Bionano Prep SP isolates high quality ultra-high molecular weight (UHMW) genomic DNA to improve research of cancer and undiagnosed disorders

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Abstract

Optical mapping of genomic DNA on the Bionano Genomics Saphyr® system for genome assembly or structural variation detection relies on starting with UHMW DNA. To achieve this, we have developed Bionano Prep SP where solution-based lysis is coupled with a purification step that leverages a novel process to bind, wash and elute UHMW genomic DNA. This entire protocol can be conducted in approximately 4 hours on a batch of six samples, allowing 12 samples to be processed in one day. The eluted material is ready to use by Day 2 and contains high quality DNA that is compatible with the Direct Label and Stain (DLS) Protocol. The resulting labeling metrics of this labeled DNA on Saphyr Chip® are comparable to labeled DNA isolated by the traditional plug lysis protocol.

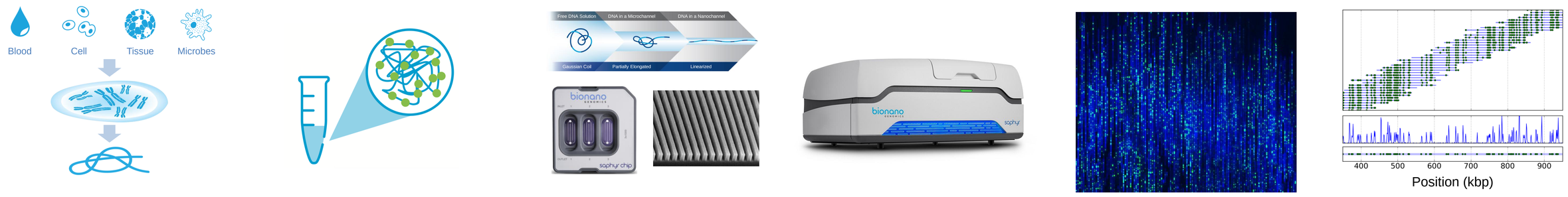
We have validated protocols for fresh/frozen human blood and cells and have further expanded utility of this solution-based method for use in research of cancer and undiagnosed disorders with development of a protocol for bone marrow aspirates. In addition, we are developing protocols with this method for use in animal tissue applications. Unlike plug lysis, this solution-based protocol provides additional value in that it is amenable to automation, providing a solution for researchers needing to purify DNA from hundreds to thousands of individuals per year.

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 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. These long labeled molecules are then *de novo* assembled into physical maps spanning the entire diploid genome. The platform provides the ability to correctly position and orient sequence contigs into chromosome-scale scaffolds and detect a large range of homozygous and heterozygous structural variants with very high efficiency.

Methods

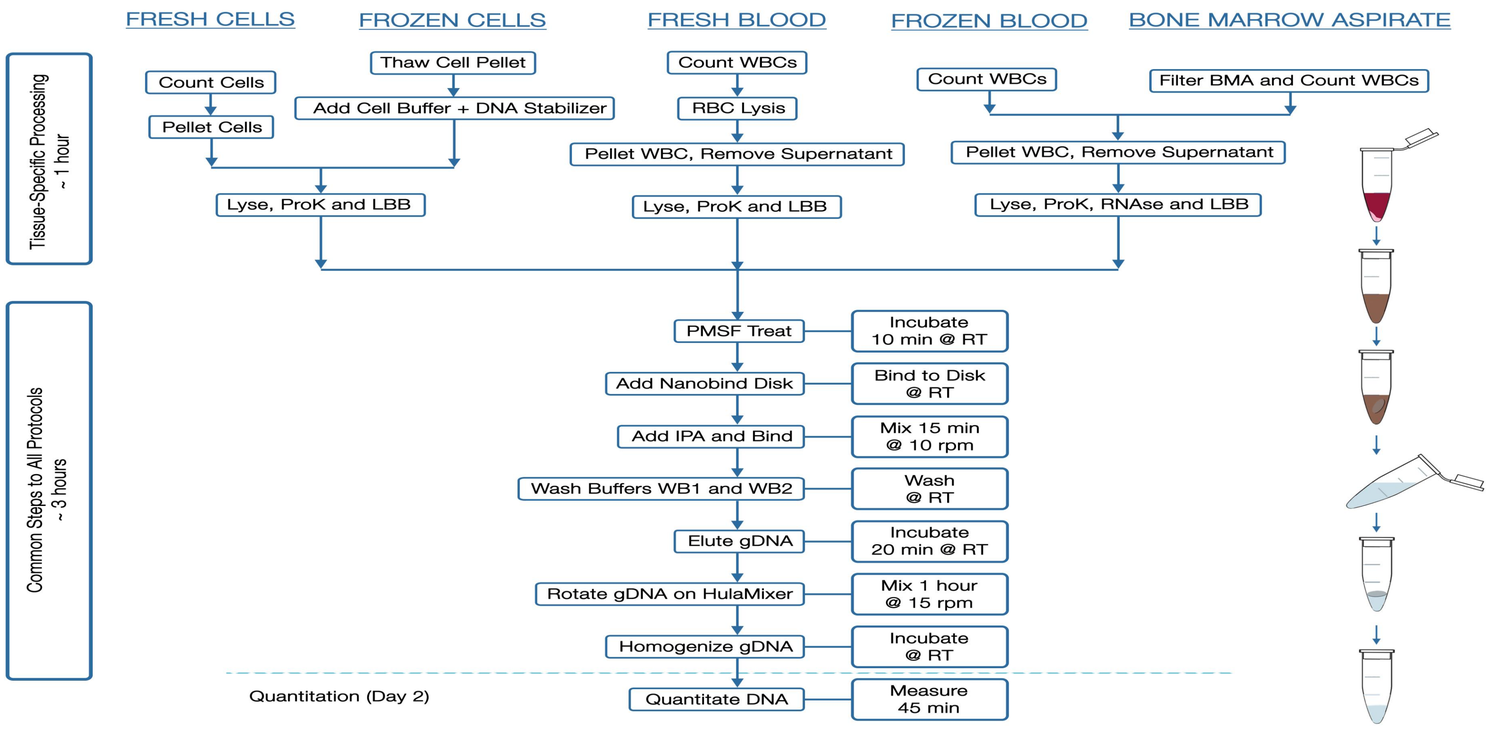
1. Extraction of long DNA molecules
2. Label DNA at specific sequence motifs
3. Saphyr Chip linearizes DNA in NanoChannel arrays
4. Saphyr automates imaging of single molecules in NanoChannel arrays
5. Molecules and labels detected in images
6. Bionano Access software assembles optical maps



(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.

Bionano Prep SP Workflows

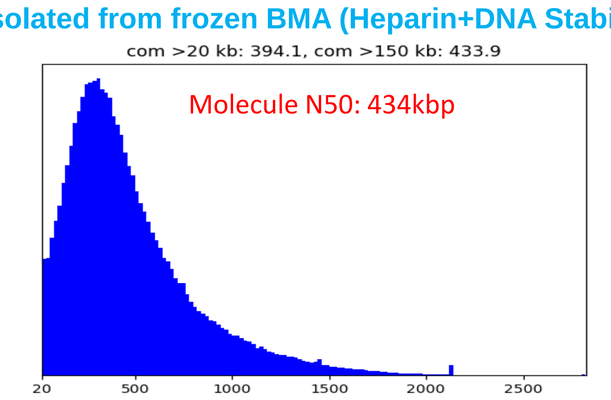
Fresh human healthy donor whole bloods (EDTA) were obtained from the San Diego Blood Bank. Upon receipt, fresh bloods were well mixed and WBC concentration was determined by Hemocue. For fresh blood processing, the volume of blood required for 1.5 million cells was subjected to differential RBC lysis in a protein lobind tube and DNA was purified by Bionano Prep SP. For frozen blood processing, following thorough mixing of the fresh blood, 1ml aliquots were frozen and stored at -80C. Following thawing, mixing and WBC concentration determination by Hemocue, the volume required for 1.5 million cells was transferred to a protein lobind tube. WBCs were pelleted by centrifugation and DNA was purified by Bionano Prep SP. Fresh GM12878 cells were obtained from the Coriell Institute, grown and cells were counted by hemocytometer. For fresh cell processing, 1.5 million cells in growth medium were pelleted, resuspended in Cell Buffer, transferred to a protein lobind tube and DNA was purified by Bionano Prep SP. For frozen cell processing, 1.5 million fresh cells were pelleted, cells were resuspended in Cell Buffer+DNA Stabilizer, and were frozen at -80C as pellets following centrifugation until use. After thawing, the cell pellet was resuspended with Cell buffer+DNA Stabilizer, transferred to protein lobind tube and DNA was purified by Bionano Prep SP. Frozen human bone marrow aspirates (BMAs) (Heparin) in which DNA Stabilizer was added to fresh BMA before freezing, were obtained from Radboud University Medical Center and stored at -80C. For frozen BMA processing, following thaw, mixing, filtering and WBC concentration determination by Hemocue, the volume required for 1.5 million cells was transferred to a protein lobind tube. WBCs were pelleted by centrifugation and DNA was purified by Bionano Prep SP. For all SP workflows, following elution from Nanobinds, gDNAs were homogenized with narrow bore shearing and end over end mixing for one hour. Following a minimum of overnight rest at room temperature, gDNA concentrations of eluted SP DNAs was determined with the Qubit BR Assay and 750 ng of gDNA was labeled with the DLS labeling chemistry.



Single Molecule DLS Metrics from SP gDNAs

Sample Type	gDNA [ ] (ng/uL)	Gbp / Scan	N50		Label Density	Map Rate
			> 20kbp	> 150kbp		
Fresh Blood (4 users, 8 bloods, 24 samples)						
Mean	59.0	36.1	233.4	300.5	15.4	89.6%
Frozen Blood (3 users, 17 bloods, 34 samples)						
Mean	57.4	27.8	203.0	264.6	15.2	90.2%
Fresh Cells (4 users, 2 cultures, 10 samples)						
Mean	113.4	31.8	283.2	317.7	14.9	93.1%
Frozen Cells (4 users, 2 cultures, 12 samples)						
Mean	106.9	21.4	264.4	319.4	15.2	89.6%
Bone Marrow Aspirate (1 user, 11 BMAs, 45 samples)						
Mean	59.0	29.9	230.0	297.9	15.2	88.3%

Size distribution of DLS labeled SP gDNA molecules isolated from frozen BMA (Heparin+DNA Stabilizer)



DLS labeled SP gDNA from a frozen BMA in Heparin+DNA Stabilizer was analyzed on the Bionano Saphyr® system and the number of accumulated DNA molecules is plotted as a function of size.

Conclusions

Bionano Prep SP provides a rapid gDNA isolation protocol from human blood, cells and bone marrow aspirate (~4 hours total time/6 samples), improving capability for research of hematologic cancers and undiagnosed disorders. The single molecule DLS labeling metrics of SP gDNAs are comparable to those from plug lysis and the Bionano Prep SP protocols are amenable to automation, less lengthy, labor intensive, and costly. In addition to Bionano Prep SP protocols being further expanded for use in research of hematologic cancers and undiagnosed disorders, further applications of Bionano Prep SP involving animal tissues as well as human solid tumors are currently being developed.

References

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