TECH NOTE

Twist Exome 2.0 Sequencing Using the Element Adept™ Workflow on the AVITI™ System

INTRODUCTION

Next-generation sequencing (NGS) enables cost-effective high-throughput DNA sequencing and is increasingly common in research and clinical settings. NGS technologies have advanced knowledge of the human genome and thus greatly impact the medical field. With researchers and clinicians now having a more comprehensive understanding of the genome, the genetic drivers behind many diseases are better understood. This has led to the development of various new techniques and technologies in diagnostics, prognostics, and personalized treatments.

Developed by Element Biosciences, the Element AVITI System offers high-quality sequencing with a flexible platform suitable for key NGS applications such as population genetics and disease research (**Figure 1**). The platform offers dual independent flow cells for flexibility in run configuration and throughput. The AVITI system also provides exceptional sequencing quality featuring %Q30 accuracy >90 and negligible duplication rates. With 150-cycle and 300-cycle kits, the AVITI System can sequence up to 24 and 32 Exome 2.0 samples on a single flow cell, respectively, at a fraction of the published cost per sequencing run on alternative platforms.[1]

Twist Bioscience generates high-performing probe panels for NGS by leveraging a proprietary semiconductor-based synthetic DNA manufacturing process featuring a high-throughput silicon platform. The Twist Exome 2.0 panel is one of Twist Bioscience's many offerings that provides efficient sequencing through its high uniformity and low off-target rate. The panel is designed to detect rare and inherited diseases, including germline cancers by covering clinically relevant regions from the ClinVar database and updated coverage of protein-coding regions from CCDS, RefSeq, and GENCODE databases. This panel is compatible with Twist Bioscience's enzymatic fragmentation and flexible hybridization solutions. The Twist Standard Hybridization Reagent Kit v2 is a standard high-performing option, while the Twist Fast Hybridization and Wash Kit can hybridize samples in as short as 15 minutes.

This technical note will highlight the compatibility and performance of the Twist Exome 2.0 panel, using both the Twist Standard Hybridization v2 and Twist Fast Hybridization workflows, when sequenced on the Element AVITI system.

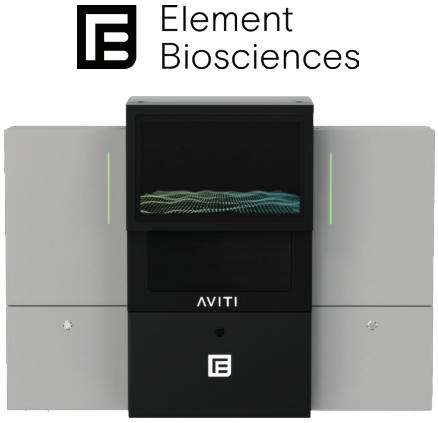

Figure 1. Element AVITI Sequencing Platform

WORKFLOW

DNA libraries were generated using the Twist Library Preparation EF Kit 2.0 with Universal Adapters, which involves enzymatic gDNA fragmentation, end repair, dA-tailing, adapter ligation, bead cleanups, and library amplification. After library preparation, an 8-plex capture using the Twist Exome 2.0 panel (panel size: 36.5 Mb) was performed with Twist Standard Hybridization v2 and Twist Fast Hybridization workflows.

Both 8-plex captures were pooled equally to yield a 30 µl pool with a 17 nM concentration. Library pools were made compatible with AVITI sequencing using the Adept Library Compatibility Kit v1.1. Libraries were loaded at 10 pM onto an AVITI 2x150 Sequencing Kit with a 5% PhiX spike-in. Sequencing was performed to generate 2x150 paired end reads. Analysis was down sampled to 150x coverage.

RESULTS

Sixteen samples were sequenced on a flow cell, resulting in a total yield of 336.7 Gb. On average, 93% of bases covered had a Q score of 30 or higher (base call accuracy at Q30 is 99.9%) and 70.2% had a Q score of 40 or higher (base call accuracy at Q40 is 99.99%).

TARGET COVERAGE

The metrics Mean Target Coverage and Percent of Target Bases at ≥30X Coverage are key metrics for assessing the effectiveness of Exome sequencing. Mean Target Coverage for Fast Hybridization and Standard Hybridization v2 was 51.3 and 48.5, respectively (**Figure 2**). For Fast Hybridization workflows and Standard Hybridization v2, the values of the Percent of Target Bases at ≥30X Coverage metric were 86% and 88%, respectively (**Figure 3**).

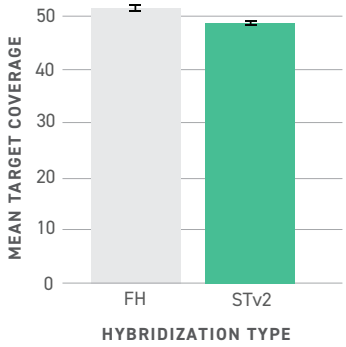

Figure 2. Mean Target Coverage

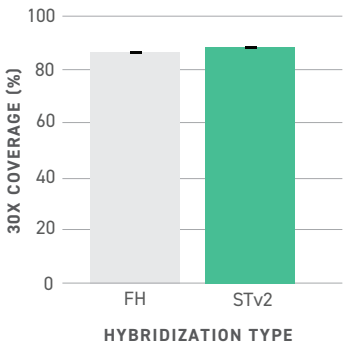

Figure 3. Percent of Target Bases at ≥30X Coverage

UNIFORMITY AND ON-TARGET RATE

The metrics On-Target Rate and Fold-80 Base Penalty help describe sequencing efficiency. On-Target Rate refers to the percentage of total reads that map to the target region. Fold-80 Base Penalty is a uniformity metric that shows how much additional sequencing is necessary to have 80% of target bases with non-zero coverage achieve the mean coverage. For an ideal target enrichment result, the On-Target Rate should be maximized and Fold-80 Base Penalty should be minimized.

For both hybridization workflows, the average On-Target Rates were high with both showing over 90% (**Figure 4**). Average Fold-80 Base Penalty was ~1.5 for Fast Hybridization, while it was ~1.4 for Standard Hybridization v2 (**Figure 5**). Both these Fold-80 Base Penalty values are within expectation for efficient exome sequencing.

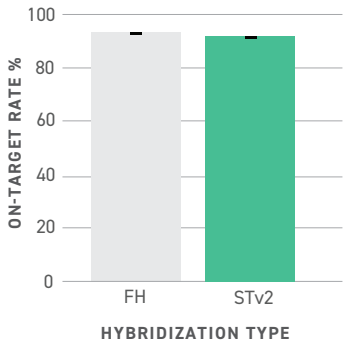

Figure 4. On-Target Rate

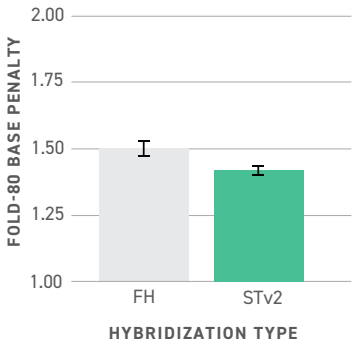

Figure 5. Fold-80 Base Penalty

FH, Fast Hybridization; STv2, Standard Hybridization v2.

COMPLEXITY

Library Size and Duplication Rate describe the complexity of a given library. Libraries need a sufficiently high level of complexity to ensure there are enough unique reads for optimal sequencing data processing. Library size refers to the number of unique DNA fragments in the library and duplication rate describes the proportion of bases found in duplicate reads. The library size for samples enriched with Fast Hybridization and Standard Hybridization v2 were 695M and 725M, respectively (**Figure 6**). The duplication rate was ~1.1% for both hybridization workflows (**Figure 7**). These values all show that the libraries have sufficient complexity for downstream analysis and a low duplication rate.

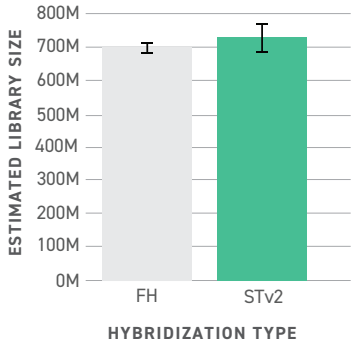

Figure 6. Library Size

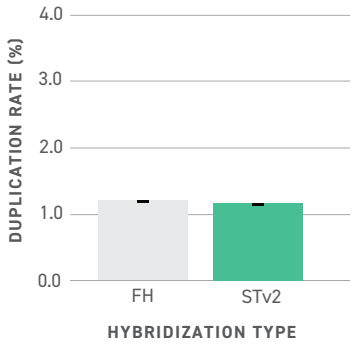

Figure 7. Duplication Rate

CONCLUSION

Twist Bioscience provides various NGS solutions that are compatible with the Element AVITI sequencing platform. The data detailed in this technical note show that the sequencing performance of the Twist Exome 2.0 panel using Twist Library Preparation EF Kit 2.0 with Standard Hybridization v2 or Fast Hybridization workflows demonstrates both high performance and compatibility with the AVITI sequencing platform.

REFERENCES

1. Ashby, M. Whole Exome Sequencing 101: Cost-effective DNA sequencing to understand genetic disease. Element Biosciences. https://www.elementbiosciences.com/blog/whole-exome-sequencing-101-cost-effective-dna-sequencing-to-understand-genetic-disease (2022).

FH, Fast Hybridization; STv2, Standard Hybridization v2.

ORDERING INFORMATION

TWIST PRODUCTS

SKU	PRODUCT	SAMPLES SUPPORTED
104445	Twist Standard Hyb and Wash Kit v2, 2 Reactions	12
104446	Twist Standard Hyb and Wash Kit v2, 12 Reactions	96
101278	Twist Fast Hybridization and Wash Kit, 2 Reactions	12
101174	Twist Fast Hybridization and Wash Kit, 12 Reactions	96
104132	Twist Exome 2.0, 2 Reactions, Kit	16
104134	Twist Exome 2.0, 12 Reactions, Kit	96
100856	Twist Universal Blockers, 2 Reactions	16
100578	Twist Universal Blockers, 12 Reactions	96

ELEMENT PRODUCTS

CATALOG NUMBER	PRODUCT DESCRIPTION
880-00001	Element AVITI System
860-00003	AVITI 2x150 Sequencing Kit Cloudbreak High Output
830-00007	Adept Library Compatibility Kit v1.1*
830-00004	PhiX Control Library, Adept**
820-00009	Adept Custom Primer Set Cloudbreak

**24 reactions with qPCR controls for quantitation*

***Spike-in for Adept Library Compatibility Workflow*

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SALES@TWISTBIOSCIENCE.COM