Sensitive and unbiased detection of clinically actionable gene fusions from FFPE tumor biopsies using the Arima-HiC platform

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Poster Abstract #84

Introduction

- Gene fusions as biomarkers have broad clinical utility in cancer patients including for accurate diagnosis, early detection, prognosis, and selection of optimal treatment regimens.
- Typically, these fusions are detected using low-resolution karyotyping, low throughput and biased fluorescence in situ hybridization (FISH) assays. Alternatively, RNA sequencing approaches can be used but present challenges due to low transcript abundance, transcript length, RNA degradation
- Formalin fixed paraffin embedded (FFPE) tissues are a critical archival and clinical sample type, but typically perform poorly in molecular assays due to DNA damage.
- Gene fusions can be detected with Hi-C technology which has been shown to have high accuracy for identifying inter- and intrachromosomal translocations and rearrangements[1,2].

Methods

To address these limitations, we developed a novel approach to identifying structural variants and gene fusions from FFPE samples using the Arima-HiC platform and Illumina short-read sequencing. We performed pan-cancer analysis on 12 FFPE adult tumor biopsies, each with gene fusions known to be clinically actionable. All cases had undergone standard of care cytogenetic (FISH) or molecular (targeted cancer NGS sequencing panel) testing.

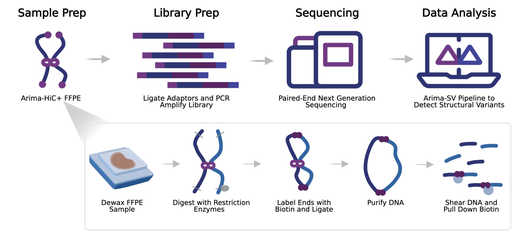

Figure 1. The Arima HiC+ FFPE Workflow[3]. Sample Prep: FFPE tissue scrolls were dewaxed, and the tissue rehydrated; then underwent chromatin digestion, end-labeling, and proximity ligation prior to DNA purification per the Arima-HiC FFPE protocol. Library Prep and Sequencing: Purified DNA was next prepared as a short-read sequencing library and sequenced on a HiSeq X.

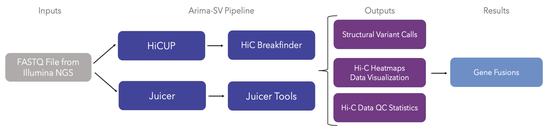

Figure 2. Data Analysis Workflow[4] FASTQ files input into the Arima-SV pipeline which enable the calling of variants, production of HiC heatmaps for identification of gene fusions.

Findings

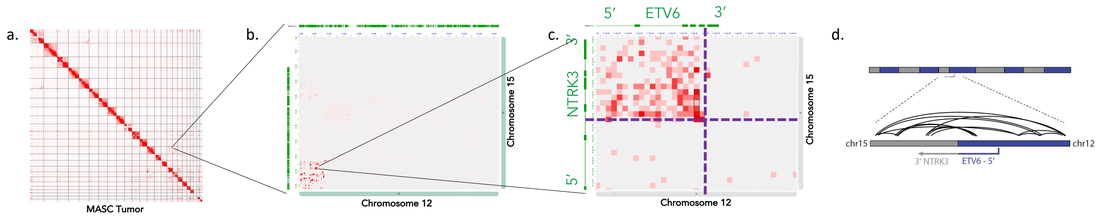

Figure 3. Leveraging 3D Genomics to Identify *NTRK* Fusions and Complex Rearrangements. **a.** genome-wide Hi-C contact matrix on a MASC (breast tumor). **b.** zoom-in Hi-C contact matrix showing 3D interactions between chr12 and chr15. The punctate signal scattered around the lower left of the matrix is indicative of complex rearrangements between a segment of chr12 and chr15, with numerous breakpoints. **c**. a zoom-in Hi-C contact matrix showing 3D interactions around a breakpoint creating an *ETV6-NTRK3* gene fusion. High 3D interaction signal is observed between the 5′ portion of *ETV6* and the 3′ portion of *NTRK3*. **d.** schematic representation of the complex rearrangement between chr12 and chr15, and a zoom in of the *ETV6-NTRK3* gene fusion.

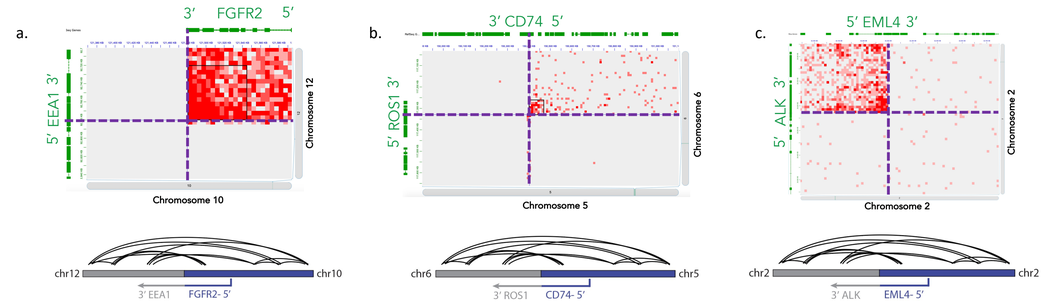

Figure 4. Leveraging 3D Genomics to Identify Clinically Actionable Gene Fusion Targets from FFPE Tumor Biopsies. Hi-C contact matrices and associated karyograms showing 3D interactions around breakpoints creating a **a.** *FGFR2-EEA1* gene fusion in a bile duct tumor biopsy. **b.** *CD74-ROS1* gene fusion in a lung lymph node tumor biopsy. **c**. *EML4-ALK* gene fusion in a lung adenocarcinoma tumor biopsy.

Gene Fusions Detected in FFPE Tumor Samples

Tumor Type	Gene Fusions Detected (Primary Result)	Gene Fusions Detected (Orthogonal Result)	Orthogonal Assay
Bile Duct Tumor	*FGFR2-EEA1*	*FGFR2-EEA1*	RNA panel
Bronchial/Lung Neoplasm	*PTRH2-ALK*	*ALK*	FISH
Liver Neoplasm	*EML4-ALK*	*ALK*	FISH
Lung Adenocarcinoma	*EML4-ALK*	*EML4-ALK*	DNA panel
Lung Adenocarcinoma	*EML4-ALK*	*EML4-ALK*	DNA panel
Lung Adenocarcinoma	*EML4-ALK*	*ALK*	FISH
Lung Lymph Node	*CD74-ROS1*	*ROS1*	FISH
Lung Neoplasm	ALK rearrangement	*ALK*	FISH
Breast MASC Tumor	*ETV6-NTRK3*	*NTRK3*	FISH
Metastatic Bronchogenic Adenocarcinoma	*EML4-ALK*	*ALK*	FISH
Synovial Sarcoma	*SS18-SSX*	*SS18*	FISH
Bladder Urothelial Carcinoma	*FGFR3-JAKMIP1*	*FGFR3-JAKMIP1*	DNA panel

Table 1. Summary Table of Technology Benchmarking Study Comparing 3D Genomics Using Arima-HiC to Standard of Care Cytogenic or Molecular Testing in FFPE Tumor Biopsies. Data showing the results of technology benchmarking study comparing Arima-HiC for FFPE and Arima-SV bioinformatics analyses to orthogonal methods for the detection of clinically actionable gene fusions across tumors types. Listed in the table from left to right is the tumor type, the gene fusion detected using Arima-HiC, the gene fusion detected using an orthogonal method, and the description of that orthogonal method (either targeted RNA-seq "RNA panel", targeted DNA sequencing "DNA panel", or FISH.

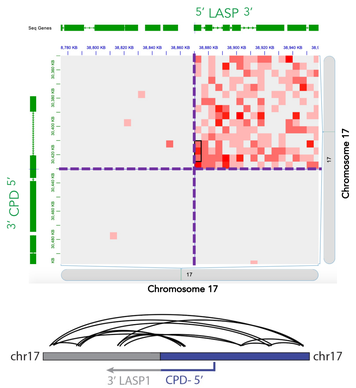

Figure 5. 3D Genomics Identifies Previously Uncharacterized Structural Variants of Potential Clinical Significance. Hi-C contact matrix and associated karyogram showing 3D interactions around a breakpoint creating a *CPD-LASP1* gene fusion in a bile duct tumor biopsy. To our knowledge, this gene fusion has not been reported before, however, *CPD* is a reported 5′ fusion partner such as with kinase *ERBB2*[5] and *LASP1* is a reported 3′ fusion partner with *KMT2A* in leukemia[6].

Conclusions

- Taken together, these findings demonstrate the analytical utility of Arima Hi-C sequencing technology to provide both chromosome-scale and gene-level resolution for the detection of structural variants in tumor biopsy samples.
- This workflow can provide improved access to critical genomic information from FFPE blocks for the identification of clinically actionable gene fusion events and other structural variants across tumor types.

References

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6. Strehl, S., et al. (2003). The human LASP1 gene is fused to MLL in an acute myeloid leukemia with t(11;17)(q23;q21). *Oncogene*, *22*(1), 157–160.

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