Expanding Sequencing Options for Arima-HiC+

High Performance Using the Element AVITI™ Sequencing Platform

Introduction

As next-generation sequencing platforms continue to emerge, it's paramount to ensure that assays and chemistries can adapt to harness the advantages of these evolving technologies. This tech note evaluates the synergy between the Element AVITI™ sequencing platform (released in March 2022) and the Arima-HiC+ FFPE kit, focusing on sequence quality, Hi-C heatmaps, and structural variant identification.

High-throughput chromatin conformation capture (Hi-C) provides insights into the 3D structure of the genome and enables the detection of structural variations (SVs). The Arima-HiC+ FFPE kit offers a streamlined approach to genome-wide Hi-C with formalin-fixed paraffin-embedded (FFPE) samples.

The integration of the Element AVITI™ benchtop sequencer into Arima-HiC+ workflows yields high-quality Hi-C performance and SV detection equivalent to the Illumina platform.

Methods

Sample Preparation

FFPE samples were sourced from Discovery Life Sciences and AMSBio. Samples described in this tech note are F10, a FFPE tumor tissue sample, and K562, a cell line embedded in FFPE.

Library Preparation

Arima-HiC+ samples were prepared using the Arima-HiC+ FFPE kit (P/N A101060). Sequencing libraries were prepared according to the Arima Library Prep kit protocol (P/N A303011). Libraries were converted into Element AVITI™-compatible libraries using the Element Adept™ Library Compatibility (P/N 830-0007) kit following manufacturer's protocols.

Next-Generation Sequencing

2 x 150 paired-end sequencing was performed using both the Illumina NextSeq™ 1000 sequencer (the sequencer we routinely use) and the Element AVITI™ sequencer following manufacturer protocols. For structural variant detection applications, we recommend sequencing a minimum of 100M reads.

Analysis

Raw sequence quality was assessed using FastQC. Sequences were sub-sampled to 100M reads for head-to-head comparisons, and analyzed using the Arima SV Pipeline for Mapping, SV Detection, and QC. This analysis incorporates Juicer to generate heatmaps and Hi-C Breakfinder for SV calling. For visualization, heatmaps were standardized to allow for direct comparison. Note: The Arima SV pipeline is now available on the cloud-based Arima Bioinformatics Platform.

Results

(A) Quality Assessment

We used the FastQC tool to assess the quality of the raw sequencing data. The quality score of reads from the AVITI™ sequencer indicated over a 99.9% base call accuracy (data not shown). We found that quality metrics (ex: percent single-end reads, percent unique valid pairs, percent intragenic pairs, signal to noise ratio for translocation calling) were similar between both sequencers, with the exception of percent duplicated pairs and the number of SVs identified (discussed below).

(B) Visual Inspection of HiC Performance Using Heatmaps

We visually inspected Hi-C interactions using Hi-C heatmaps of entire chromosomes. As shown in the example below, we found that the heatmaps generated from both sequencers showed similar patterns of inter-chromosomal and intra-chromosomal interactions (Figure 1). The heatmaps generated from the AVITI™ sequencer reads had a slightly higher signal intensity than that from the NextSeq™ possibly due to a slightly higher number of unique valid pairs from the AVITI™ sequencer reads.

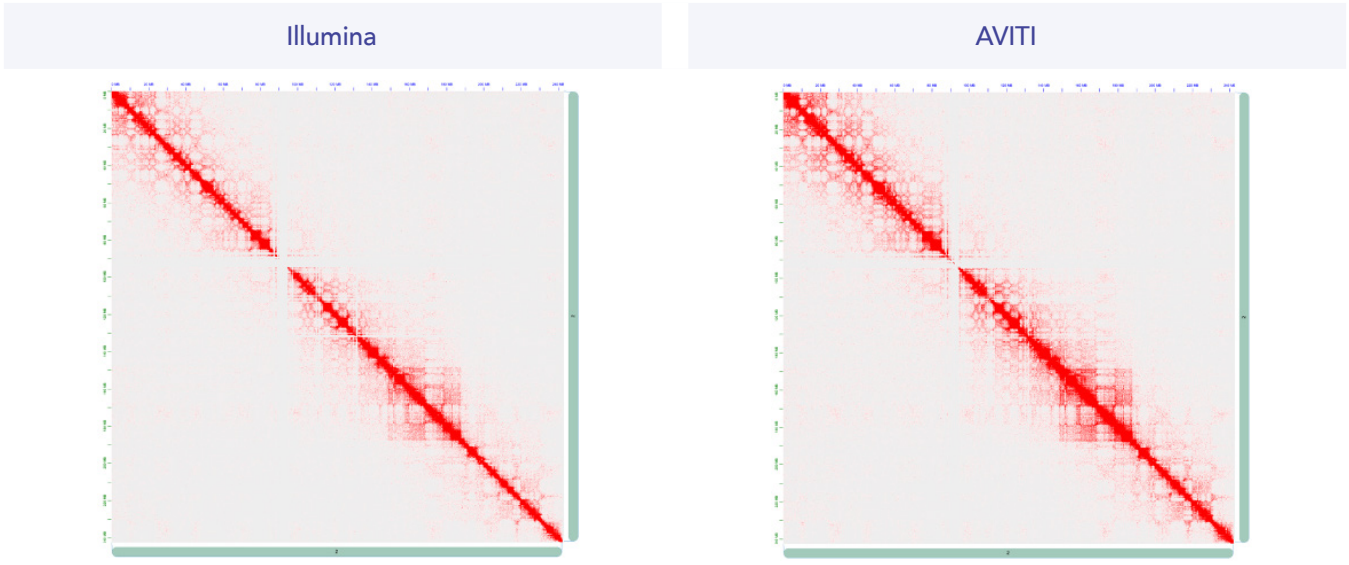

Figure 1: Hi-C interactions within chromosome 2 from sample K562 sequenced with the NextSeq™ (left) and the AVITI™ (right).

(C) SV Calling

Across the samples we examined, HiC Breakfinder identified similar SVs between reads from the AVITI™ and the NextSeq™ sequencers. In the example below, we identified the same inter-chromosomal SVs at the chromosome 9:chromosome 22 boundary from both sequencers (Figure 2).

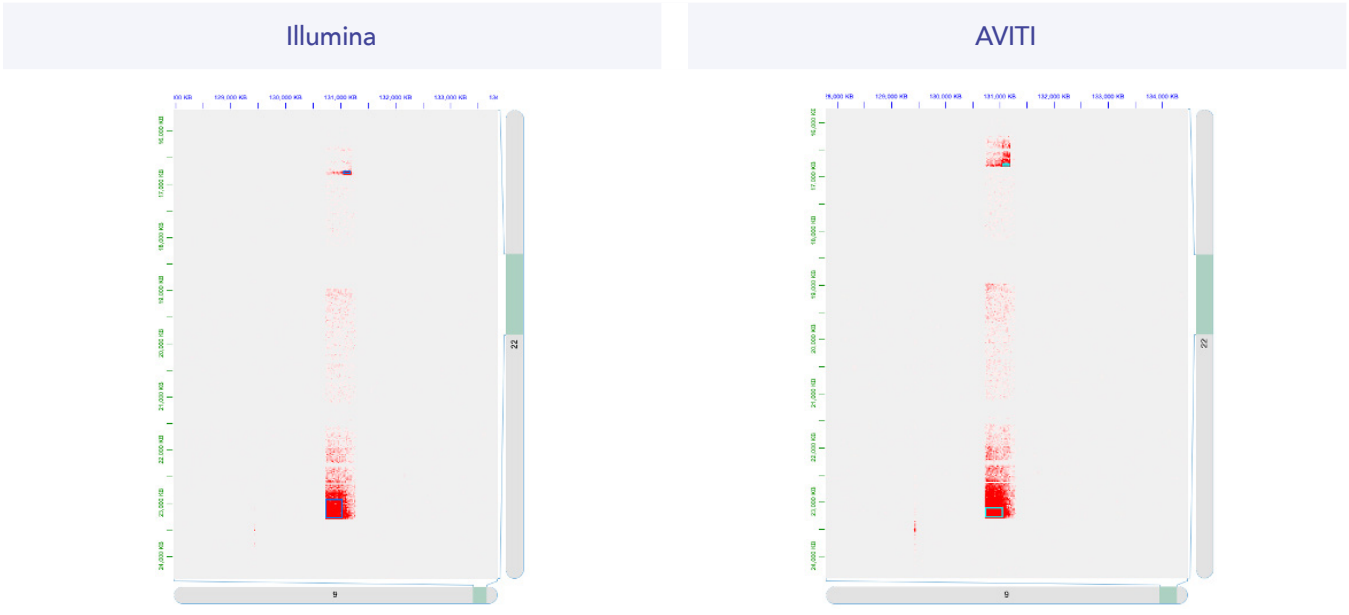

Figure 2: Hi-C heatmap of an inter-chromosomal interaction between chromosome 9 and chromosome 22. SVs are noted by the blue boxes on each heatmap.

However, there were some cases where SV identified from the AVITI™ reads were not found from the NextSeq™ reads. Among 7 samples we examined in total, there were an average of 29 SVs per sample identified from the AVITI™ reads while there was an average of 21 SVs per sample from NextSeq™ reads. For example, we identified an SV based on AVITI™ reads within chromosome 17 that was not called based on the NextSeq™ data (Figure 3). This increase in SV calling could be due to the slight increase in number of unique valid pairs and the slight decrease in percent duplicate pairs from the AVITI™ sequencer vs. NextSeq™.

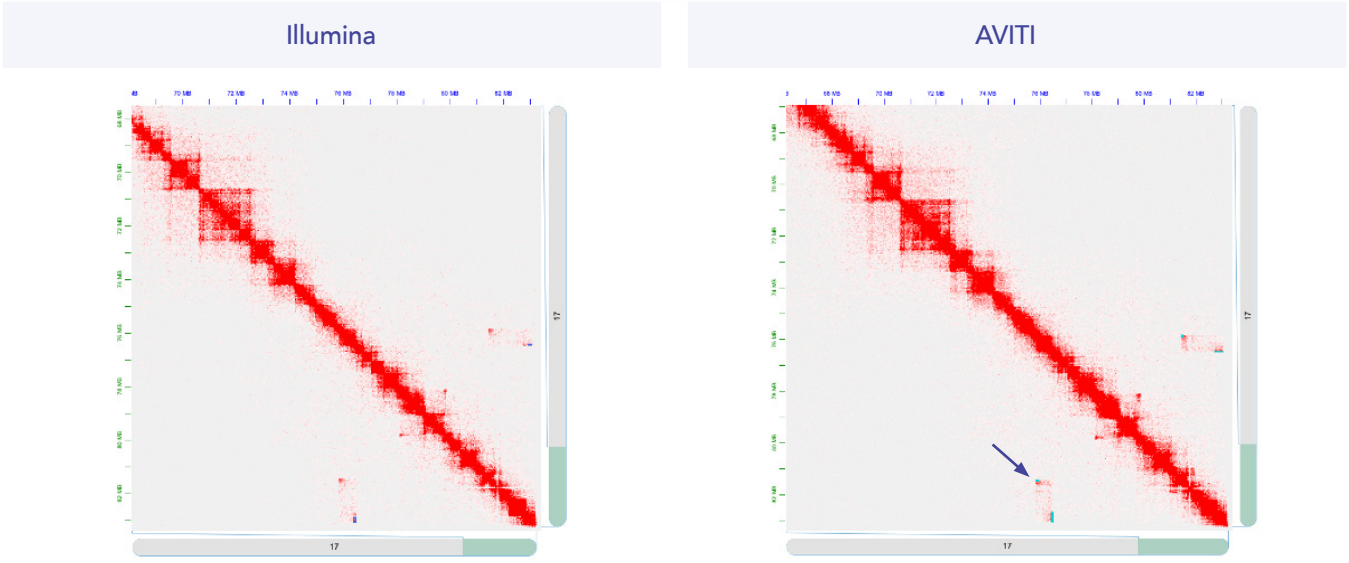

Figure 3: Hi-C heatmaps of intra-chromosomal interactions in chromosome 17 based on NextSeq™ reads (left) and AVITI™ reads (right). SVs are noted by the blue boxes on each heatmap.

Conclusions

We found that the AVITI™ sequencer can be used successfully with the Arima-HiC+ chemistry to detect structural variants in the genome. While we did not specifically test the detection of chromatin structures and interactions, the same overall procedures can be applied to detect chromatin loops, topologically associating domains, and compartments. The ability of our Hi-C kit to perform on multiple sequencing platforms opens doors for researchers to use a wider array of sequencers with confidence.

References

1. User Guide: Library Preparation for the Arima HiC+ for Genome Wide HiC Kit. Arima Genomics.

2. Arima SV Bioinformatics Pipeline. Arima Genomics.
Note: A cloud-based version of this pipeline is now available. Visit www.arimagenomics.com to learn more.

Connect with an Arima Genomics scientist to plan your next project.