Bionano Hybrid Scaffold Assemblies Provide High Contiguity and Accuracy
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Abstract
High quality genome assembly of novel genomes has gained momentum in recent years as a result of the advancement of new technologies. To disambiguate homologous regions of these novel genomes, long reads and linked reads are used to assemble contigs. Scaffolding these sequences into chromosome arm or full chromosome length can only be accomplished using Bionano Genomics’ long read mapping or one of the Hi-C based methods. In comparing the assemblies of these scaffolding technologies, we demonstrate that Bionano can correct sequence errors and orientation errors generated by other technologies.

Bionano genome mapping of physically intact molecules of hundreds of kilobases, is unique and generates tens of megabases 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.

Methods
1. Extraction of long DNA molecules
2. Label DNA at specific sequence motifs
3. Saphyr Chip linearizes DNA in NanoChannel arrays
4. Saphyr automates imaging of single molecules in NanoChannel arrays
5. Molecules and labels detected in images
6. Bionano Access software assembles optical maps

(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.

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 2). 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 3).

Conclusions
Bionano is a rapid, cost effective and highly accurate method for chromosome level de novo assembly that can complement other genomic methods. Bionano’s high-quality de novo assemblies have been a major part of most published genome finishing projects in recent years.

Reference
2) Cao, H., et al., Rapid detection of structural variation in a human genome using NanoChannel-based genome mapping technology. Gigascience (2014); 3(1):34