Fully automated hybridization-based targeted sequencing sample prep with superior uniformity in 6 hours: A Point-n-Seq® workflow on Opentrons OT-2.

ABSTRACT

Automation has become increasingly vital to accommodate the growing demands of sample throughput, reliability, and reduced hands-on-time. As an industry first, automating Avida's Point-n-Seq® (PnS) hybridization-based targeted sequencing chemistry on the Opentrons® OT-2 liquid handler provides unparalleled processing efficiency in NGS sample prep library construction and target enrichment, without compromising NGS performance data.

Key features

- Automating Avida's Point-n-Seq targeted sequencing sample prep on OT-2 results in a workflow that can be completed within 6 hours, with 10 to 15 minutes of hands-on time**.**
- The automated Point-n-Seq workflow shows comparable sequencing performance with the manual workflow.
- Avida's Point-n-Seq 500 (PnS 500) assay accurately detects oncology variants in both FFPE and cfDNA samples. It outperforms a market-leading oncology assay with less total turnaround time, better uniformity and automation flexibility.

INTRODUCTION

Avida's Point-n-Seq technology is a new target enrichment chemistry essentially based on an interlocked, three-dimensional structure, designed specifically for synergistic indirect capture of intended DNA targets. This highly efficient DNA capture chemistry offers a higher specificity and faster binding speeds compared to conventional hybridization capture chemistry. Particularly, this hybridization chemistry enables a 1-hour hybridization-based target enrichment without any DNA input PCR amplification. This eliminates the need for a number of process steps, such as PCR, purification, quantification, which saves time and reduces DNA loss associated with each step. Here, we show that the Point-n-Seq targeted sequencing (TS) sample preparation workflow can be fully automated on OT-2. The OT-2 system is an affordable, open-source liquid handling platform, with a modular setup supporting many molecular biology workflows. Automating the Point-n-Seq workflow on OT-2 provides consistent performance with dramatically reduced hands-on time, while maintaining detection accuracy. A sequencing-ready target enriched library is prepared in less than 6 hours with just 10 to 15 minutes of hands-on time. The automated workflows were tested using various inputs and capture panels, including genomic DNA, FFPE genomic DNA and cell-free DNA (cfDNA), with panels ranging from 20 Kb to 2 Mb.

MATERIALS AND METHODS

Overview of the Point-n-Seq (PnS) TS workflow on the Opentrons OT-2 platform

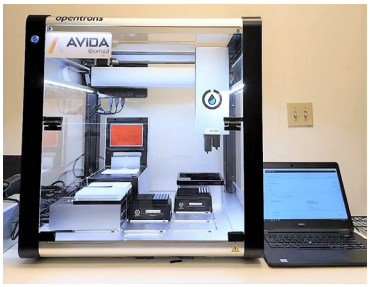
The Point-n-Seq TS protocol consists of reagent setup, library prep and target capture, and index PCR amplification and purification **(Figure 1).** Processing times and hands-on time for each step are listed in **Table 1**.

Schematic of OT-2 deck layout for the Point-n-Seq TS protocol

The OT-2 deck layout for the Point-n-Seq TS protocol includes the modules, labware, and library prep reagents **(Figure 1)**. No manual intervention is required once the run is set up and started.

A

PNS Workflow on OT-2	Reagent Setup	Library Prep + Target Capture	Indexing PCR and Purification
Process Time	15 min	270 min	60 min
Hands-on Time	10 min	0 min (walk-away)	15 min



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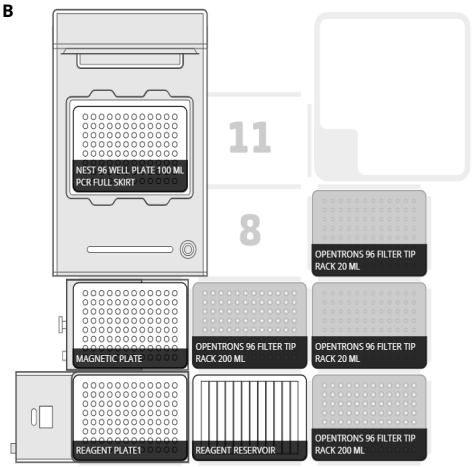

Figure 1. Point-n-Seq TS workflow on OT-2 (A), and robot configuration for NGS sample prep and target enrichment (B).

RESULTS

Point-n-Seq TS workflow automation reduces hands-on time, provides a quicker turnaround time, and improves LOD.

Compared to conventional hybridization based target enrichment, processing time for automated Point-n-Seq TS workflow reduced the turnaround time from 2 days to 6 hrs **(Table 1)**. LOD was improved from 5% to 1% for Avida Point-n-Seq 500 panel compared to a leading oncology assay panel **(Table 2)**.

Automation provides an accurate LOD of SNV

DNA isolated from reference genomes -NA12878 and NA24385 were mixed to mimic alternative allelic frequencies of 1% for SNV detection. All libraries were enriched with a human sample ID panel targeting a 14 kb region of highly polymeric SNPs. Accuracy of variant calling was assessed over 12 loci **(Table 3)**. 12/12 loci were detected with highly reproducible data across 8 wells in a column (wells A to H).

Reliable variant detection on oncology FFPE and cfDNA reference DNA

Automated target enrichment was performed using 30 ng of FFPE reference (severely damaged Formalin-Compromised DNA, Horizon, HD803) **(Table 4)** and 40 ng of cfDNA (Horizon, HD833) **(Table 5)** as input. Variant allele frequencies (AF) were detected and compared to known frequencies to confirm the reliability of variant detection using the automated Point-n-Seq workflow.

		REAGENT SET UP	LIB PREP TARGET CAPTURE	INDEX PCR PURIFICATION
Point-n-Seq OT-2 Automation	Process time	15 min	270 min	60 min
	Hands on time	10 min	0 min	15 min
Conventional	Process time	2 days		
	Hands on time	6 hr		

Table 1. Automation of Point-n-Seq TS on Opentrons OT-2 reduces processing time and hands-on time. Processing times and hands-on time were compared for automated PnS workflow and conventional hybridization based target enrichment.

TARGET CAPTURE PRODUCT	AUTOMATION INSTRUMENT COST	TOTAL TIME	HANDS-ON TIME	DNA INPUT	LOD
Avida PNS500 (on OT-2)	$25,000	6 hr	25 min	40 ng	1%*
Other oncology assay panel	$200,000	2 days	2.5 hr	40 ng	5%

*Liquid Biopsy product with 0.1% LOD in development

Table 2. Comparison of Avida PNS 500 to a leading oncology assay panel

	CHROM:POS	EXPECTED VAF %	DETECTED VAF% A1	DETECTED VAF% B1	DETECTED VAF % C1	DETECTED VAF % D1	DETECTED VAF % E1	DETECTED VAF % F1	DETECTED VAF % G1	DETECTED VAF % H1
HID02	chr1:89388944	2.00	2.61	2.86	2.66	2.62	2.41	2.25	2.07	2.28
HID07	chr2:60000720	1.00	0.70	1.04	0.72	0.35	0.60	0.34	0.57	0.54
HID15	chr4:46329655	1.00	1.54	1.53	1.41	1.01	0.55	0.58	1.12	1.14
HID16	chr4:58706855	1.00	1.24	1.30	1.75	1.71	2.06	1.37	1.64	2.24
HID27	chr6:164782889	2.00	3.88	4.38	2.70	3.70	3.32	2.17	2.38	2.43
HID30	chr7:105679551	1.00	1.13	1.19	1.10	1.65	1.77	1.39	1.78	1.98
HID33	chr8:11718528	1.00	1.23	1.26	1.18	1.51	1.56	2.02	2.07	0.99
HID39	chr9:137417308	1.00	1.59	1.12	1.66	0.92	1.58	2.26	1.28	1.83
HID48	chr12:130761696	1.00	1.34	1.66	1.36	1.37	1.16	1.16	1.38	1.86
HID52	chr15:25057315	1.00	1.59	1.79	1.81	1.66	1.63	1.82	1.95	1.46
HID61	chr18:23417133	1.00	1.58	1.46	1.20	1.35	0.96	1.36	1.48	1.92
HID71	chr22:33559508	1.00	1.41	1.66	1.16	1.55	1.51	2.06	1.97	1.45

Table 3. SNV accuracy for target captures 20 ng of DNA -NA12878 and NA24385 (Genome In A Bottle -GIAB) was mixed at 2% to mimic alternative allelic frequencies of 1% for SNV detection.

GENE	VARIANT	HORIZON AF%	AVIDA PNS 500 AF%
BRAF	V600E	13.6	14.6
cKIT	D816V	9.5	11.6
EGFR	ΔE746 - A750	2.27	3.1
EGFR	L858R	3.67	5.1
EGFR	T790M	1.28	0.7
EGFR	G719S	24	20.2
KRAS	G13D	13.5	22.5
KRAS	G12D	5.41	4.7
NRAS	Q61K	13.1	15.4
PIK3CA	H1047R	19.5	20.2
PIK3CA	E545K	6.79	11.6

Table 4. Reliable variant detection on oncology FFPE reference DNA Allele frequency (AF) detection was conducted with Horizon Multiplex Severely Formalin Compromised DNA (DIN: 1.9) using 40 ng as input. 11/11 of variants were detected with the AF close to expected values.

GENE	VARIANT	HORIZON AF%	PNS 500
ALK	c.*61_*64dup-CAAT	8.3	4.0
APC	p.T1493T	35.2	33.5
BRAF	p.V600E	9.8	9.1
BRCA2	p.K1691fs*15	32.4	29.7
CTNNB1	p.S33Y	36.8	35.7
CTNNB1	p.S45del	10.5	7.8
EGFR	p.E746_A750_delELREA	2.54	1.9
EGFR	p.G719S	23.8	24.4
EGFR	p.L858R	2.7	2.4
EGFR	p.Q787Q	14.8	14.6
EGFR	p.T790M	1.06	0.3
FBXW7	p.S668fs*39	32.8	34.2
FLT3	p.P986fs*8	10.2	9.1
KIT	p.D816V	10.8	8.1
KIT	p.L862L	6.5	5.5
KRAS	p.G12D	5.7	7.4
KRAS	p.G13D	15.7	12.3
MET	p.A1357A	6.2	6.6
MET	p.L238fs*25	6.5	5.3
NOTCh1	p.P668S	30.1	26.6
NRAS	p.Q61K	12.4	11.7
PIK3CA	p.E545K	16.1	9.6
PIK3CA	p.H1047R	8.3	17.3
RET	p.L769L	64.0	66.9
TP53	p.P72R	92.85	92.5

Table 5. Reliable variant detection on oncology cfDNA reference DNA Allele frequency (AF) detection was conducted with Horizon OncoSpan cfDNA Reference Standard using 30 ng as input. 25/25 of variants were detected with the AF close to expected values.

SNVs, small deletions and insertions were detected matching the expected AF, down to 1%, which was determined by the manufacturer using ddPCR.

Better uniformity for Avida Point-n-Seq 500 assay compared to a market leading oncology assay

Coverage across a wide spectrum of GC content was assessed and the results showed better uniformity in the Avida Point-n-Seq 500 panel than in a market-leading comprehensive oncology assay **(Figure 2)** under the same sequencing reads.

Automated Point-n-Seq workflow provides comparable performance with manual Point-n-Seq workflow

Same input DNA (cfDNA10 ng, or gDNA 100 ng) were subjected to either manual or automated (OT-2) workflow. For cfDNA a focused small panel (20 kb) and for gDNA a relatively larger comprehensive panel (~2 Mb) were used for target enrichment. Library yield, on-target rate, dedup read, duplication read, mapability, insert length, and uniformity were compared to show comparable sequencing results from manual Point-n-Seq workflow and automated workflow on OT-2 **(Table 6).**

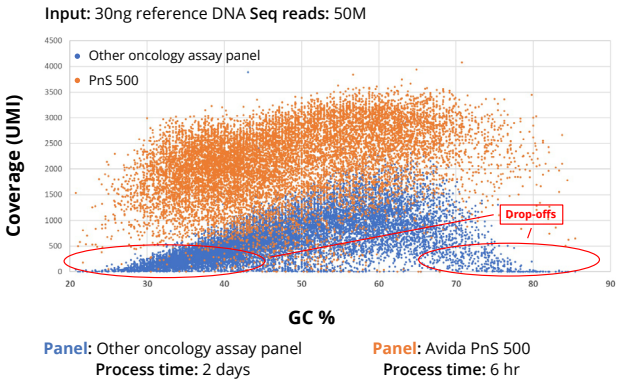

Figure 2. Avida PnS 500 panel demonstrates superior performance compared to a market-leading oncology panel. Coverage across GC content was assessed and showed better uniformity compared with another commercially available comprehensive oncology assay. Input: 30 ng of reference DNA; sequenced reads: 50 M.

CONCLUSION

A comprehensive oncology assay powered by Avida's Point-n-Seq technology can be automated on the Opentrons OT-2 liquid handling robot, providing a walk-away solution for rapid and accurate variant detection.

This automated solution greatly accelerates the development of fast, simple, and accurate genomic profiling assays for current and future clinical applications.

cfDNA 10 NG

20KB PANEL	LIBRARY YIELD MEAN	LIBARY YIELD SD	DEDUP MEAN	DEDUP SD	MAPPING MEAN	MAPPING SD	ON-TARGET MEAN	ONTARGET SD	0.2X MEAN	0.2X SD	0.5X MEAN	0.5X SD
Manual n=4	22.9	1.5	3288	162	99.7	0.1	69.4	1.9	100	0	100	0
Auto n=8	20.4	1.4	3382	101	99.7	0.2	74.2	1.7	100	0	100	0

gDNA 100 NG

2MB PANEL	DEDUP MEAN WITH 25M READ	DEDUP SD	DUPLI-CATION MEAN	DUPLI-CATION SD	MAP-PING MEAN	MAP-PING SD	INSERT MEAN	INSERT SD	0.2X MEAN	0.2X SD	0.5X MEAN	0.5X SD	FOLD80 MEAN AT 150X	FOLD80 SD AT 150X
Manual n=8	1214	14	23.8	0.9	99.9	0	216.3	1.0	97.9	0.0	92.4	0.1	1.46	0.01
Auto n=8	1142	22	26.6	0.9	99.9	0	211.3	1.9	98.2	0.1	94.5	0.1	1.38	0.05

Table 6. Comparable performance to manual Point-n-Seq workflow. Library yield, on-target rate, dedup read, duplication read, mapability, insert length, uniformity were assessed for PNS 500 (2 Mb) on 100 ng NA12878 gDNA (sheared to 200 bp peak length), and Pan-Cancer (20 kb) on 10 ng cfDNA (n = 4).