Arima Hi-C Sequencing Accurately Detects Clinically-relevant Structural Variants in Pediatric Leukemia Samples

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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, or low throughput and limited fluorescence in situ hybridization (FISH) assays. Alternatively, RNA sequencing approaches can be used but present challenges due to low transcript abundance or RNA degradation.
- Gene fusions can be detected with 3D genomics. Specifically, Hi-C technology, which has been shown to have high accuracy for identifying inter- and intra-chromosomal translocations and rearrangements[1,2].

Methods

- Five archived pediatric acute myeloid leukemia (AML) samples (archival period range: 1-4 years) known to be fusion-positive via prior clinical cytogenetic testing (i.e., karyotyping, FISH, and/or microarray) were analyzed for clinical concordance.
- Another 13 archived pediatric leukemia samples (n=3 AML, n=10 acute lymphoblastic leukemia (ALL)) without detectable fusions from prior cytogenetic and molecular (a targeted cancer NGS sequencing panel) testing, were analyzed in the discovery set.
- All samples were subject to Arima Hi-C sample prep, Illumina sequencing (~10X coverage per sample), and analysis using the Arima-SV pipeline.

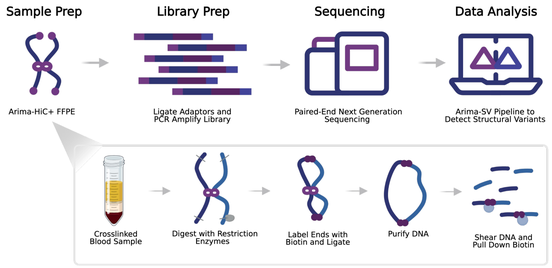

Figure 1. The Arima HiC+ Workflow[3]. Sample Prep: Blood samples were crosslinked followed by chromatin digestion, end-labeling, and proximity ligation prior to DNA purification per the Arima Hi-C protocol for blood. Library Prep and Sequencing: Purified DNA was next prepared as a short-read sequencing library and sequenced on an Illumina HiSeq X.

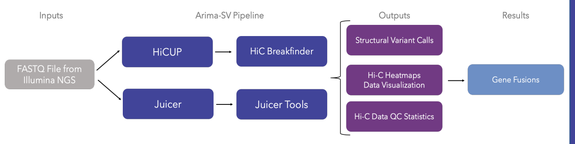

Figure 2. Data Analysis Workflow[4]. FASTQ files were inputted into the Arima-SV pipeline, which enabled the gene fusion detection and produced Hi-C heatmaps for visualization of gene fusions.

Findings

Disease	Cytogenetics	Arima Hi-C
AML	*KMT2A-MLLT4*	*KMT2A-MLLT4*
AML	*RUNX1-RUNX1T1*	*RUNX1-RUNX1T1*
AML	*RUNX1-RUNX1T1*	*RUNX1-RUNX1T1*
AML	*CBFB-MYH11*	*CBFB-MYH11*
AML	*CBFA2T3-GLIS2*	*CBFA2T3-GLIS2*

Table 1. Result summary of clinical concordance study. Table showing concordance between clinical cytogenetic testing (karyotyping, FISH and microarray) and 3D genomics-based Arima Hi-C in 5 pediatric AML cases.

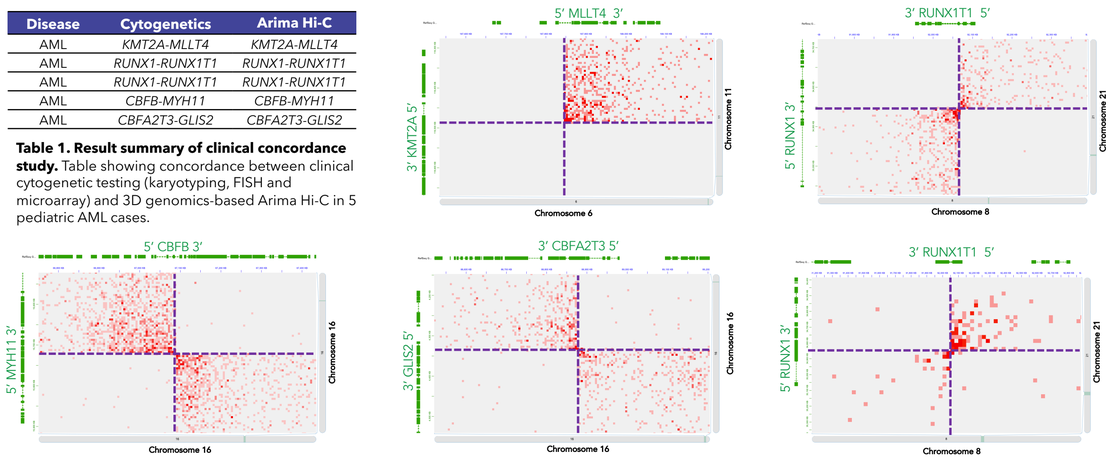

Figure 3. 3D Genomics is 100% concordant with clinical cytogenetics for detection of clinically significant gene fusions in pediatric leukemia. Hi-C contact maps showing 3D interactions around the breakpoints of each clinically relevant gene fusion in all 5 pediatric AML cases. For each Hi-C contact map, the breakpoints are indicated by dashed purple lines, the genes are annotated in green with respective gene orientations, and the chromosomes are labeled on each axis.

Disease	Cytogenetics & Molecular	Arima Hi-C
AML	*Negative*	*KMT2A-MLLT10*
ALL	*Negative*	*ZNF384-EP300*
ALL	*Negative*	*ZNF384-EP300*
ALL	*Negative*	*SKAP2-CDK6*
ALL	*Negative*	*ABHD17B-PTK2B*
ALL	*Negative*	*KRAS-r*
ALL	*Negative*	*EGFR-r*
ALL	*Negative*	*CDK8-r*
ALL	*Negative*	*IgH deletion*
AML	*Negative*	*Negative*
AML	*Negative*	*Negative*
ALL	*Negative*	*Negative*
ALL	*Negative*	*Negative*

Table 2. Result summary of gene fusions and other gene rearrangements detected by Arima Hi-C in leukemias with prior negative clinical cytogenetic and molecular testing. Table showing gene fusions or other notable gene rearrangements detected using 3D genomics-based Arima Hi-C, all of which were not detected by prior clinical cytogenetic testing (karyotyping, FISH and/or microarray) or molecular testing (clinical NGS panel sequencing). Note that "-r" indicates a gene rearrangement that does not create a fusion oncoprotein.

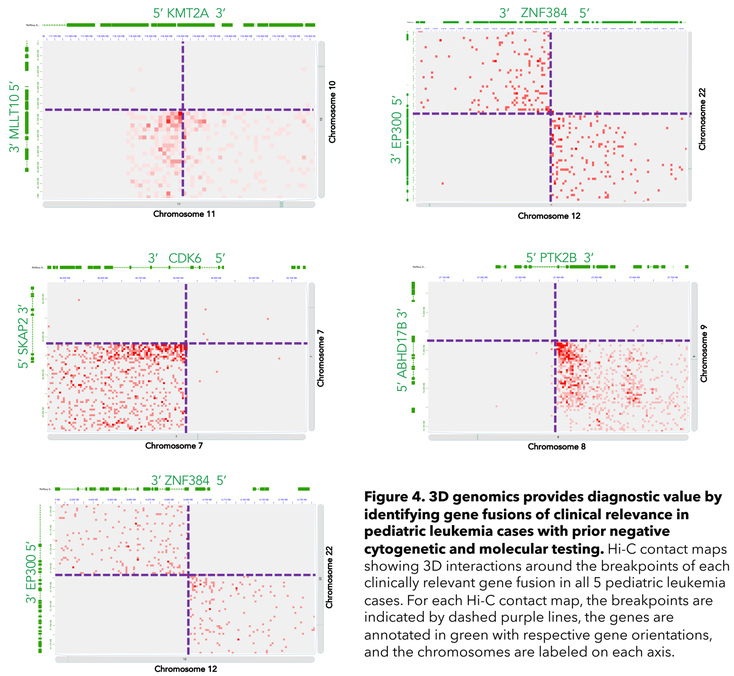

Figure 4. 3D genomics provides diagnostic value by identifying gene fusions of clinical relevance in pediatric leukemia cases with prior negative cytogenetic and molecular testing. Hi-C contact maps showing 3D interactions around the breakpoints of each clinically relevant gene fusion in all 5 pediatric leukemia cases. For each Hi-C contact map, the breakpoints are indicated by dashed purple lines, the genes are annotated in green with respective gene orientations, and the chromosomes are labeled on each axis.

Findings

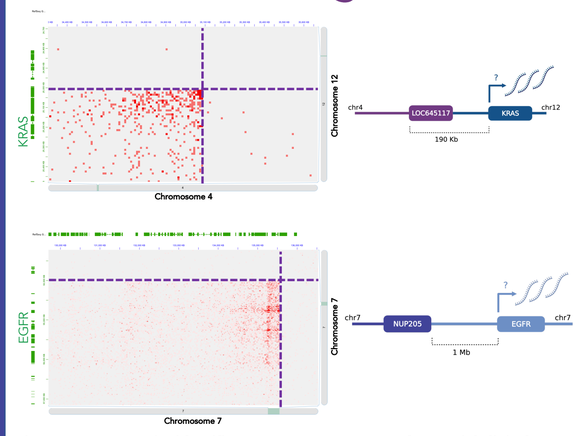

Figure 5. 3D genomics identifies gene rearrangements of potential clinical relevance in pediatric leukemia cases with prior negative cytogenetic and molecular testing. Hi-C contact maps showing 3D interactions around the breakpoints of each gene rearrangement, whereby the breakpoints are outside of the oncogene body and would not produce a fusion oncoprotein. For each Hi-C contact map, the breakpoints are indicated by dashed purple lines, the genes are annotated in green, and the chromosomes are labeled on each axis. For each case, a schematic depiction illustrates the gene rearrangement even, which we hypothesize may induce full-length oncogene expression.

Conclusions

- 3D genomics is 100% concordant with clinical cytogenetic testing (karyotyping, FISH, and/or microarray) for detecting clinically relevant gene fusions in pediatric AML cases.
- 3D genomics detected gene fusions with established clinical relevance in 23% (3/13) of pediatric leukemia cases that has previously undergone cytogenetic and molecular testing and where a genetic driver and/or gene fusion had not been identified.
- 3D genomics detected gene fusions or gene rearrangements of potential clinical relevance in an additional 38% (5/13) of pediatric leukemia cases that have previously undergone cytogenetic and molecular testing and where a genetic driver and/or gene fusion had not been identified, including gene fusions involving novel partners or gene rearrangements not creating fusion oncoproteins (e.g., KRAS).
- Overall, this study demonstrates how Arima Hi-C sequencing can provide diagnostic value in pediatric leukemia specimens via the identification of clinically relevant gene fusions and rearrangements.

References

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2. Harewood, L., et al. (2017). Hi-C as a tool for precise detection and characterisation of chromosomal rearrangements and copy number variation in human tumours. Genome Biology, 18(1), 125.
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