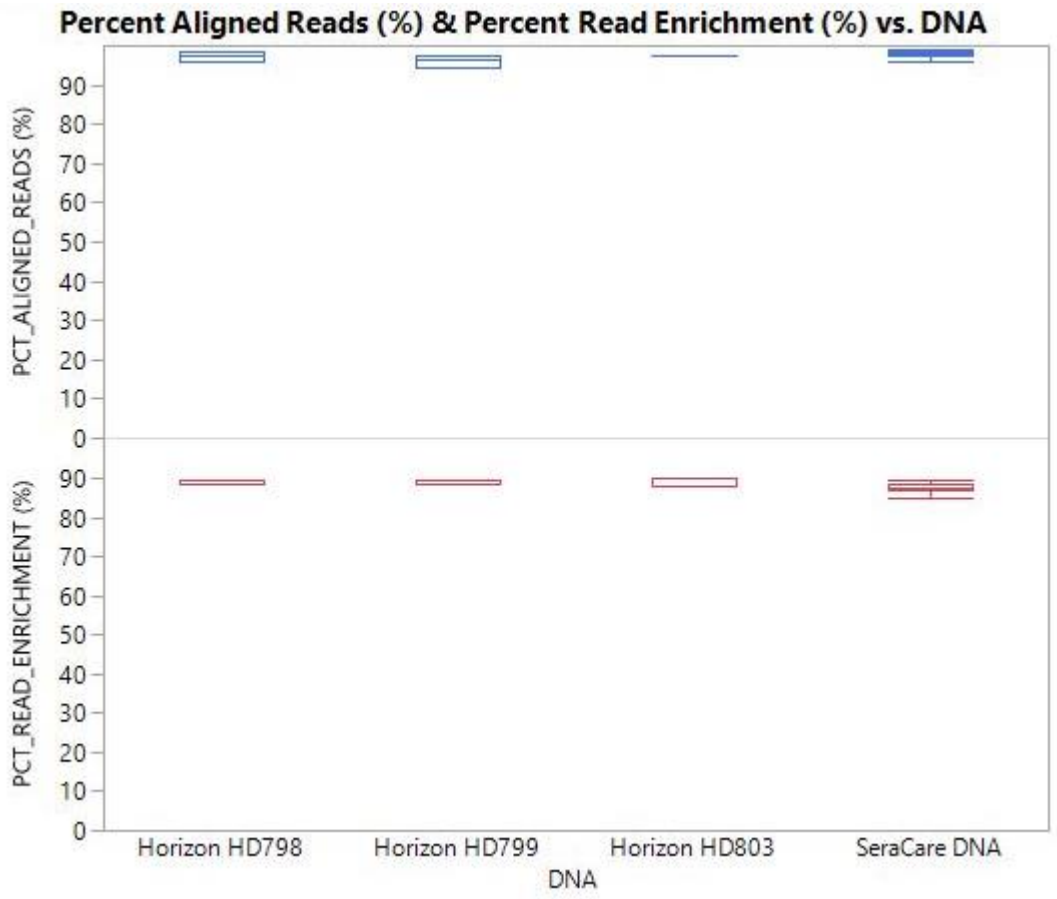
The sequencing run generated 205,866,836 pass filter reads (92.66% of total reads), of which 85% of reads were successfully identified. 94.35% of all bases were Q30 or higher. Total sequencing output was 88.25 Gb. Sequencing results should have 80% of reads pass filter with 80% of bases Q30 or higher.

TSO500 DNA Only 9-Sample and 4-Sample Demo Runs

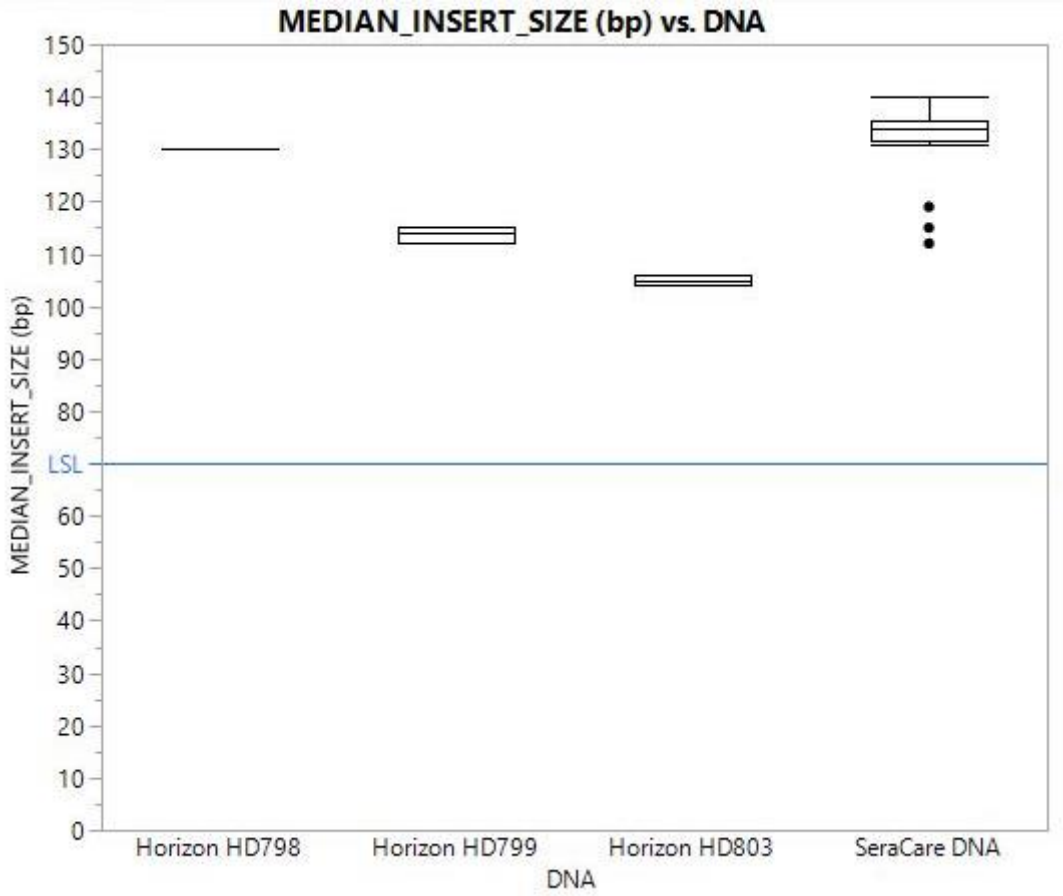
The sequencing run generated 271,579,046 pass filter reads (86.54% of total reads), of which 87.29% of reads were successfully identified. 91.37% of all bases were Q30 or higher. Total sequencing output was 115.45 Gb. Sequencing results should have 80% of reads pass filter with 80% of bases Q30 or higher.

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.

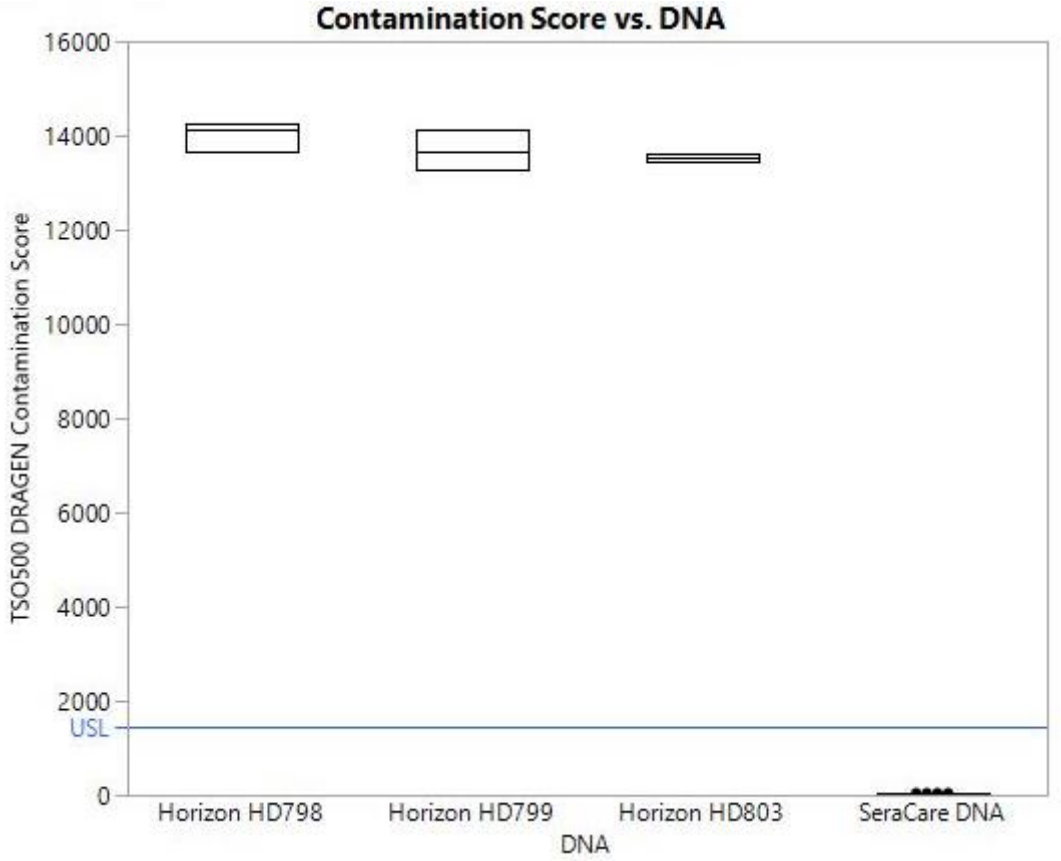
DNA Metrics


Percent Aligned Reads (%) & Percent Read Enrichment (%) vs. DNA
PCT_ALIGNED_READS (%)
PCT_READ_ENRICHMENT (%)
90
80
70
60
50
40
30
20
10
0
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

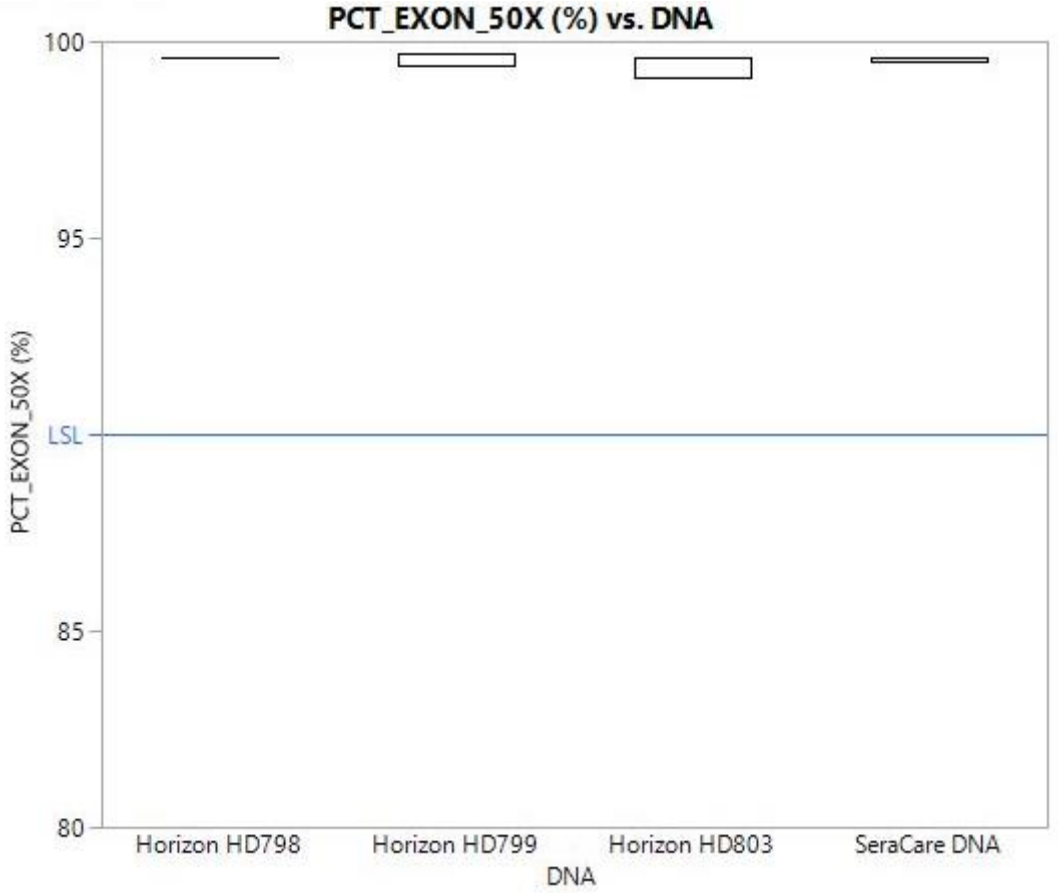
DNA Metrics


MEDIAN_INSERT_SIZE (bp) vs. DNA
MEDIAN_INSERT_SIZE (bp)
150
140
130
120
110
100
90
80
LSL
60
50
40
30
20
10
0
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

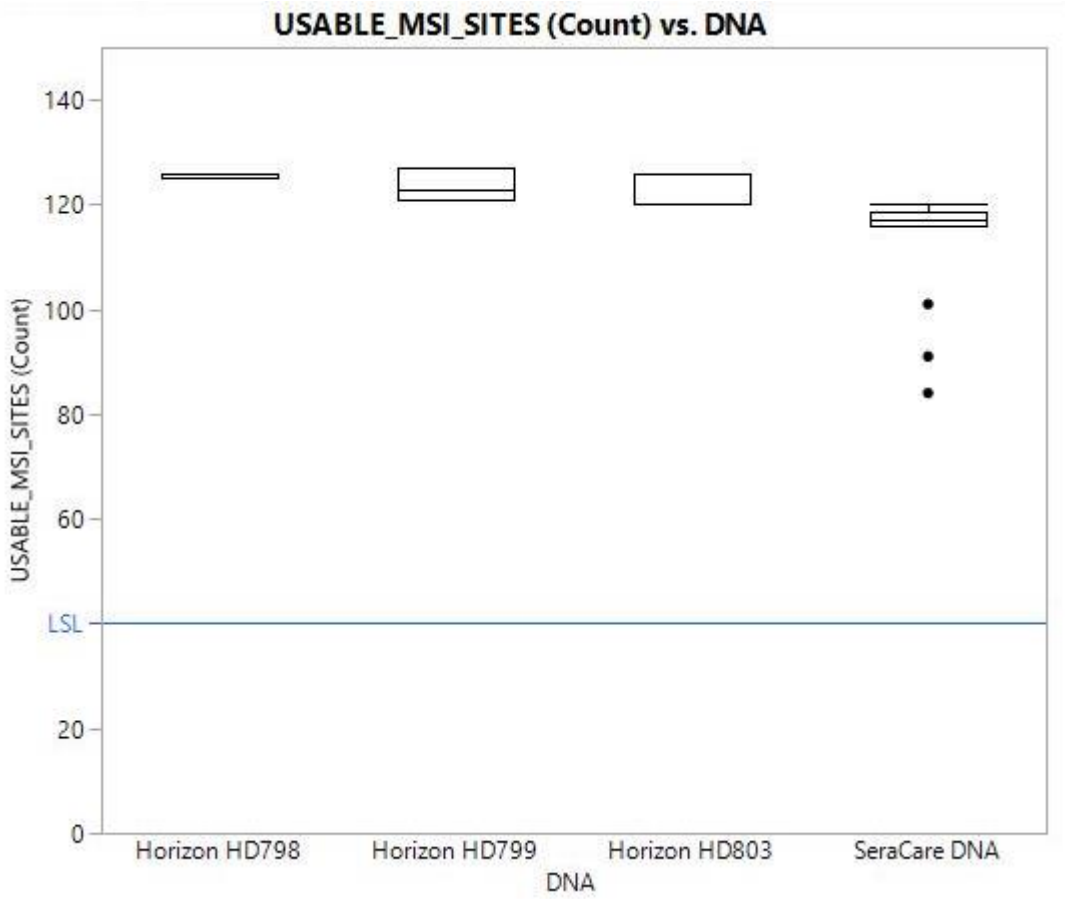
DNA Metrics


Contamination Score vs. DNA
TSO500 DRAGEN Contamination Score
16000
14000
12000
10000
8000
6000
4000
2000
USL
0
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

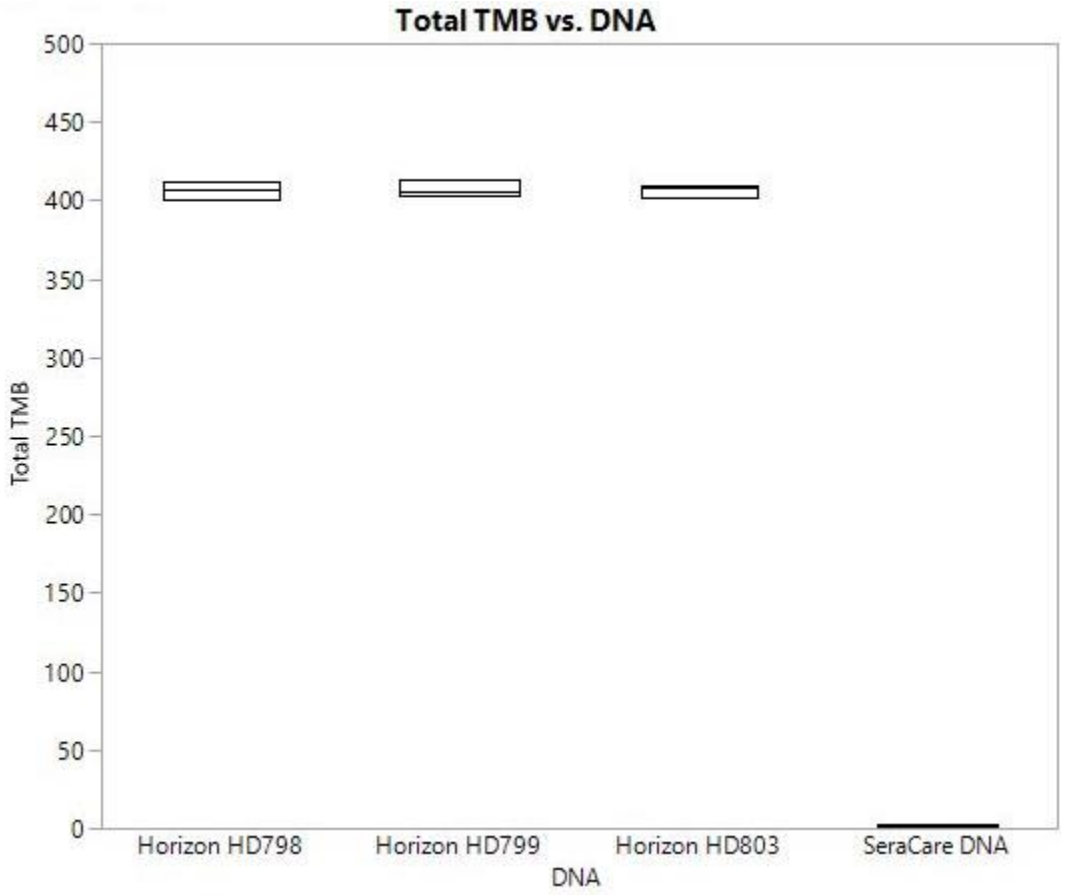
DNA Metrics


PCT_EXON_50X (%) vs. DNA
100
95
LSL
85
80
PCT_EXON_50X (%)
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

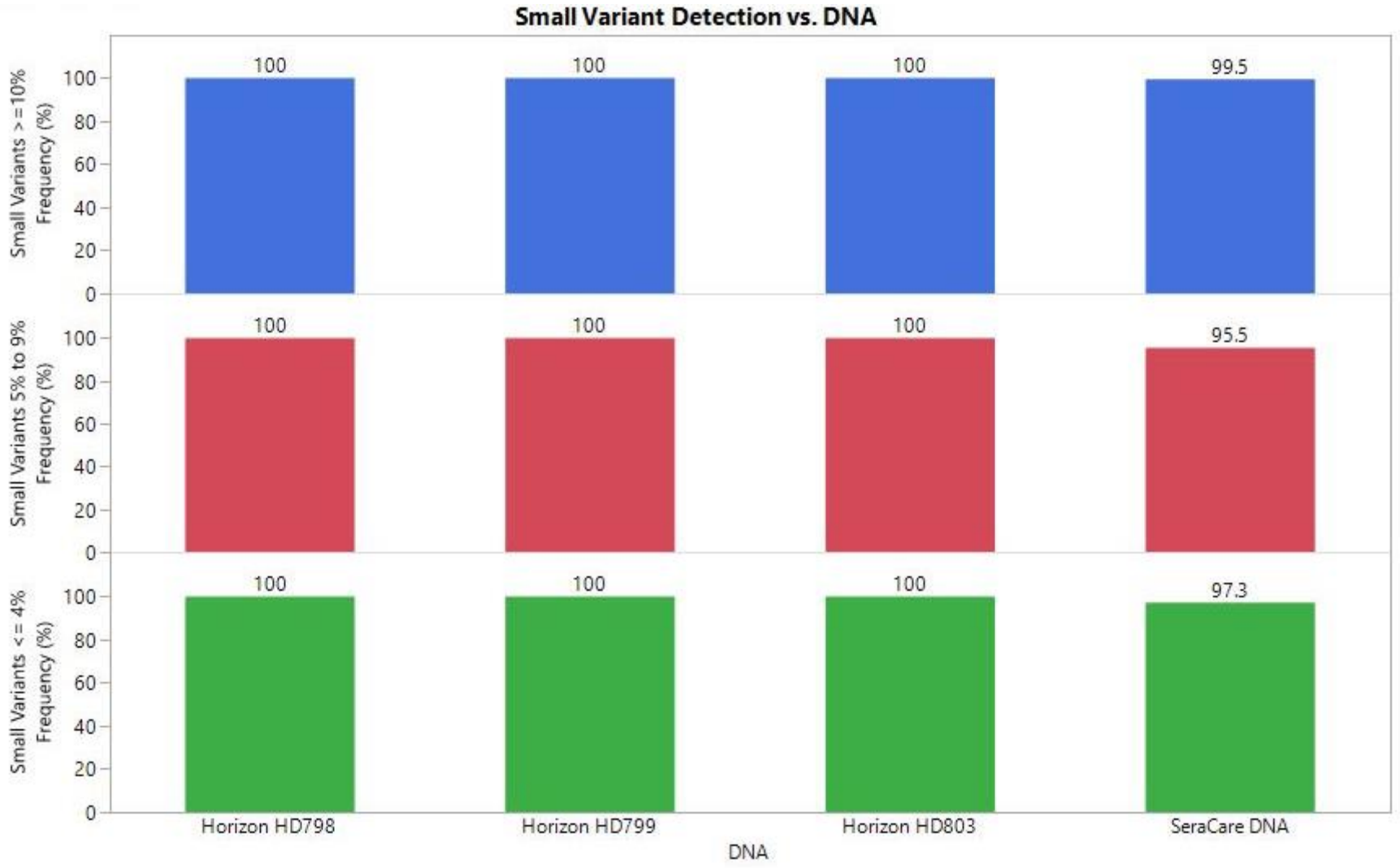
DNA Metrics


USABLE_MSI_SITES (Count) vs. DNA
USABLE_MSI_SITES (Count)
140
120
100
80
60
LSL
20
0
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

DNA Metrics


Total TMB vs. DNA
500
450
400
350
300
250
200
150
100
50
0
Total TMB
Horizon HD798
Horizon HD799
Horizon HD803
SeraCare DNA
DNA

DNA Metrics: Sera Care and Horizon Discovery Small Variants



Metric Results

Results Metrics	DNA Libraries
Pre-Sequencing: Pre-library normalization yield should be 3ng/ul or higher as determined by Qubit.	Passed (26/26)
Post Sequencing: Sequencing run percent pass filter reads should be 80% or higher	Passed (85% and 87.2%)
Post Sequencing: Contamination Score should be less than 1457	Passed (26/26)*
Post Sequencing: Median Insert Size should be greater than or equal to 70bp	Passed (26/26)
Post Sequencing: Median Exon Coverage (Count) should be greater than or equal to 150X	Passed (26/26)
Post Sequencing: Percent Exon Coverage 50% should be greater than or equal to 90%	Passed (26/26)
Post Sequencing: Usable MSI Sites (Count) should be greater than or equal to 40	Passed (26/26)
Post Sequencing: Gene_Scaled_MAD (Count) should be less than 0.134	Passed (26/26)
Post Sequencing: Median Bin Count CNV Target (Count) should be greater than or equal to 1	Passed (26/26)
Post Sequencing: 5% Variant Allele Frequency (VAF) for small variants	Pass. Variants as low as 4% frequency detected
Post Sequencing: 2.2X fold-change for Copy Number Variants (CNV)	Passed (26/26)
Post Sequencing: Analytical sensitivity greater than 96% for all variant types at 5% VAF or higher	Pass (98.2% of all 10% and 7% variants identified)

*Horizon Discovery samples are known to have higher contamination scores than the 1457 upper limit due to the mix of cell lines used per Illumina.

Considerations

General automation considerations

- 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
  - Sample Purification Beads (SPB) and Streptavidin Magnetic Beads (SMB)
- 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
- Prepare samples while Biomek NGeniuS System is aliquoting reagents
  - Avoids sample evaporation while Biomek NGeniuS System is preparing run

Conclusion

Illumina TruSight Oncology 500 DNA Only Libraries prepared on the Biomek NGeniuS Next Generation Library Prep System meets all specifications for successful sequencing.