Abstract

Bionano genome mapping traditionally images long, megabase sized molecules that are fluorescently labeled using nicking endonucleases followed by incorporation of fluorescent nucleotides at nick sites. One inherent limitation of nickase-based labeling is the introduction of systematic double-stranded breaks in regions containing sequence motifs in close proximity on opposite strands.

In order to avoid the inherent limitation of these fragile sites, we have developed an enzymatic direct labeling approach that allows fluorescent labeling of a specific sequence motif on native double stranded DNA. The direct labeling enzyme shows very high single molecule sequence specificity and efficiency. By not introducing DNA nicks, direct labeling enables the assembly of genome maps with N50 measurements that now exceed 60 Mbp. DNA labeling and cleanup can be achieved in approximately 5 h with low hands on time.

We present the workflow for direct labeling of ultra-high molecular weight DNA for running on the Saphyr™ system and a comparison of the results between this new labeling method and the nickase-based labeling. The data obtained using the direct labeling method can be combined with data from the nickase-based approach in hybrid scaffolding applications for creating high quality genome references. Bionano genome mapping using this new assay drastically improves SV detection and hybrid scaffolding results all for < $1,000 per sample.

Methods

1. Comparison of NLRS and DLS Workflows
2. PFGE Results Comparing NLRS and DLS
3. DLS Molecules are Long
4. DLS (Direct Label and Stain) Labeling Chemistry
5. Full Chromosome Arms of human Chr3 assembled with DLS
6. De novo Assembly of Diverse Genomes with DLS
7. DLS Assembly across the human Chr6 MHC locus
8. In silico SV Detection with Bionano Maps (human)

References


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