# Solution-based Isolation of Ultra-High Molecular Weight Genomic DNA from 0.2-0.5 mL of Frozen Human Blood (1.5 million WBCs) in less than 3 Hours

H. B. Sadowski, C. Proskow, A. Files, K. Pham, G. Pljevaljcic and M. Borodkin

Bionano Genomics, San Diego, California, United States of America

## Abstract

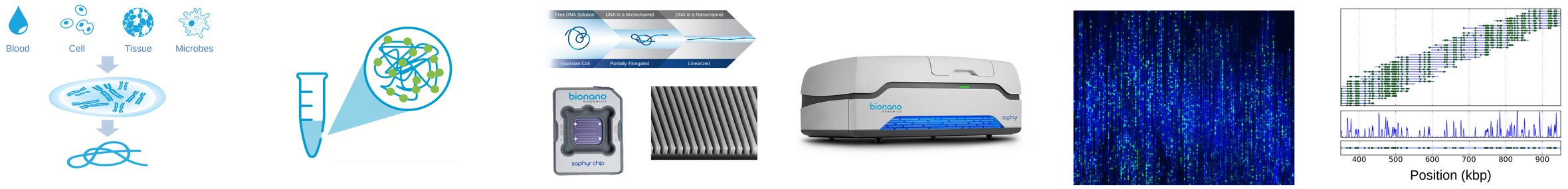
Successful optical mapping of genomic DNA using the Bionano Genomics Saphyr® system for genome assembly or structural variation detection relies on starting with ultra-high molecular weight (UHMW) DNA. To achieve this, we have established methods for the isolation of UHMW DNA from various sources that involve the embedding of material in agarose plugs with overnight lysis and digestion *in situ* with detergent and Proteinase K. Following extensive washing on Day 2, the agarose plug is melted, treated with beta agarase to digest agarose polymers to monomers, and the liberated DNA is subjected to drop dialysis. The UHMW DNA is ready for quantification and labeling on Day 3. This plug lysis DNA isolation method is extremely robust, but it is also labor intensive, difficult to automate, lengthy and expensive. To address these shortcomings, we have developed a solution-based lysis method with a purification step leveraging a novel process to bind, wash and elute UHMW genomic DNA. This entire protocol can be completed in less than three hours on a batch of six samples. The eluted DNA is pure and of high quality for subsequent enzymatic labeling using direct labeling and staining (DLS). The resulting single molecule metrics of this labeled DNA using the Saphyr system are comparable to labeled DNA isolated by the traditional plug lysis protocol. We have a validated protocol for frozen blood and have working protocols in development for fresh blood, cultured cells and frozen cell pellets. This rapid solution-based isolation method is very attractive compared to plug lysis because it is amenable to automation, is less cost prohibitive and provides a time saving 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 *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.

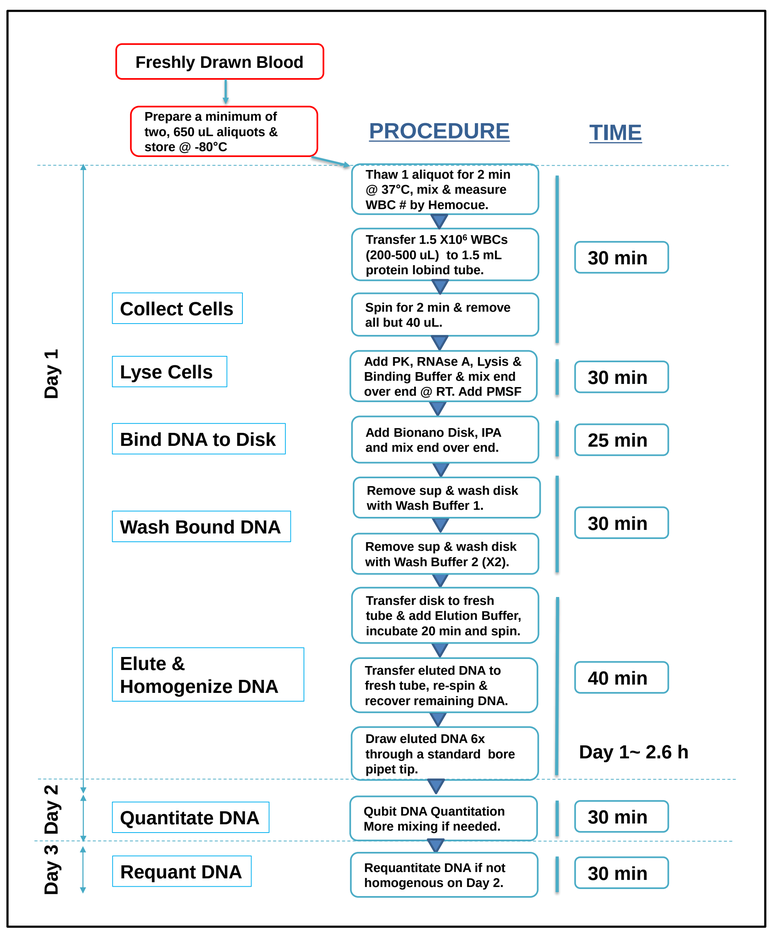
## Methods

1. 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

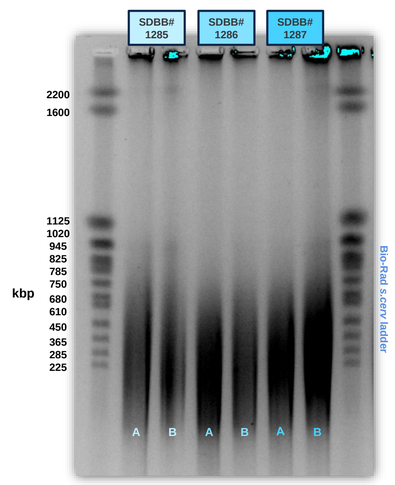



**(1) Long molecules of DNA are labeled with Bionano reagents by (2) incorporation of fluorophores at a specific sequence motif throughout the genome. (3) The labeled genomic DNA is then linearized in the Saphyr Chip using NanoChannel arrays (4) Single molecules are imaged by Saphyr and then digitized.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

## Solution-based Isolation of UHMW DNA from 0.2-0.5 mL of Frozen Human Blood (1.5 million WBBCs) in <3 Hours




## Solution-based Isolation of UHMW DNA from Frozen Human Blood: Analysis by PFGE




~500ng DNA loaded per well.

UHMW DNAs (A & B) were isolated from aliquots of three frozen bloods using solution-based methods. Equal amounts of each DNA was subjected to PFG analysis. (1% agarose, 0.5X TBE PFG, 20 hr run time 2 state, 6V/cm, 120˚ included angle, 90 sec initial & final switch times with linear ramping. Stained with SYBR Gold.

## Isolation of UHMW DNA from Frozen Human Blood: Assembly & SV Metrics

| Assembly Metrics | SDBB2250 |
|---|---|
| Data Collected (Gbp>150 kbp) | 458.9 |
| Assembly Size (Gbp) | 5.85 |
| Molecule N50 (kbp) | 264 |
| Label Density (/100 kbp) | 16.2 |
| Coverage of the Reference | 148x |
| Diploid Genome N50 (Mbp) | 59.8 |
| Coverage of the Assembly | 78x |
| **SV Summary** | |
| Deletions | 1439 |
| Insertions | 3795 |
| Duplications | 80 |
| Inversion Breakpoints | 194 |
| Translocation Breakpoints | 0 |

## Single Molecule Metric Comparison of Solution-based and Plug Lysis Methods for Isolation of UHMW DNA from Frozen Human Blood

### Solution-based DNA Isolation with 12 Frozen SDBB Samples (1.5 x10$^6$ cells)

| Donor ID | Sample ID | DNA [ ] ng/µl | N50 >20kbp | N50 >150kbp | Map Rate | Throughput Gbp DNA/scan |
|---|---|---|---|---|---|---|
| SDBB1920 | 1 | 67 | 247 | 303 | 92.5% | 13.1 |
| SDBB2244 | 2 | 49 | 178 | 238 | 91.5% | 32.7 |
| SDBB2249 | 3 | 59 | 208 | 270 | 92.2% | 22.9 |
| SDBB2250 | 4 | 95 | 208 | 263 | 90.4% | 16.2 |
| SDBB2251 | 5 | 112 | 165 | 247 | 86.8% | 20.8 |
| SDBB2252 | 6 | 95 | 189 | 269 | 88.3% | 14.1 |
| SDBB2253 | 7 | 88 | 219 | 270 | 90.6% | 21.7 |
| SDBB2255 | 8 | 89 | 195 | 263 | 89.5% | 23.8 |
| SDBB2247 | 9 | 67 | 191 | 252 | 92.8% | 27.9 |
| SDBB2254 | 10 | 59 | 211 | 267 | 93.6% | 44.5 |
| SDBB2248 | 11 | 55 | 202 | 258 | 90.9% | 16.6 |
| SDBB2246 | 12 | 61 | 246 | 291 | 91.3% | 31.3 |
| Mean ±SD | 1-12 | 74.7 ± 20.1 | 205 ± 24 | 266 ± 18 | 90.9 ± 1.9 | 23.8 ± 9.1 |

### Bionano Prep® Plug Lysis DNA Isolation with 6 Frozen SDBB Samples (200 µL blood)

| Donor ID | Sample ID | DNA [ ] ng/µl | N50 >20kbp | N50 >150kbp | Map Rate | Throughput Gbp DNA/scan |
|---|---|---|---|---|---|---|
| SDBB1209 | 1 | 70 | 191 | 248 | 85.8% | 12.5 |
| SDBB1210 | 2 | 97 | 172 | 243 | 85.9% | 12.1 |
| SDBB1211 | 3 | 55 | 194 | 292 | 80.3% | 8.8 |
| SDBB1209 | 4 | 102 | 222 | 262 | 90.7% | 14.2 |
| SDBB1211 | 5 | 82 | 235 | 297 | 91.2% | 12.1 |
| SBDD1449 | 6 | 89 | 205 | 259 | 88.2% | 13.3 |
| Mean ±SD | 1-6 | 82.5 ± 17.6 | 203 ± 22.7 | 267 ± 22.6 | 87.0 ± 4.0 | 12.2 ± 1.8 |

UHMW DNA was isolated from aliquots of frozen bloods (San Diego Blood Bank) using either the solution-based or Plug Lysis Isolation method. The UHMW DNAs were subjected to DLS labeling and run on the Saphry system. Representative results are shown above.

## Conclusions

- We have developed a solution-based Isolation protocol to obtain UHMW DNA from frozen human blood.
- We present data demonstrating that the size and DLS single molecule metrics for DNAs isolated by the solution-based isolation protocol are very similar to DNAs isolated by the Bionano Prep Plug Lysis Protocol.
- In addition, assembly and SV metrics for the solution-based isolation protocol samples are right in line with previous results using plug lysis DNA from frozen blood.
- DNAs from 6 blood samples can be isolated in less than three hours allowing for the generation of DNAs from 12 blood samples in a single day by a single lab technician.
- This novel rapid isolation method and workflow provide greater value to researchers relative to traditional plug lysis because it is amenable to automation, is less cost prohibitive, and provides a time saving solution for sample prep.

## References

1. Cao, H., et al., Rapid detection of structural variation in a human genome using NanoChannel-based genome mapping technology. Gigascience (2014); 3(1):34
2. Hastie, A.R., et al. Rapid genome apping in NanoChannel rrays for highly complete and accurate *de novo* sequence assembly of the complex *Aegilops tauschii* genome. PLoS ONE (2013); 8(2): e55864.
3. Lam, E.T., et al. Genome mapping on NanoChannel arrays for structural variation analysis and sequence assembly. Nature Biotechnology (2012); 10: 2303