Bionano hybrid scaffold assemblies provide high contiguity and accuracy

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Abstract

With the advancement of new technologies, high quality assemblies of novel genomes have gained momentum in recent years. To disambiguate homologous regions in these novel genomes, long reads and linked reads are used to assemble contigs. Scaffolding these sequences into chromosomal-arm or full chromosome length can only be accomplished using Bionano Genomics optical mapping or one of the Hi-C based methods. In comparing the assemblies of these scaffolding technologies, we demonstrate that Bionano can correct sequence and orientation errors generated by other technologies while providing having superior contiguity.

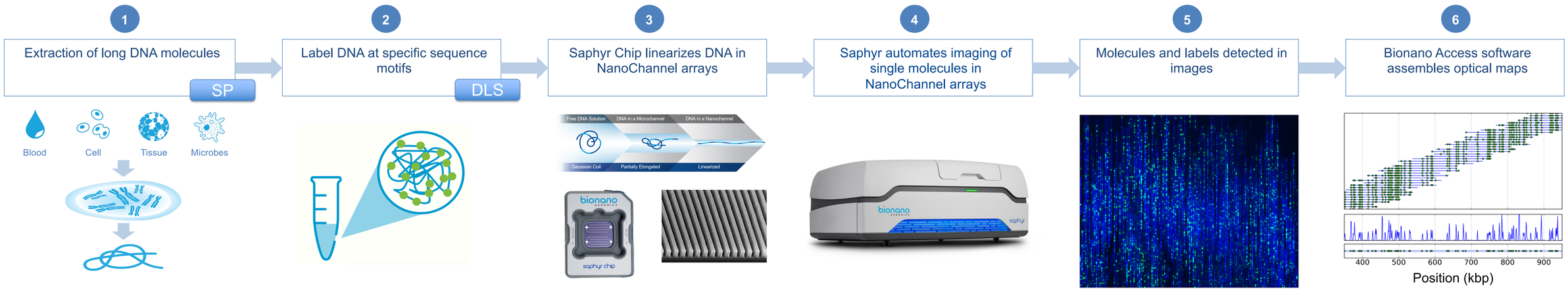
Chromosome assemblies can often be achieved by using Bionano's new DLS chemistry. Bionano Genomics, with its physically intact molecules that have N50 length on average >250kbp, is unique and can generate megabase-long contiguous assemblies with the well understood overlap-layout-consensus algorithms. These assemblies are then used to scaffold sequences into chromosome or chromosome arm length assemblies by the Bionano Hybrid Scaffold pipeline. Alternatively, Hi-C based methods leverage crosslinking of DNA that is in close-proximity *in vivo* through chromatin folding, which is then sequenced using short read sequencing. Since the long-range interaction in Hi-C is based on cells at different stages of dynamic biological connections and is encoded by short reads, significant inference is required to reconstruct the interaction information.

We present here a few case studies of scaffolding plant and animal genomes using Hi-C and Bionano maps. We examine some of the challenges, suggest an effective workflow in generating high-quality reference-graded assemblies.

Background

Generating high-quality finished genomes replete with accurate identification of structural variation and high completion (minimal gaps) remains challenging using short read sequencing technologies alone. The Bionano Saphyr™ system provides direct visualization of long DNA molecules in their native state, bypassing the statistical inference needed to align paired-end reads with an uncertain insert size distribution. Ultra long DNA molecules can be isolated using Bionano Prep Solution Phase (SP) DNA isolation kit or a conventional plus-lysis method. DNA can be labelled in a non-destructively with Direct Label Stain (DLS) chemistry. These long-labeled molecules are *de novo* assembled into physical maps spanning the entire diploid genome. The resulting provides the ability to correctly position and orient sequence contigs into chromosome-scale scaffolds and detect a large range of homozygous and heterozygous structural variation with very high efficiency.

Methods



(1) Long molecules of DNA are labeled with Bionano reagents by (2) incorporation of fluorophores at a specific sequence motif throughout the genome. (3) The labeled genomic DNA is then linearized in the Saphyr Chip using NanoChannel arrays (4) Single molecules are imaged by Saphyr and then digitized. (5) Molecules are uniquely identifiable by distinct distribution of sequence motif labels (6) and then assembled by pairwise alignment into *de novo* genome maps.

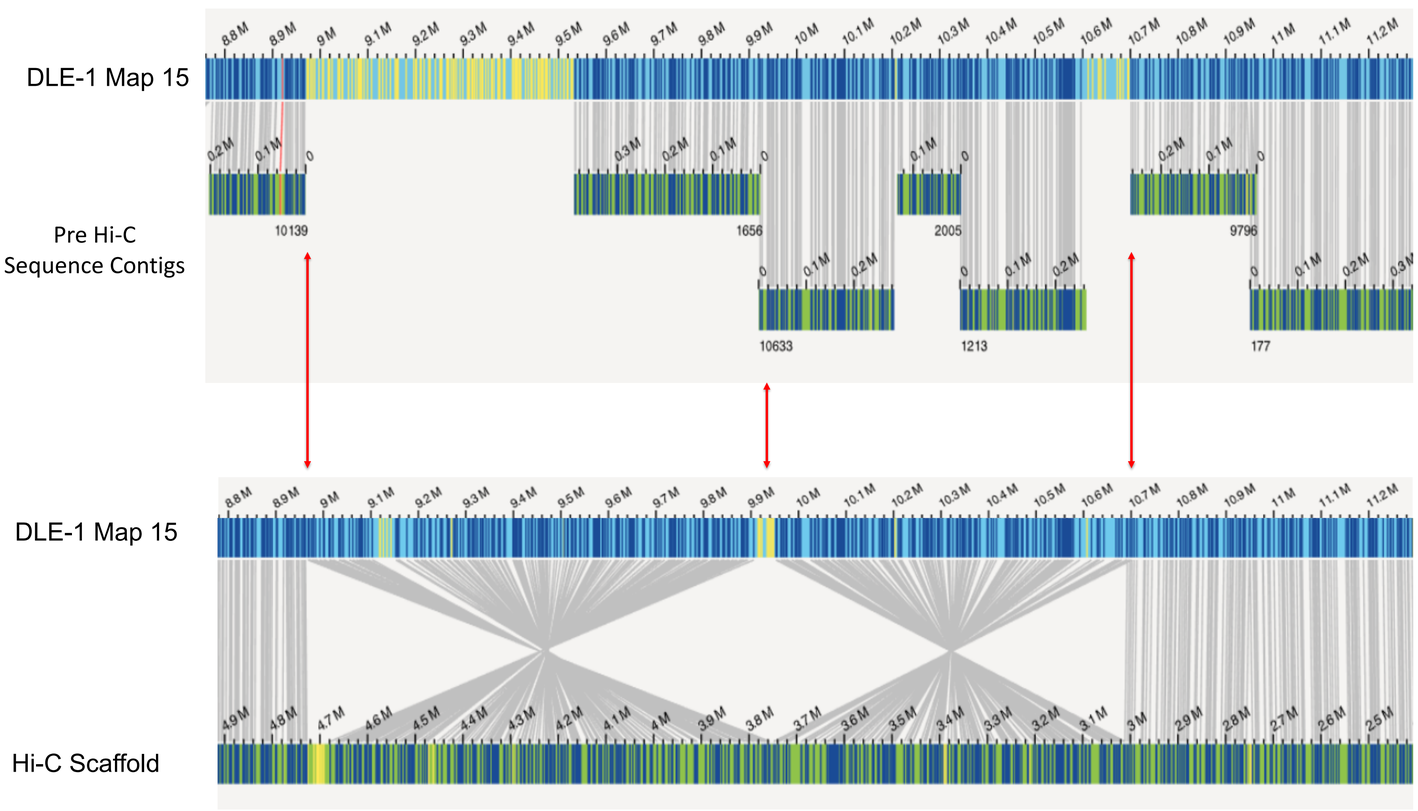


Figure 1. Discrepancies between Bionano maps and sequence assemblies were introduced after Hi-C scaffolding. Bionano corrects errors in sequence scaffolds and also provides superior contiguity.

Results

In comparing Hi-C scaffolds with Bionano DLE Maps, many discrepancies were found. Inverted and rearranged segments of various sizes were identified when aligning the Hi-C scaffolds to the Bionano DLE Maps. Aligning pre-Hi-C scaffolded sequences with Bionano DLE maps also shows that most of the divergent breakpoints were at ends of the pre-Hi-C scaffolded contigs, suggesting that orientation and arrangement discrepancies were introduced during Hi-C scaffolding (Figure 1). Since the Bionano method leveraged native intact molecules, spanning breakpoints of these segments by Bionano molecules suggests mis-orientations in the Hi-C scaffolds. At regions of discrepancies, a third long read sequencing technology also agrees with Bionano structure, further supporting the accuracy of the Bionano assembly (Figure 2).

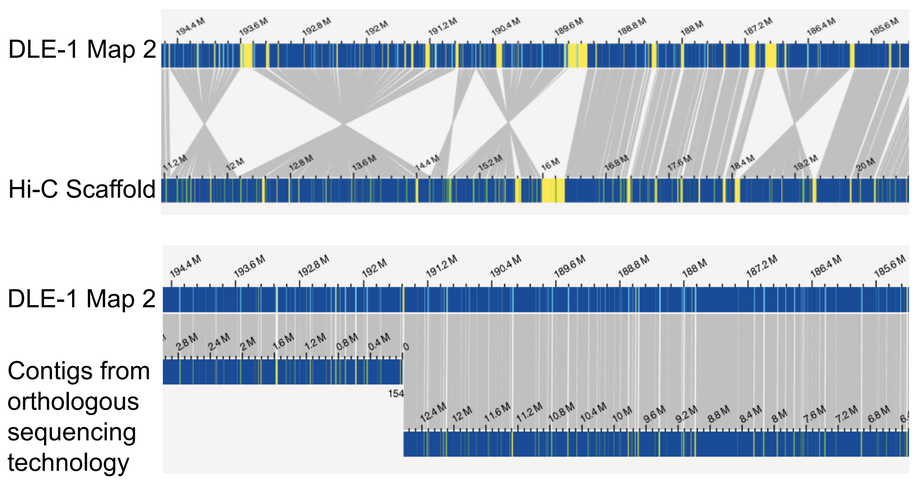


Figure 2. At discrepancy regions between the Bionano and Hi-C scaffolds, Bionano's structure is supported by a third technology of lower contiguity.

Conclusions

Bionano genome mapping is a rapid, cost effective and highly accurate method for chromosome level *de novo* assembly that can complement other genomic methods. With Bionano's non-inferred method of assembling chromosome-arm length *de novo* assemblies, it is the golden standard for generating high-quality genomes in the era of precision medicine.

Reference

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