INTRODUCTION OF A WALK-AWAY AUTOMATED ROCHE NGS WORKFLOW SOLUTION: INTEGRATED KAPA LIBRARY PREPARATION, KAPA TARGET ENRICHMENT AND THE AVENIO EDGE INSTRUMENT

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AVENIO Edge instrument is currently in development. The data presented here are not intended for diagnosis or patient management. The AVENIO Edge instrument can be used for Research Use Only (RUO) workflows. The compatible workflows, materials and AVENIO Edge Designer software are RUO only, not for use in diagnostic procedure.

INTRODUCTION

The AVENIO Edge system* will provide a walk-away automated NGS (next generation sequencing) library preparation and target enrichment solution with QC (quality control) from extracted nucleic acid with minimal hands-on time and flexibility for customer modified workflows.
Features include:

- 8 pipetting channels
- Robotic gripper arm with an attached 2D barcode scanner
- Integrated plate reader (Quant Module) which performs DNA quantification
- On Deck Thermal Cycler
- Cooling station for reagent tubes
- Heated Shaker
- Magnetic bead separation processing station for DNA / PCR clean-up steps
- Touch screen
- UV light
- Hand-held barcode scanner

AVENIO Edge Instrument

Quant Module

The AVENIO Edge instrument* will be launched with a hybridization-based capture workflow application using pre-kitted reagents.

MATERIALS AND METHODS

AVENIO Edge Hybridization-based Capture Workflow Reagents and samples used in verification studies:

A

B

Figure 1: Capture Workflow Reagent Kits. A)** Automation friendly, pre-kitted reagents will be available with the option of AVENIO Edge HyperPrep or HyperPlus Library preparation and the option to use HyperPlex or HyperPlex UMI adapters. **B)** The fully automated AVENIO Edge instrument encompasses the AVENIO Edge Designer Software to set up a protocol. The protocol will be loaded on the AVENIO Edge Software and in turn will list all consumables and reagents needed per the protocol loaded. Once the load check is complete, the run will proceed to completion.

Sample	Information
Cell line blend FFPE blocks (Horizon)	HD789 (high quality)
Cell line genomic DNA (RMSCC/Coriell Institute)	NA12878 (Genome in a bottle)

Panel	Primary target region
<40Mb sized panel	530kb
≥40Mb sized panel	41Mb

Figure 3: Samples and Panels used in Verification Studies. Various high quality samples from cell line, FFPE DNA, MP24 and MP96 extracted DNA from whole blood were used during verification studies. Two assay panels varied in size were tested and results from pre capture and post-capture workflows were compared.

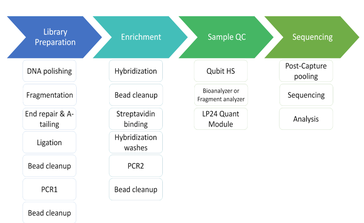


Figure 2: Hybridization Capture Workflow.** This figure shows the individual steps involved in the hybridization capture workflow. QC is optional after PCR1 for post capture workflow or needed for pre-capture pooling. After Target enrichment and PCR2 quantification, pooling was performed on deck using concentration and DNA fragment size input to get equimolar sequencing ready pooled libraries. Sequencing was done using Illumina NextSeq and NovaSeq sequencers.

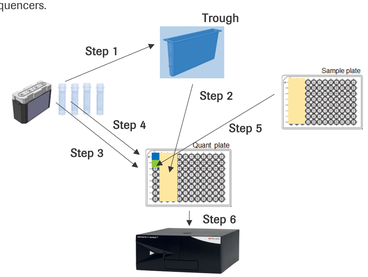


Figure 4: AVENIO Edge Quantification Module Setup and hands-free protocol:
Step 1: Dilute quant dye from Tube 1 in trough with 1X TE to create master mix.
Step 2: Dispense master mix into Quantification plate.
Step 3: Pipette high standard from Tube 2 into Quant plate well.
Step 4: Pipette low standard from Tube 2 into Quant plate well.
Step 5: Pipette DNA samples into Quant plate respective wells.
Step 6: Load Greiner Quant plate into Quant Module for concentration readings (ng/ul units).

RESULTS

AVENIO EDGE WORKFLOW PERFORMANCE USING OPEN PARAMETERS

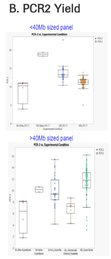
Workflows using different open parameters show equivalent performance across 8,16,24,48 reaction library preparations and Target Enrichment.

A

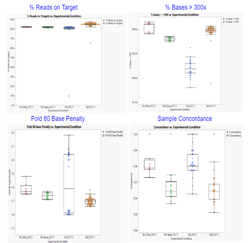
Table showing representative verification runs with various open parameters used

Run ID	Sample size	Sample type	Input mass (ng)	Adapter type	Panel	Reagent Lot#	Pooling strategy	Sequencer(s)
1	8	FFPET(HD789)	50	HyperPlex UMI	P5.7.1 (530 Kb- internal panel)	Lot#1	Pre-capture, 2-Plex	Nextseq (HO 300 cycle)
2	16	FFPET(HD789)	50	HyperPlex UMI	P5.7.1 (530 Kb- internal panel)	Lot#1	Pre-capture, 8-Plex	Nextseq (HO 300 cycle)
3	24	MP24/96 Whole Blood DNA	50	HyperPlex UMI	P5.7.1 (530 Kb- internal panel)	Lot#1	Post-capture	Nextseq (HO 300 cycle)
4	48	FFPET(HD789)	50	HyperPlex UMI	P5.7.1 (530 Kb- internal panel)	Lot#1	Post-capture, 1-Plex	Novaseq (S1 300 cycle)
5	8	Cell line: NA12878	100	HyperPlex	KAPA HyperExome (41 Mb)**	Lot#1	Pre-capture, 2-Plex	Novaseq (S1 300 cycle)
6	16	Cell line: NA12878	100	HyperPlex	KAPA HyperExome (41 Mb)**	Lot#1	Pre-capture, 8-Plex	Novaseq (S1 300 cycle)
7	24	Cell line: NA12878 (HyperPlus)	1000	HyperPlex	KAPA HyperExome (41 Mb)**	Lot#2	Post-capture, 1-Plex (volumetric TE input)	Novaseq (S1 300 cycle)
8	24	Mechanically sheared cell line: NA12878 (HyperPrep)	100	HyperPlex	KAPA HyperExome (41 Mb)**	Lot#2	Pre-capture, 4-Plex	Novaseq (S1 300 cycle)
9	48	Cell line: NA12878	100	HyperPlex	KAPA HyperExome (41 Mb)**	Lot#1	Post-capture, 1-Plex	Novaseq (S1 300 cycle)

B. PCR2 Yield



C. Sequencing Metrics using <40Mb sized panel



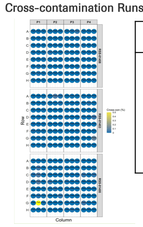
D. Sequencing Metrics using ≥40Mb sized panel

Figure 3: A) Nine representative verification runs are listed using various open parameters for the capture workflow. **B)** Demonstrates PCR2 library yields which will be pooled in equimolar concentrations for sequencing runs. **C)** and **D)** Shown are representative sequencing performance metrics based on panel size as the workflow is panel agnostic.

CROSS-CONTAMINATION / CARRY OVER CONTAMINATION AND REAGENT STABILITY STUDIES

Cross-contamination (intra-plate) and carry over (inter-run) contamination were evaluated during Verification.

Cross-contamination Runs



Carry-over Contamination Runs

Instrument	carry-over observed in...	Carry-over contamination from...	Carry-over Contamination Detected >0.1%
Repeated on three different AVENIO Edge Instruments	Plate 1	N/A	N/A
	Plate 2	Plate 1	None
	Plate 3	Plate 1	None
		Plate 2	None
	Plate 4	Plate 1	None
		Plate 2	None
		Plate 3	None

Figure 4: Carry-Over and Cross-contamination evaluation
Carry-over and Cross-contamination results for four subsequent runs on the same AVENIO Edge Instrument* are shown. Each reaction well contained a known input genomic DNA sequence and was barcoded using unique pre-kitted primers across 4 subsequent runs. Each set of 4 runs was replicated on 3 different instruments. Results indicate less than 0.1% cross contamination or carry-over contamination across runs and within each run.

Reagent Stability

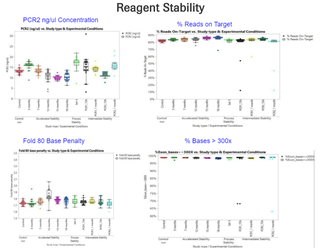
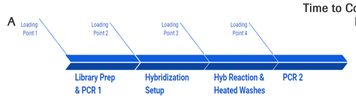


Figure 5 Stability studies performed on capture workflow reagents.
Reagents were stressed for i) shelf life dating (Accelerated stability studies up to 18 months) ii) manufacturing/process stability and iii) library on-deck stability. All metrics were compared to the control (Unstressed reagents). Results demonstrated reagents were stable for 18 months and could withstand manufacturing stress and libraries were stable up to 72 hr on-deck or for a month at -20^{0}C.

Time to Completion and Kit sizes



B

# of Samples	Average Run Time
24	~31 hours
48	~35 hours

C

Figure 6: Capture workflow during Feasibility Phase and Turn-around time. A) The workflow will be split into five Test Definition Files (TDFs) namely 1) DNA sample input 2) Library Prep (LP) and Target Enrichment (TE) 3) Normalization and Pooling 4) Only Library Prep and 5) Only Target Enrichment. **B)** Turn-around time was determined by the total time it took to begin and finish each TDF. Desired time to process up to 48 samples is ≤ 72 hours. **C)** There will be 8 and 24 reaction kit sizes and can load double of the reagents to increase the reactions that can be run per TDF.

AVENIO EDGE WORKFLOW REPRODUCIBILITY

Inter-instrument variation (five AVENIO Edge instruments) and intra-instrument (five runs on the same AVENIO Edge instrument*) verification data show comparable results.

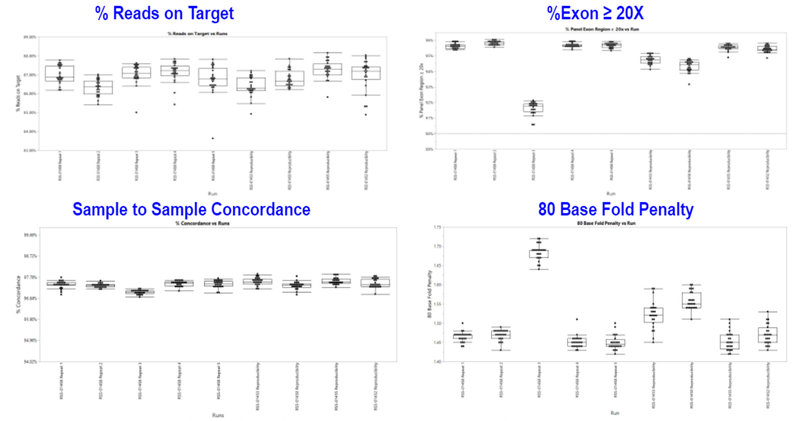


Figure 7: Using the AVENIO Edge instrument,* the end-to-end, 2 day workflow, using open parameter for >40Mb sized panel was run with DNA NA12878 and the KAPA Hyper Exome panel** (41Mb). This same run was executed five times using the same instrument (the first five column data)and this same run was repeated using four additional AVENIO Edge instruments (the last 4 column data). Data show comparable results for inter-instrument operability and intra-instrument operability.

AVENIO EDGE QUATIFICATION KIT

AVENIO Edge Quantification kit** performance results show robust, reproducible results

A **Accuracy ≥85% using Cross Talk data**

Run	%Accuracy= (Measured - Expected) / Expected			
	Min	Median	Mean	Max
Replicate run 1 (n=24)	0.3862%	3.6839%	4.1874%	11.3915%
Replicate run 2 (n=24)	0.006455%	1.8713%	3.8371%	10.6319%

B Crosstalk between Blank Wells

Run	Blank well readout with 100ng neighboring wells using a checkerboard pattern (ng)			
	Min	Median	Mean	Max
Replicate run 1 (n=24)	0.0001	0.001244	0.001087	0.002589
Replicate run 2 (n=24)	0.0001	0.0001	0.003211	0.06630

C %CV for across replicate runs

Replicate runs (100ng printed in 24 / 48 wells)	CV% <15%
Replicate run 1 (n=48)	3.9844%
Replicate run 2 (n=48)	4.4657%
Replicate run 3 (n=24)	4.0227%
Replicate run 4 (n=24)	4.3088%
	Reliability
Run level reliability	100%

Figure 8: AVENIO Quantification kit Performance. A)** Results demonstrate accuracy is >85% during verification across two replicate plates with 100ng in . **B)** 48 wells were interspersed with blank wells and wells with 100ng DNA in a checkerboard manner with minimal crosstalk observed across the blank wells. **C)** % CV was <15% across four plates, two plates were printed with 100ng / well in 48 wells and two replicates had 100ng/well for 24 wells.

SUMMARY AND CONCLUSIONS

Hybridization-based Capture Workflow Performance: Verification studies using the AVENIO Edge* runs (end-to-end runs, 2 complete days) were executed to show the performance of the capture workflow using a representative set of panels (<40Mb and >40Mb sized panels) and with different DNA sample input types and different concentration inputs (50ng-1ug). Pre-capture and post-capture performance data was shown to be comparable. 8,16,24 and 48 sample open parameter capture workflow runs also show equivalent performance. PCR1 data showed that yields exceeded the desired 20ng/uL concentrations. PCR2 data showed that yields exceeded the desired 1ng/uL concentrations which is adequate for multiple sequencing runs. Workflow Performance was also equivalent when a capture workflow using parameters for >40Mb sized panel (KAPA Hyper Exome**) was compared across five different AVENIO Edge instruments and run five times on the same AVENIO Edge instrument.* In addition, the processing time for 24 samples was 31 hours and 48 samples was 35 hours meeting the desired processing time of less than or equal to 72 hours for 48 samples.

Cross-contamination / Carry over contamination and Reagent Stability Studies: For cross-contamination and carry over evaluation, two studies were performed. Shown are results for study 1 where known synthetic DNA sequences were used as input and four consecutive runs were performed. During four consecutive runs, the sequences were barcoded with unique primers (run1: 1-24 primers; run 2:25-48 primers; run 3: 49-72 primers; run 4: 73- 96 primers). Cross contamination was assessed for each run and cross contamination was assessed after every run. <0.1% carry over and cross contamination was detected. Second study used the entire end-to-end capture workflow with male and female DNA samples and carry-over or cross contamination was assessed for the presence of the Y-chromosome (male samples) in the female DNA containing wells. Results of the second study are as shown:

	Test	Replicate	Subsample	Estimated crosscon/carryover			
				Min	Median	Mean	Max
Male+Female plate1	Crosscon	Rep1	100M	0.00%	0.01%	0.01%	0.06%
Male+Female plate2	Crosscon	Rep2	100M	0.00%	0.01%	0.01%	0.02%
All Female plate1	Carryover	Rep3	100M	0.00%	0.00%	0.00%	0.02%
All Female plate2	Carryover	Rep2	100M	0.00%	0.01%	0.00%	0.02%

Stability studies performed using the workflow reagents showed that the reagents were stable up to 18 months, could withstand manufacturing / process stress, and libraries generated were stable up to 72 hr on the AVENIO Edge instrument deck or for one month at -20^{0}C.

AVENIO Edge Quantification Module and Kit:** Verification results provide robust data for the fluorometric quantification kit using the integrated plate reader on the AVENIO Edge system.

*AVENIO Edge System is a Class 1 IVD instrument and is intended for downstream diagnostic applications.
**AVENIO Edge reagents, AVENIO Edge workflows, and KAPA reagents are for Research Use Only. They are not intended for diagnostic applications.

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