AVENIO Edge DNA Hybridization User-defined workflow using the KAPA HyperExome Probes

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Date of publication: April 2022

Introduction

Uptake and utilization of automation in the clinical laboratory is growing at a rate of over 30% per year. This is accelerating the development of new technologies and helps drive the rapid adoption of new NGS applications into labs.

Automated systems can provide a large variety of benefits for the sequencing laboratory, including:

- Minimal hands on time for higher staff productivity
- Better sample and workflow traceability
- Reduced user error and sample contamination
- Consistent and reproducible results
- Rapid deployment of new workflows

Demand for clinical next-generation sequencing (NGS) is expected to increase by 350% (~14% CAGR)[1] during the next decade, and hybrid capture-based methods remain the first choice for the majority of laboratories. Roche offers a variety of products to support both manual and automated hybrid capture workflows, including the KAPA HyperExome Probes, Roche's Whole Exome Sequencing solution.

The AVENIO Edge System is a fully automated system for NGS library preparation, target enrichment, quantification and sample pooling, all empowered by the award-winning KAPA technology. Experience high quality results from absolute automation, thanks to validated protocols, integrated components and end-to-end control.[2] The AVENIO Edge System is designed to optimize resources, from staffing and labor to reagents and consumables, breaking down many of the obstacles laboratories face today. Finally, the laboratory personnel have the freedom to walk away from repetitive tasks and spend time where it matters. The AVENIO Edge System simplifies the NGS sample prep workflows by providing:

- Intuitive system software for sample tracking and consumables inventory
- On-deck capabilities such as thermocycling and quantification
- Hassle-free, ready-to-use reagents
- Efficient, consistent, high-quality results

This Application Note demonstrates the high performance and reliability of KAPA HyperExome Probes on the automated AVENIO Edge System. Testing included 287 samples of cell line DNA, with equivalent results observed for small (8 sample), medium (16 to 24 sample) and large (48 sample) batch sizes.

AVENIO Edge System delivers highly reproducible results

- Sample concordance routinely exceeds 96.8%
- Consistent post-capture library fragment size of ~323bp

KAPA HyperExome Probes delivers high performance on AVENIO Edge System

- High specificity with >86% (see Figure 4) of reads on-target
- High uniformity with Fold-80 base penalty as low as 1.4

Materials and Methods

System and Software: The AVENIO Edge System is a liquid handling robot designed to perform input control, library preparation, target enrichment, pooling, and quantification all on-deck. The output are pools of libraries ready to be sequenced. The AVENIO Designer Software is used to generate user-defined workflow templates where the user has the flexibility to design their own workflows with open parameters and run up to 48 samples in a single run.[3]

Input DNA: Cell line genomic DNA, NA12878, was obtained from Coriell Biocell Repositories. This Genome in a Bottle high quality control extracted DNA stock was buffer exchanged prior to performing the study runs on the AVENIO Edge System. Samples were prepared from 100 ng input following the product Instructions for Use.

Reagents: The AVENIO Edge DNA Hybridization User-defined Workflow (UDW) consists of 9 reagent kit boxes. For more detailed information on the reagent kits, talk to a Roche sales representative.

- Box 1: AVENIO Edge Quantification Kit
- Box 2: AVENIO Edge DNA HyperPlus Kit
- Box 3: AVENIO Edge Target Enrichment Kit, A
- Box 4: AVENIO Edge Target Enrichment Kit, B
- Box 5: AVENIO Edge Primer Plate A
- Box 6: AVENIO Edge HyperPure Beads
- Box 7: AVENIO Edge HyperPlex Adapter Kit
- Box 8: AVENIO Edge UDI Primer Mixes, 1-96
- Box 9: AVENIO Edge Solution A

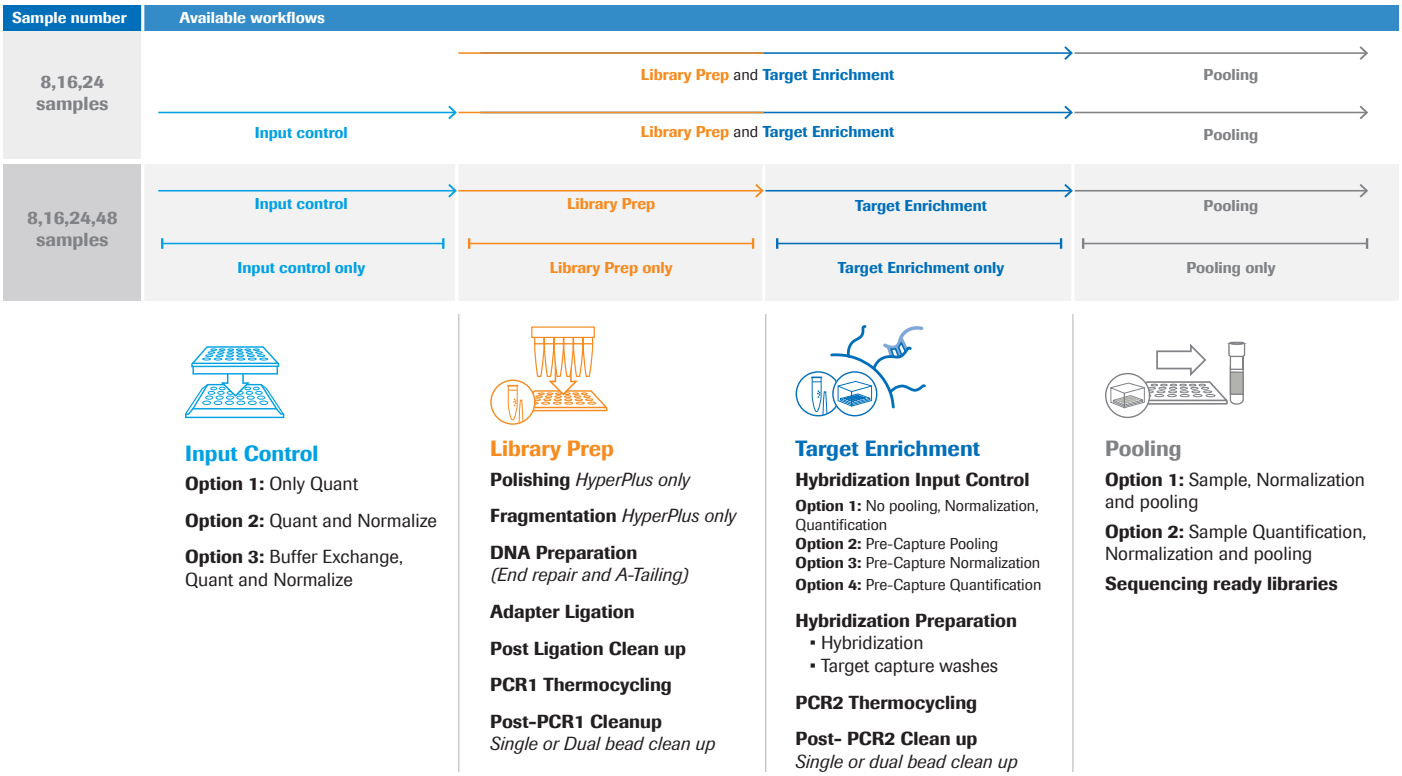
Four empty panel tubes are provided to the customer, providing the flexibility to perform capture with their desired panel(s) for up to four panels in parallel captures per run. Commercially available KAPA HyperExome Probes (43 Mb in capture target size) were used throughout this study.

Workflow: The AVENIO Edge DNA Hybridization UDW provides 6 main user-defined workflow templates: Input Control, LPTE 8/16S, LPTE 24S, LP 48S, TE 48S, and Post-Capture Pooling (see Figure 1). These templates were converted to run protocols using the AVENIO Designer Software and uploaded onto the AVENIO Edge System.[4] This study tested all 6 baseline workflow templates, which include quantification, pre-capture and post-capture pooling of the DNA libraries on the instrument. For more detailed information on these templates, including the recommended open parameter values used, please refer to the **Appendix**.

Sequencing: Pools generated on the AVENIO Edge System were normalized by the system to 4 nM final molarity. The pools were prepared for sequencing using the NovaSeq™ 6000 System Denature and Dilute Libraries Guide. Sequencing reagents were prepared as per the guide and all libraries were sequenced on the Illumina NovaSeq™ 6000 System.

Analysis: Illumina System Suite Version 2.2.0 was used to monitor and determine quality of the libraries being sequenced. After completion of the sequencing runs, data analysis was performed following the recommended pipeline in the *How to evaluate Roche Target Enrichment data for germline variant research* White Paper.[5]

Figure 1. Diagram of AVENIO Edge DNA Hybridization UDW templates



The AVENIO Edge System is a Class 1 IVD and Class A CE IVDR and is intended for downstream diagnostic applications.
Reagents and kits mentioned are for Research Use Only. They are not intended for diagnostic procedures.
KAPA products are for Research Use Only. Not for use in diagnostic procedures.

Results

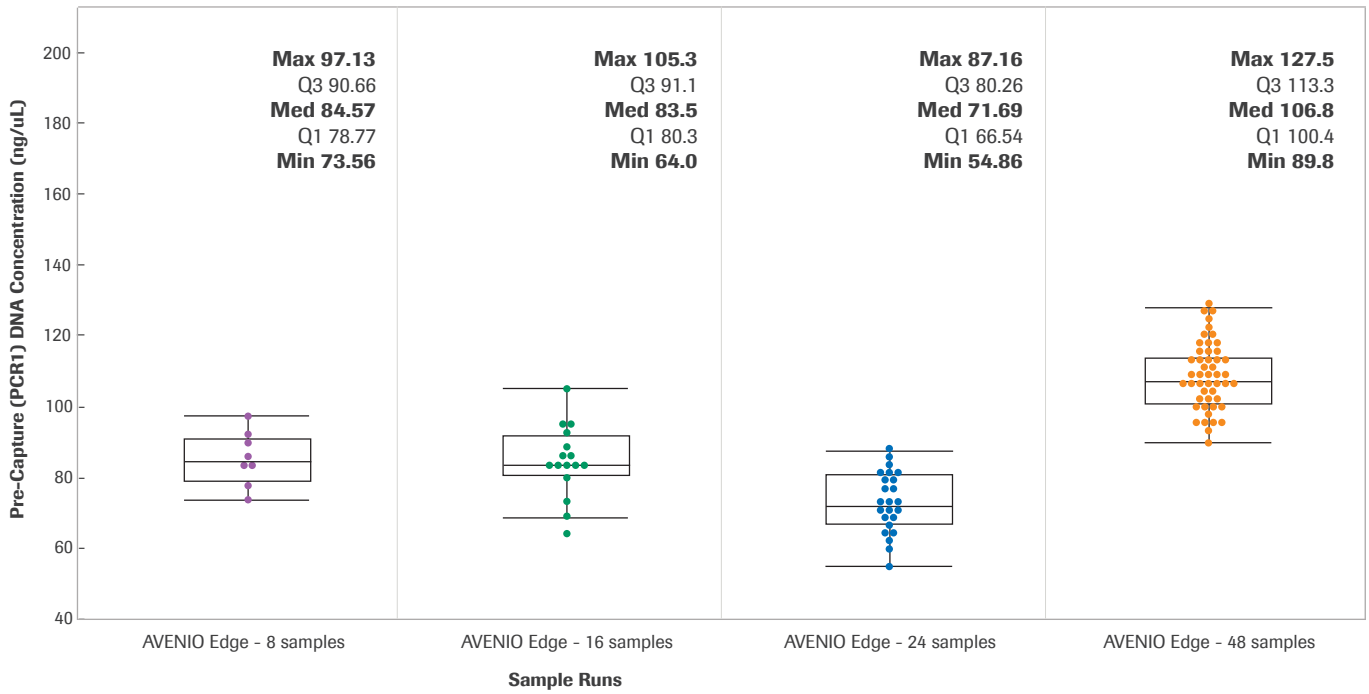
The study included 4 test workflows with varying batch sizes (8, 16, 24 or 48 samples) per run. Each of the workflows included AVENIO Edge User-defined templates for Input control, core Library Preparation and Target Enrichment (LPTE) template(s), and Post-capture pooling. Every workflow followed the recommended baseline conditions for ≥ 40 Mb panels (capture target; see **Appendix**). Different strategies for pre-capture and post-capture pooling were tested, as outlined in the table below.

Run size	Core LPTE template(s) used	Pre-capture pooling strategy	Post-capture pooling strategy
8 samples	LPTE 8/16S	2-plex on-deck with 1500 ng target mass, resulting in 4 pre-capture pools	All pools combined on-deck into 1 output tube for sequencing
16 samples	LPTE 8/16S	8-plex on-deck with *3000 ng target mass, resulting in 2 pre-capture pools	All pools combined on-deck into 1 output tube for sequencing
24 samples	LPTE 24S	Singleplex	All samples combined on-deck into 1 output tube for sequencing
48 samples	LP 48S and TE 48S	Singleplex	Combined on-deck into 2 pools of 24 samples each; 2 pools combined manually into 1 output tube for sequencing

**A 3000 ng target mass has been internally validated at Roche for 8-plex pre-capture pooling strategies on the AVENIO Edge System*

Sample Quality Metrics

Figure 2. Pre-Capture (PCR1) DNA Concentration (ng/uL)



8 sample run: DNA concentrations had an average of 85.02 ng/µL.

16 sample run: DNA concentrations had an average of 84.11 ng/µL.

24 sample run: DNA concentrations had an average of 72.4 ng/µL.

48 sample run: DNA concentrations had an average of 107.68 ng/µL.

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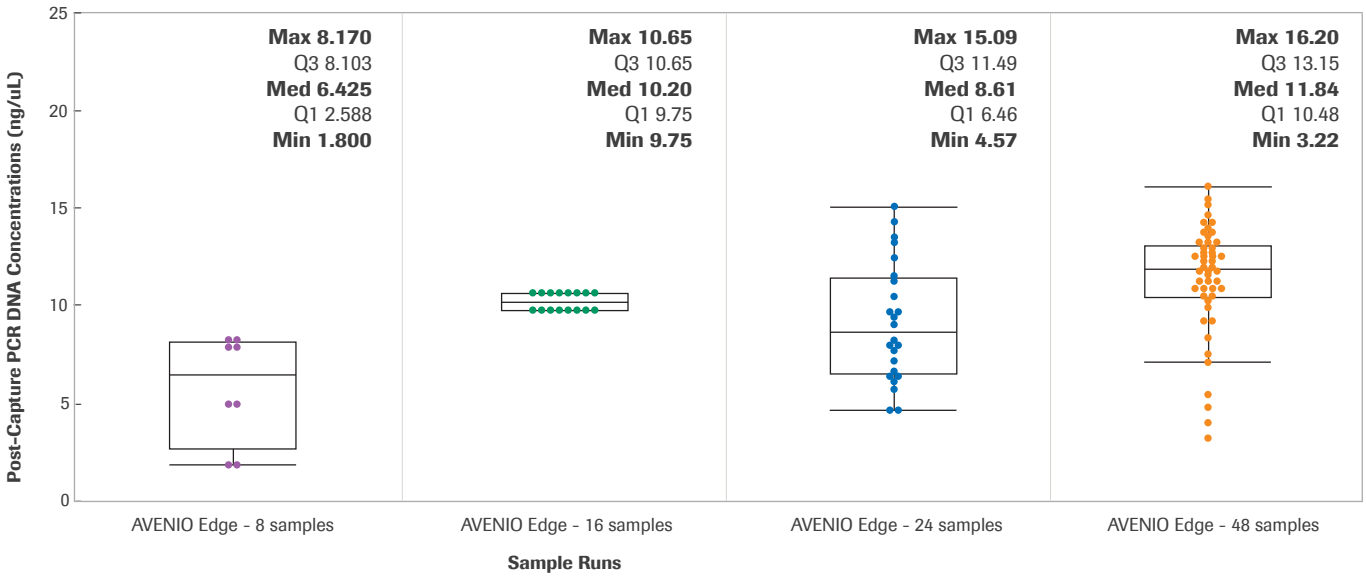
Sample Quality Metrics (continued)

Figure 3. Post-Capture PCR DNA Concentrations (ng/uL)



8 sample run: 8 samples were 2-plexed and DNA concentrations averaged 5.71 ng/µL.

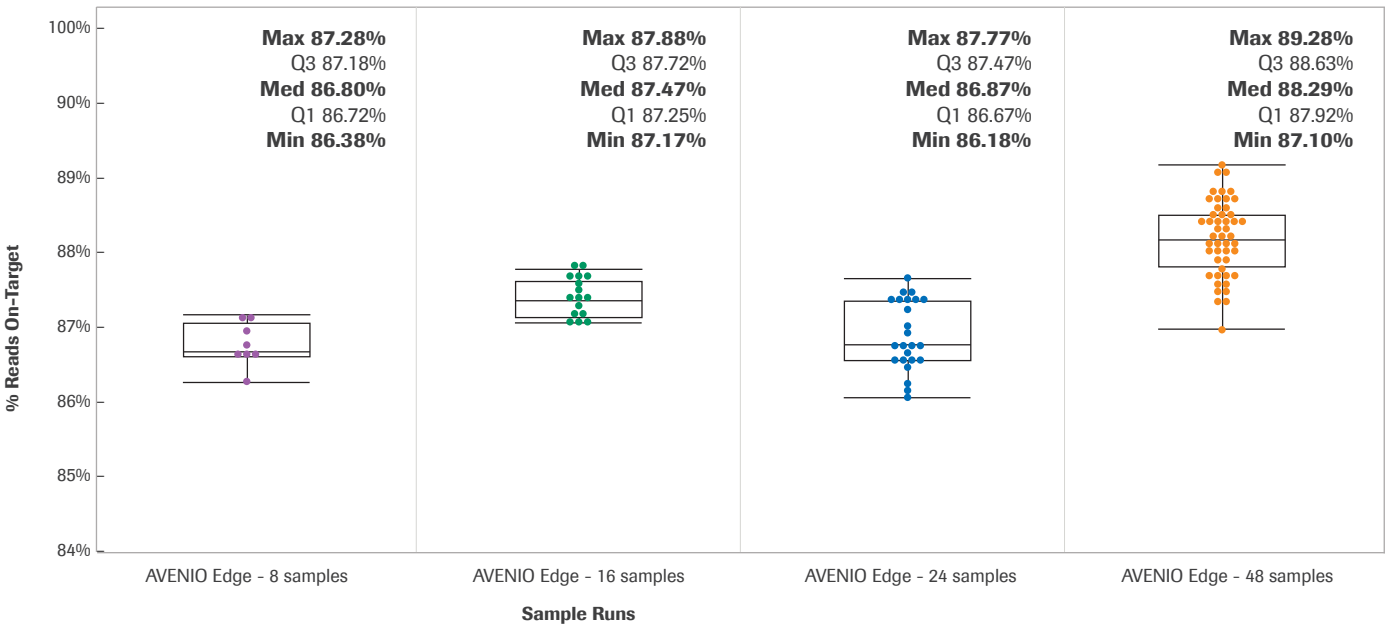
16 sample run: 16 samples were 8-plexed and DNA concentrations averaged 10.20 ng/µL.

24 sample run: 24 samples (singleplex) DNA concentrations averaged 9.11 ng/µL.

48 sample run: 48 samples (singleplex) DNA concentrations averaged 11.35 ng/µL.

Sequencing Metrics

Figure 4. % Reads On-Target



8 sample run: On-target reads had an average of 86.87%.

16 sample run: On-target reads had an average of 87.50%.

24 sample run: On-target reads had an average of 87.00%.

48 sample run: On-target reads had an average of 88.28%.

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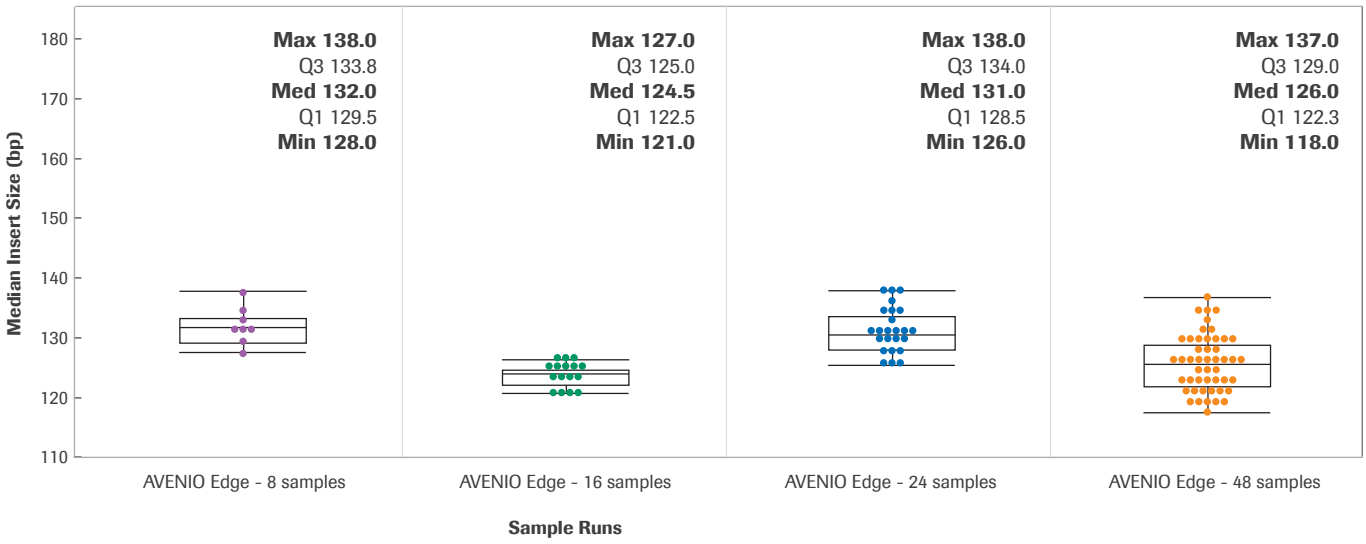
Sequencing Metrics (continued)

Figure 5. Median Insert Size (bp)



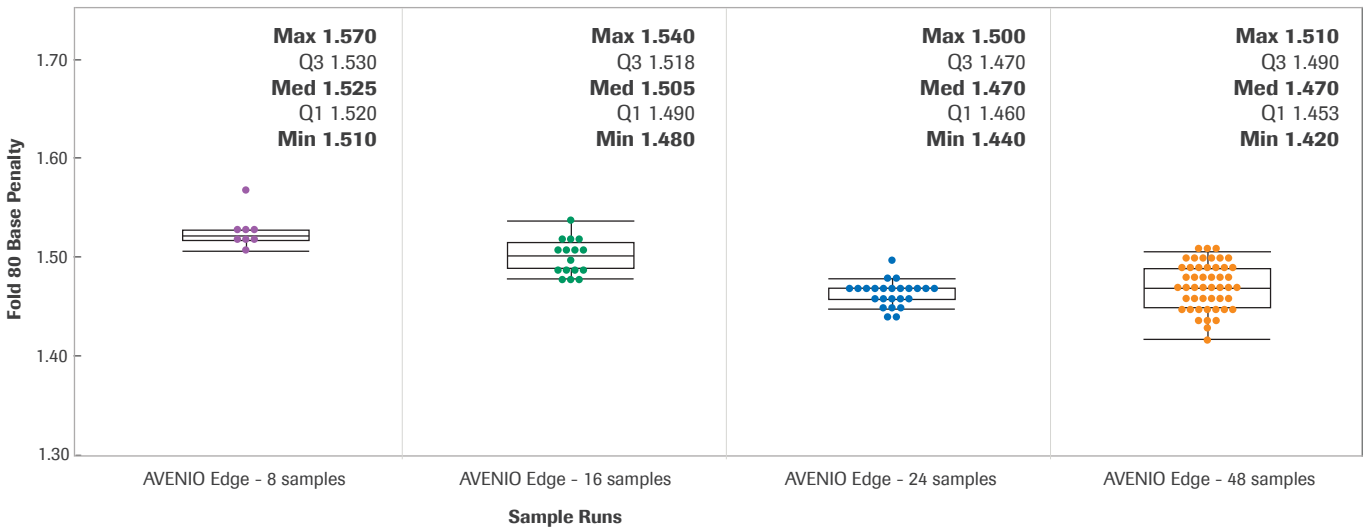
8 sample run: Median insert size had an average of 132 bp.

16 sample run: Median insert size had an average of 124 bp.

24 sample run: Median insert size had an average of 132 bp.

48 sample run: Median insert size had an average of 126 bp.

Figure 6. Fold 80 Base Penalty



8 sample run: Fold 80 base penalty ranged from 1.51 - 1.57 with an average of 1.53.

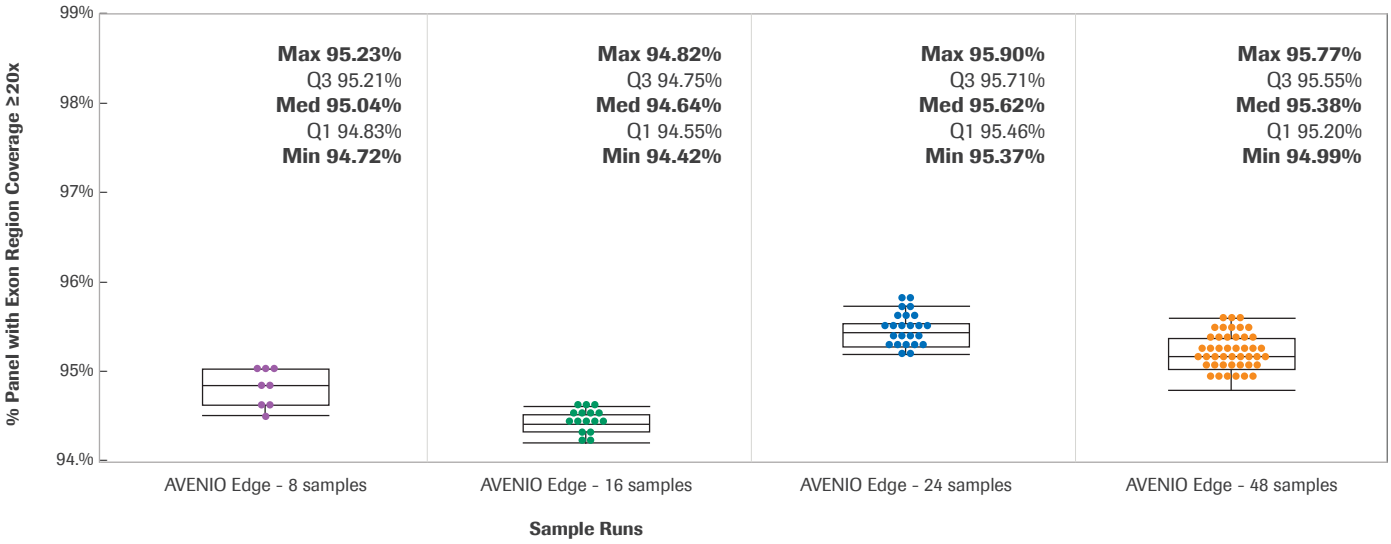
16 sample run: Fold 80 base penalty ranged from 1.48 - 1.54 with an average of 1.50.

24 sample run: Fold 80 base penalty ranged from 1.44 - 1.5 with an average of 1.47.

48 sample run: Fold 80 base penalty ranged from 1.42 - 1.51 with an average of 1.47.

Sequencing Metrics (continued)

Figure 7. % Panel with Exon Region Coverage ≥20x



8 sample run: Panel exon region coverage ≥20x ranged from 94.72 - 95.23% with an average of 95.01%.

16 sample run: Panel exon region coverage ≥20x ranged from 94.42 - 94.82% with an average of 94.65%.

24 sample run: Panel exon region coverage ≥20x ranged from 95.37 - 95.90% with an average of 95.61%.

48 sample run: Panel exon region coverage ≥20x ranged from 94.99 - 95.77% with an average of 95.37%.

Discussion

The aim of these studies is to demonstrate performance of the AVENIO Edge User-defined Workflow using the KAPA HyperExome Probes on the AVENIO Edge System for 8, 16, 24, and 48 sample runs. AVENIO Edge specific reagents were used, which includes quantification, library preparation, and target enrichment kits. Multiple user templates have been validated for the system to cover the various batch size needs for different labs (see appendix section for LP/TE template versions). The generated libraries were compatible with, and ran on, the Illumina NovaSeq™ 6000 System. The recommended parameters for each template are provided in the **Appendix**.

We used the KAPA HyperExome Probes with the AVENIO Edge System and generated high performing target enriched libraries from 100 ng of a coriell cell-line gDNA (NA12878). As shown in Figures 2-7 the AVENIO Edge System with the KAPA HyperExome Probes enable high-quality sequencing data with high specificity (% reads on-target) and high uniformity (Fold-80 base penalty), regardless of the run, pooling scheme (pre or post capture) or batch size used.

- **High specificity with % reads on-target greater than 86%**
- **High uniformity with Fold-80 base penalty as low as 1.4**
- **Concordant high performance across various run templates**
- **Consistent post-capture library fragment size of ~323 bp***

Conclusions

The AVENIO Edge System is an intelligently designed, all-in-one, pre-analytical platform that minimizes manual touchpoints, includes ready-to-load reagents, validated protocols, on-board capabilities, and intuitive software to manage your NGS sample workflows as efficiently as possible.

The AVENIO Edge System delivered high performance and high quality results while in addition offers:

- **Full walk-away solution that boosts personnel's productivity**
- **Intuitive system software for sample tracking and consumables inventory**
- **On-deck thermocycling and quantification capabilities**
- **Hassle-free, ready-to-use reagents**
- **Efficient, consistent, high-quality results**

When the AVENIO Edge System is used with the high performing KAPA HyperExome Probes it prepares high quality target enriched NGS libraries for efficient, reproducible and uniform whole exome sequencing.

** Data on file.*

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Appendix

The following protocol templates were used during this study:

1. Input control: This template provides input sample preparation for extracted DNA samples ("Input control") prior to starting a library preparation workflow. This template contains multiple options for sample quantification and normalization, along with direct sample transfer and a buffer exchange with bead cleanup option for samples with EDTA or other inhibitory substances.

- Run size: 1 to 96 samples (for Quantification options: maximum of 94 samples plus 2 standards)
- Estimated run time: 0.5 to 2 hours
- Minimum sample input volume: 50 µL
- Final sample output volume: 32.5 – 41.5 µL

Option	Process	Recommended Applications	User Requirements
Quantification with No Dilution	Sample Quantification	For customers who only want to determine sample concentrations without normalization. This option is recommended for samples with expected concentrations from 0.2 to 50 ng/µL.	The maximum number of samples that can be quantified is 94, as standards need to be present. 5 µL of each sample will be used for quantification.

2. Library Preparation and Target Enrichment (LPTE): These templates are designed to prepare DNA sample libraries for sequencing using a seamless library preparation and target enrichment workflow. The templates provide options for pre-capture quantification and sample pooling.

Full or Standalone LPTE Templates

LPTE 8/16S (Run size 1 to 16 samples)
- Minimum input volume: 32.5 µL
- Final sample output volume: 50 µL
- Estimated run time: 30 to 36 hours

LPTE 24S (Run size 1 to 24 samples)
- Minimum input volume: 32.5 µL
- Final sample output volume: 50 µL
- Estimated run time: 30 to 36 hours

LP 48S (Library Prep Only Run size 1 to 48 samples)
- Minimum input volume 32.5 µL
- Final sample output volume: 40 µL
- Estimated run time: 8 to 10 hours

TE 48S (Target Enrichment Only Run size 1 to 48 samples)
- Minimum input volume 30 to 40 µL
- Final sample output volume: 50 µL
- Estimated run time: 22 to 30 hours

Library Preparation step	Parameters values used for this study
Polishing, fragmentation, DNA preparation	▪ Polishing temperature and time: 37°, 1800 seconds ▪ Fragmentation temperature and time: 37° C, 1800 seconds ▪ End-repair and A-tailing temperature and time: 65° C, 1800 seconds
Adapter ligation and post ligation cleanup	▪ Adapter volume: 10 µL ▪ Ligation temperature and time: 20° C, 3600 seconds ▪ Cleanup beads volume: 55 µL (1X)
PCR1 thermocycling	▪ Initial denaturation temperature and time: 98° C, 45 seconds ▪ Denaturation temperature and time: 98° C, 15 seconds ▪ Annealing temperature and time: 60° C, 30 seconds ▪ Extension temperature and time: 72° C, 30 seconds ▪ PCR1 Cycles: 7 cycles ▪ Final extension temperature and time: 72° C, 60 seconds ▪ Final hold temperature and time: 4° C, 30 seconds ▪ Estimated completion time: 1200 seconds
Post-PCR1 cleanup	Cleanup type selection: Dual beads cleanup ▪ 1st cleanup beads volume: 50 µL ▪ 2nd cleanup beads volume: 50 µL

Appendix (continued)

Target Enrichment step	Parameter values used for this study
Hybridization input control	▪ For 24 sample and 48 sample run testing: No pooling, normalization, quantification: Fixed volume of 30 µL for each sample into Hybridization ▪ For 8 sample and 16 sample run testing: Pre-capture pooling: ▪ Number of samples per pool: Combine between 2 and 8 samples per pool ▪ Target mass into Hybridization: 1500 ng for up to 4 samples per pool; 3000 ng for 8 samples per pool ▪ Volume into Hybridization: 30 µL per pool
Hybridization preparation	▪ Hybridization bead cleanup: 2X ▪ Component H concentration for hybridization Mix: 15% ▪ Cleanup beads volume: 100µL (2X) ▪ Panel Volume: 5µL
Hybridization and target washes	▪ Initial Temperature and Hold Time: 95° C, 600 seconds ▪ Hybridization Temperature and Time: 55° C, 57,600 seconds (16 hrs) ▪ SA Bead volume: 75 µL ▪ Heater Shaker Temperature: 58° C
PCR2 Thermocycling	▪ Initial denaturation temperature and time: 98° C, 45 seconds ▪ Denaturation temperature and time: 98° C, 15 seconds ▪ Annealing temperature and time: 60° C, 30 seconds ▪ Extension temperature and time: 72° C, 30 seconds ▪ PCR1 Cycles: 7 cycles ▪ Final extension temperature and time: 72° C, 60 seconds ▪ Final hold temperature and time: 4° C, 30 seconds ▪ Estimated completion time: 1200 seconds
Post-PCR2 cleanup	▪ Cleanup type selection: single beads cleanup ▪ Cleanup beads Volume: 50 µL (1X)

3. Post-capture pooling: This template is designed to quantify and prepare 4 nM pool groups of final DNA sample libraries prior to sequencing. This template provides options for using external QC metrics (including concentration and average peak size) when performing pooling.

- Run size: 1 to 48 samples
- Estimated run time: 0.5 To 1 hour
- Sample input volume: 50 µL
- Final output: up to 4 pooling group tubes containing 35 µL of each sample input
- Target pool concentration is fixed at 4 nM
- Maximum of 24 samples per pooling group tube

For this study, all runs were performed using the Pooling Group option for Sample Quantification, Normalization and Pooling. The preprogrammed default value was used for Average Library Peak Size (bp). Samples were pooled into 1 or 2 sample pooling tubes for sequencing.

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References

1. *Internal market research data*
2. *AVENIO Edge System User Assistance v1.0 or later*
3. *AVENIO Designer Software User Assistance, v1.0 or later*
4. *AVENIO Edge User-Defined Workflow - DNA Hybridization Workflow Instructions for Use, v1.0 or later*
5. *How to evaluate Roche target enrichment data for germline variant research - Roche White Paper (MC--08067)*

Published by:

Roche Sequencing Solutions, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588

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MC--09114 05/2022