Preliminary Evaluation of Hi-C as a Novel Diagnostic Technology for Detecting Genomic and Chromosomal Structural Variants in Constitutional Disorders

He Fang, PhD[1], Yu Wu, PhD[1], Stephen Eacker, PhD[2], Yajuan J. Liu, PhD[1]*
[1]Department of Laboratory Medicine and Pathology, University of Washington, [2]Phase Genomics, Seattle, Washington, USA

Abstract

Detection and precise characterization of chromosomal rearrangements (CRs) and copy number aberrations (CNAs) have critical implications in diagnosis, prognosis, and therapeutic management of human genetic diseases. Conventional cytogenetics has specific limitations and sequential application of these different tests adds time and cost to diagnosis. Thus, revolutionary technologies that can alleviate the need for multiple technologies and improve resolution and sequence specificity are needed.

The NGS strategy of using Hi-C, a technology originally designed for delineating the spatial genome organization, has been demonstrated in a research setting to be better than other NGS methods at characterizing balanced and unbalanced chromosome rearrangements, and at a significantly lower cost. Yet, the application of Hi-C for clinical diagnostic workup has seen limited adoption so far.

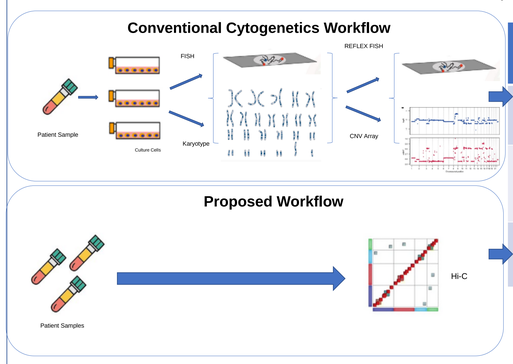
Here we evaluate the ability of Hi-C to detect both balanced and unbalanced chromosomal rearrangements in constitutional chromosomal disorders, with the potential to define chromosome breakpoints to bp resolution. We also demonstrate that copy number profiles and loss of heterozygosity profile can be obtained with high accuracy.

Introduction

Conventional cytogenetic tools have specific limitations with each method, such as karyotyping is a single cell whole genome assay but having limited resolutions, FISH is a targeted assay with limited coverage, and cytogenomic microarray analysis can detect CNAs and copy neutral LOH (cnLOH) with high resolution but not able to detect balanced rearrangements.

Some NGS methods also have limitations, such as low pass whole genome has low resolution due to limited read length and depth [1] and not able to detect copy neutral LOH nor balanced rearrangements while long-read sequencing technology is limited by the requirement for high molecular weight DNA, relatively high error rate, lack of mature computational analysis tools and high sequencing cost [2].

Hi-C is a chromatin conformation analysis to capture chromatin contacts within the nucleus by proximity ligation followed by NGS, which can detect both CRs and CNAs with potential for improving test accuracy with high resolution, eliminating reflex testing, and significantly shortening the turn-around time [3] (Figure 1). Here we assess the performance of Hi-C in the detection of simple and complex constitutional chromosomal aberrations of clinical relevance. Based on this study, we aim improve the workflow within our clinical laboratories for the accurate and timely detection of CRs & CNAs.

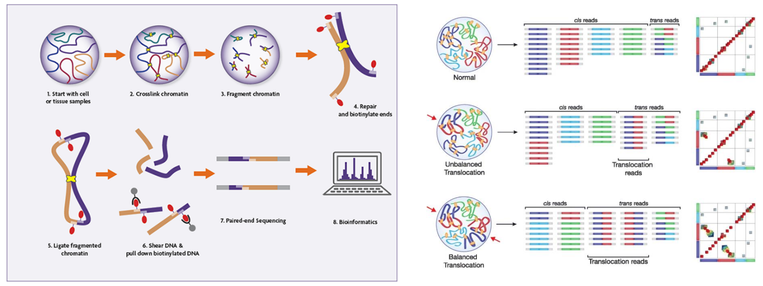


Balanced and Unbalanced SVs	Precise Break-points	Through-put	Cost	TAT
✓	NO	low	high	weeks
✓	✓	high	low	days

Figure 1: Schematics of conventional and proposed workflows with performance indicated in the table at right. The three conventional tests are shown as been done sequentially. However, the exact order of test is not always as shown.

Methods and Materials

We assessed the performance of Hi-C to detect chromosomal rearrangements and copy number alterations with high resolution in clinical samples with constitutional aberrations. We performed Hi-C on 113 samples. These samples include 31 normal specimens as control and 82 abnormal specimens representing various cell types (peripheral blood, amniotic fluid, chorionic villi), and various chromosomal aberrations (translocations, inversions, deletions, duplications, and loss of heterozygosity).



Results

1. Sample types and chromosomal variants of individuals with constitutional disorder used in Hi-C study

Sample Type	Number of Samples	Sex	Structural variants
Prenatal	56	Male:29	Triploidy, Trisomy, Monosomy Duplication, Deletion, Loss of Heterozygosity
		Female:27	
Postnatal	57	Male:29	Triploidy, Trisomy, Monosomy Duplication, Deletion, Loss of Heterozygosity
		Female: 28	

2. Detection of trisomy and monosomy by Hi-C

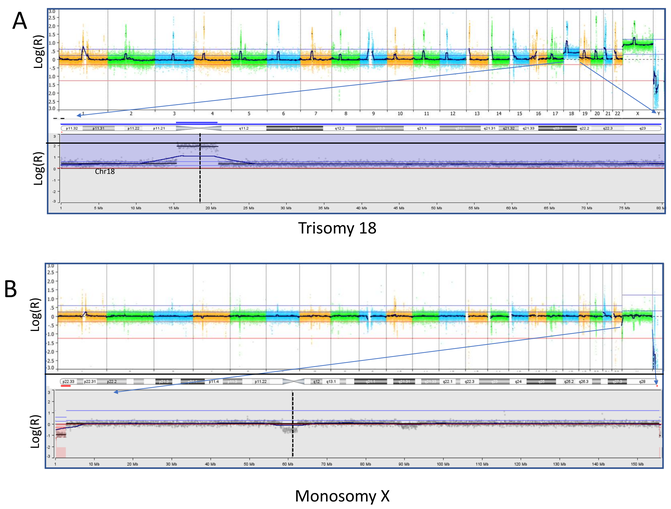


Figure 2: Trisomy and monosomy detected by Hi-C. (A) Detection of trisomy and monosomy by Hi-C. The log(R) ratio showing trisomy 18 5x coverage. (B) Detection of trisomy and monosomy by Hi-C. The log(R) ratio showing monosomy at 5x coverage.

3. Detection of copy number alterations and cnLOH by Hi-C

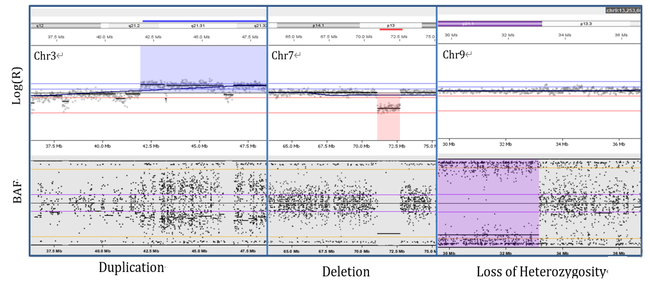


Figure 3: Detection of duplication, deletion and copy neutral heterozygosity of chromosomal segments by Hi-C. The log(R) ratio (upper panel) and B allele frequency (lower panel) showing regions of duplication on chr 3 (blue), deletion on chr 7 (pink) and cnLOH on chr 9 (purple).

4. Detection of translocations by Hi-C

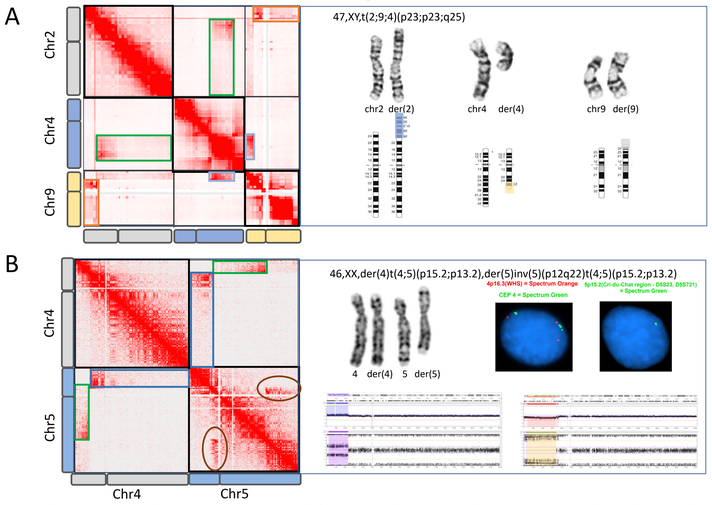


Figure 3: Translocations detected by Hi-C at 8x coverage. (A) Heatmap showing translocations between chr 2, chr 4 and chr 9. The colored boxes outline interactions. Right panel shows the karyotype results. (B) Heatmap showing a reciprocal translocations between chr 4 and chr 5. The colored box outlines interactions within chr 4 and chr 5. The brown circles outlines inversions within chr 5. Right panel shows the corresponding karyotype, FISH and/or SNP array results.

Conclusions

- We demonstrated the use of Hi-C as a tool for detection of chromosomal rearrangements and copy number alterations in constitutional disorders.
- Specifically, Hi-C can detect both balanced rearrangements (such as translocations and inversions) and genomic imbalanced (such as deletion and duplications) at a high resolution.
- Loss of homozygosity and mosaicism detection can also be achieved with Hi-C with deeper coverage.

References

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3. Harewood L, Kishore K, Eldridge MD, Wingett S, Pearson D, Schoenfelder S, Collins VP, Fraser P. Hi-C as a tool for precise detection and characterization of chromosomal rearrangements and copy number variation in human tumours. Genome Biol. 2017 Jun 27;18(1):125.