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

H. Sadowski1, C. Proskow1, A. Files1, K. Pham1, D. Carroll1, H. Barnes1, Y. Zhang1, D. Primich1, G. Pijevajlic1, M. JPL Stevens-Kroell1, A. Hoischen2 and M. Oldakowski1

1Bionano Genomics, San Diego, California, United States of America, 2Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

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. We validate 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.

Methods

1. Extraction of long DNA molecules
2. Labeling 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

Bionano Prep SP Workflows

Fresh human healthy donor whole blood (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 lobe and DNA was purified by Bionano Prep SP. For frozen blood processing, following thawing the mixture of fresh blood, 1ml aliquots were frozen and stored at -80°C. After thawing, mixing and WBC concentration determination by Hemocue, the volume required for 1.5 million cells was transferred to a protein lobe 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 lobe tube and DNA was purified by Bionano Prep SP. For frozen cell processing, 1.5 million fresh cells were collected, cells were resuspended in Cell Buffer+DNA Stabilizer, and were frozen at -80°C as pellets following centrifugation until use. After thawing, the cell pellet was resuspended with Cell Buffer+DNA Stabilizer, transferred to protein lobe 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 -80°C. For frozen DNA processing, following thaw, mixing, filtering and WBC concentration determination by Hemocue, the volume required for 1.5 million cells was transferred to a protein lobe tube. WBCs were pelleted by centrifugation and DNA was purified by Bionano Prep SP. For all SP workflows, following elution from Nanobind, 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 DNA was determined with the QuantIT BR Assay and 970 ng of gDNA was labeled with the DLS labeling chemistry.

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 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

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

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