Inter-site workflow performance of a fully automated sample preparation system for NGS applications: *integration with KAPA HyperExome probes* for whole exome sequencing, the AVENIO Edge† instrument, and navify® Mutation Caller#*

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navify Mutation Caller is not for sale/use in the United States. † The AVENIO Edge System and navify® Mutation Caller are for Research Use Only workflows. * Reagents and kits mentioned are for Research Use Only. Not for use in diagnostic procedures.

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Background and Goals

- Next generation sequencing (NGS) sample preparation automation systems, for library preparation and target enrichment, are used by laboratories to drive scalability in precision medicine research.[1-3] The AVENIO Edge System† is a fully automated NGS sample preparation system for library preparation, target enrichment, pooling, normalization and quantification.

This study evaluated the inter-site performance of the AVENIO Edge System† automated workflow for whole-exome sequencing (WES) with the KAPA HyperExome Probes* in comparison to the manual workflow at four sites.

1. Results – Part 1

- For the baseline runs, percent reads on-target +/- SD were 87.3±0.5 (A) and 87.4±1.1 (B), mean depth of coverage +/- SD 55.7±1.4 (A) and 54.8±1.7 (B), and percent total duplication +/- SD 4.8±1.5 (A) and 4.9±1.6 (B). Percent reads on-target +/- SD were 86.4±0.7 (A) and 86.3±1.2 (B) [50ng], 87.2±0.4 (A) and 87.2±0.6 (B) [8-plex], and 81.4±1.7 (A) and 83.2±0.9 (B) [manual]. Six manual run (A) samples did not meet acceptance criteria (>60M reads for panel ≥40 Mb).

A %On-target

Avg	Run1	Run2	Run3	Run4	Run5	Run6
Site A	87.38	87.29	87.36	87.15	86.42	83.99
Site B	86.31	88.11	87.76	87.21	86.25	83.25

B Coverage depth

Avg	Run1	Run2	Run3	Run4	Run5	Run6
Site A	56	56	55	58	58	71
Site B	55	54	56	58	58	60

C %Duplication

Avg	Run1	Run2	Run3	Run4	Run5	Run6
Site A	5.88	4.59	3.91	3.47	4.62	6.98
Site B	5.71	3.21	5.83	3.68	5.18	3.42

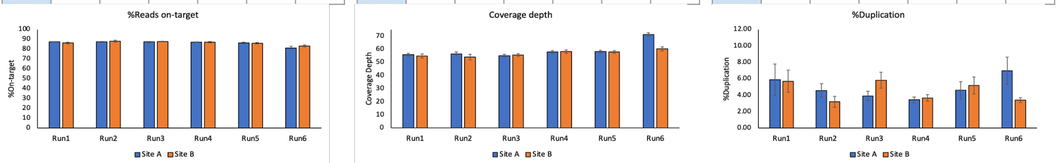

Figure 1: AVENIO Edge† workflow performance and manual workflow comparison: Percent reads on-target (**A**), mean depth of coverage (**B**), and percent total duplication (**C**).

2. Results – Part 2

- Automated workflow recall, precision and concordance was greater than 99.1% (A) and 99.1% (B) (**Table** 1). Eight samples [six Run6 (A), one Run2 (B), one Run5 (B)] that did not meet acceptance criteria were removed from variant calling analysis since low coverage or base quality can result in wrong calls or a refusal to call.

Table 1: Workflow recall, precision and concordance by **navify**® Mutation Caller#

		AVENIO Edge† Automation					Manual
		Run 1 (baseline)	Run 2 (baseline)	Run 3 (baseline)	Run 4	Run 5	Run 6
Site A	% Recall	99.19	99.25	99.25	99.27	99.28	99.43
	% Precision	99.16	99.21	99.24	99.31	99.24	99.49
	% Concordance	99.10	99.16	99.19	99.26	99.19	99.45
Site B	% Recall	99.24	99.15	99.23	99.26	99.26	99.36
	% Precision	99.20	99.14	99.22	99.27	99.30	99.35
	% Concordance	99.13	99.07	99.16	99.22	99.24	99.30

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Disclosures

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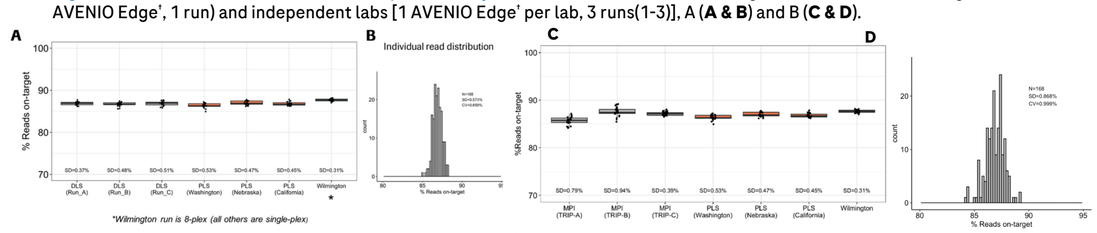
Abbreviations

gDNA, genomic DNA; NA, nucleic acid; Rep, replicate; SD, standard deviation; SNP, single nucleotide polymorphism; WES, whole exome sequencing

3. Results – Part 3

- Consistent inter-instrument reproducibility was observed across each instrument site (A/B and two Roche sites; seven runs, five instruments) (**Figure 2**). All AVENIO Edge† instruments achieved >85% reads on-target (**Fig 2A & 2C**). Individual read distribution for 24 replicates across 7 instrument runs (168 samples) showed average percent reads on-target ± SD, 88±0.57 (A) (**Fig 2B**) and 86±1.62(B) (**Fig 2D**).

Figure 2: Inter-site and inter-instrument reproducibility: Roche Pleasanton (3 AVENIO Edges†, 3 runs), Roche Wilmington (1 AVENIO Edge†, 1 run) and independent labs [1 AVENIO Edge† per lab, 3 runs(1-3)], A (**A & B**) and B (**C & D**).



Methods

- Two independent labs (site A and B) each prepared 24 replicates (NA12878 human gDNA) for each run (**Table 2**). Five automated runs evaluating nucleic acid inputs (100 ng and 50 ng) and pooling (singleplex and 8-plex) conditions were conducted following AVENIO Edge System† instructions for use with KAPA HyperExome probes* (Roche) and compared against a (manual) KAPA HyperCap v3.0 workflow* (Roche) run (100 ng, singleplex) with Illumina sequencing. At each lab, repeatability and well-to-well comparison was measured across three baseline instrument runs (100 ng, singleplex) using percent reads on-target, mean depth of coverage, and percent total duplication and standard deviation (SD) of each measure. Percent reads on-target were also assessed for the 50ng and 8-plex pooling instrument runs, and the manual run. Variant calling was performed with **navify**® Mutation Caller# (Roche) to determine the variant detection performance of the automated workflow as compared to NA12878 gold standard with 24,901 high confidence SNPs in the KAPA HyperExome* target region. Inter-site and inter-instrument reproducibility was evaluated against two Roche sites.

Table 2. Study design: AVENIO Edge† and manual study runs and conditions tested by each independent lab

Run	Workflow	Sample Type	Replicates	Nucleic Acid Input	Pooling	Description	
1	Automated	NA12878	24	100 ng	Singleplex	Rep1 (baseline)	Evaluate AVENIO Edge System† uniformity and reproducibility, samples prepared in triplicate. Also, used for inter-site reproducibility (**Figure 2**)
2			24	100 ng	Singleplex	Rep2 (baseline)	
3			24	100 ng	Singleplex	Rep3 (baseline)	
4			24	100 ng	8-plex	Pre-capture pooling performance	
5			24	50 ng	Singleplex	Low NA sample input performance	
6	Manual		24	100 ng	Singleplex	Compare AVENIO Edge† automated runs to a manual run	

Conclusions

- AVENIO Edge System' for NGS sample preparation automation integrated with **navify**® Mutation Caller# for variant calling demonstrated robust inter-site workflow and variant detection performance as compared to a manual workflow for WES using KAPA HyperExome Probes* to enable improved laboratory efficiency and ensure result confidence.

References

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