Isolating High-Quality Ultra-High Molecular Weight (UHMW) Genomic DNA (gDNA) from 5-10 mg of Fresh Frozen Animal and Human Tissues

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Abstract

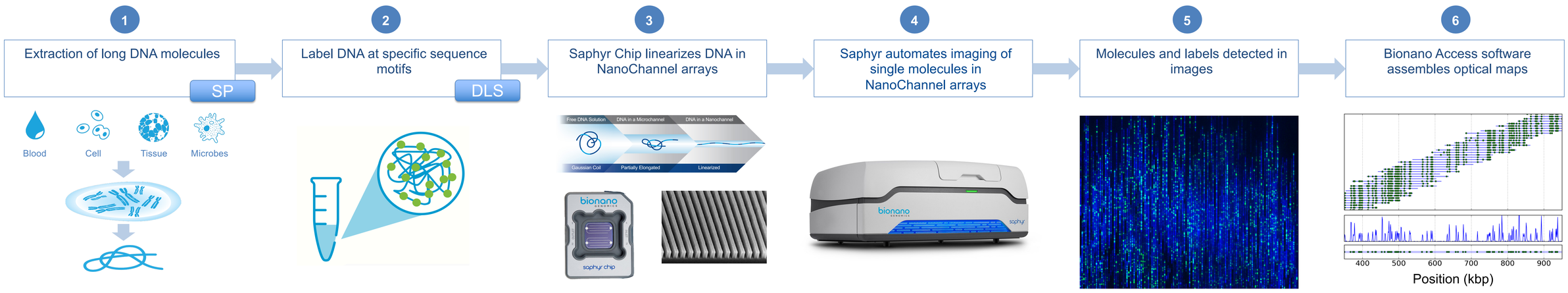
Optical mapping of genomic DNA on the Bionano Genomics Saphyr® system for genome assembly or structural variation detection relies on starting with ultra-high molecular weight (UHMW) gDNA. To achieve this, we have developed a streamlined Bionano Prep SP Animal Tissue DNA Isolation Protocol to isolate ultra-high molecular weight (UHMW) gDNA from solid tissues of animal and human origin. The protocol begins with a simplified front end tissue homogenization step for both soft and fibrous tissue types. It then utilizes Bionano Prep SP where solution-based lysis is coupled with a purification step leveraging a novel process to bind, wash and elute UHMW genomic DNA. This entire protocol can be conducted in less than 6 hours on a batch of 6 - 8 samples. The eluted material is ready to use by Day 2 and contains high-quality UHMW gDNA that is compatible with the Direct Label and Stain (DLS) Protocol. When run on a Saphyr Chip® DNA purified by this protocol gives consistently high throughputs (1.3 Tbp per sample) with excellent single molecule metrics of DNA size and map rate, sufficient for use in genome finishing, structural variant comparative analysis and cancers.

We have validated the protocol from a variety of fresh frozen animal and human tissues. Unlike plug lysis, this solution-based animal tissue DNA isolation protocol provides additional value in that it is amenable to automation, providing a solution for researchers who must 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 Bionano 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. Ultra long DNA molecules can be isolated using Bionano Prep Solution Phase (SP) DNA isolation kit or a conventional plus-lysis method. DNA can be labelled in a non-destructively with Direct Label Stain (DLS) chemistry. 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) 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

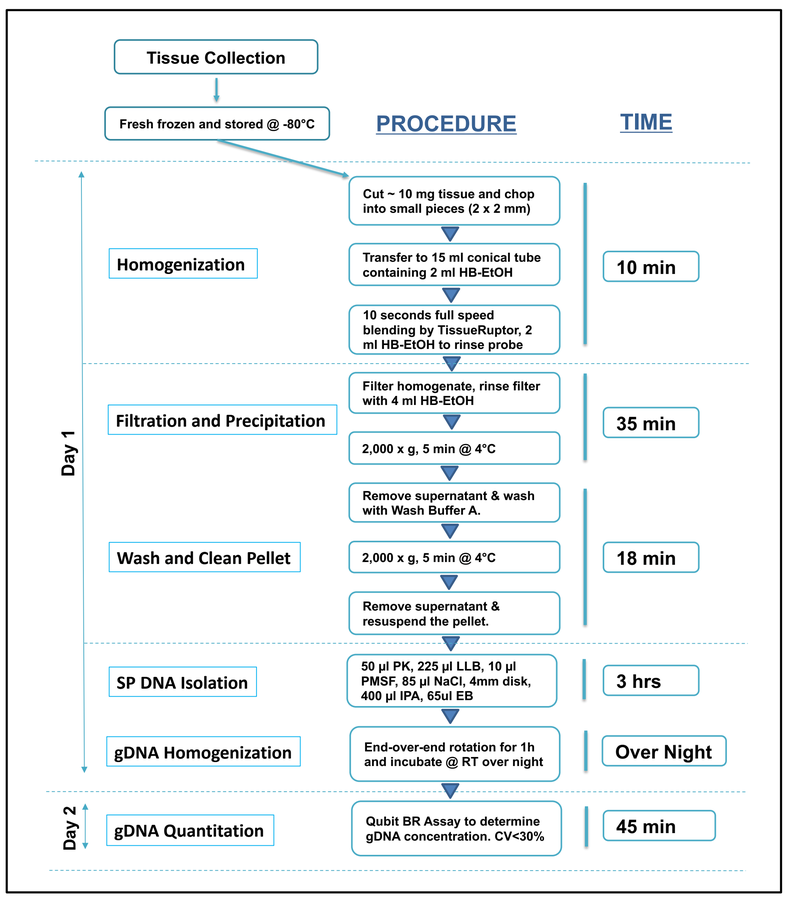


Figure 1. UHMW gDNA SP Isolation from 10 mg of Fresh Frozen Tissue

Table 1. Single Molecule DLS Metrics from Fresh Frozen Brown Norway Rat Tissues and Human Solid Tumor Tissues

Tissue Type	No. of Reps	DNA Con. [ng/µl]	DNA Yield [µg/10mg tissue]	Post stain [ng/µl]	N50 [>20Kbp]	N50 [>150Kbp]	Map Rate [%]	Label Density [/100Kbp]	Throughput [Gbp/scan]
Lung - R	20	254	17	12.2	206	289	75.5 *	16.3	45
Bladder - R	2	179	12	10.2	171	266	71.6 *	15.9	46
Liver - R	11	205	13	10.1	171	268	66.9 *	16.0	36
Colon - R	22	200	13	11.0	159	264	66.5 *	15.8	37
Uterus - R	28	199	13	15.2	158	261	71.6 *	16.5	36
Kidney - R	4	225	15	9.7	235	323	74.5 *	16.9	48
Ovary - R	8	142	9	11.5	174	272	72.1 *	16.5	43
Testes - R	3	152	10	9.6	191	278	70.2 *	15.6	38
Prostate - R	4	202	13	11.3	221	302	71.4 *	16.5	55
Lung - H	3	153	10	13.0	261	309	92.4 **	15.2	50
Bladder - H	3	199	13	9.3	311	355	92.2 **	15.2	66
Liver -H	3	196	13	17.4	264	306	90.0 **	14.9	84

R: Rat; H: Human

* The maximum mapping rate we have achieved for Brown Norway rat is ~80% due to the quality of the reference genome.

** The theoretical maximum mapping rate for human genome is ~94% due to centromeres and telomeres.

Figure 1 shows the workflow of Bionano Prep SP Animal protocol, which consolidated the two existing Bionano Prep Soft/Fibrous Animal Tissue DNA Isolation Protocols with a simplified single front-end tissue homogenization step.

Using the new Bionano Prep SP Animal protocol, we have successfully isolated UHMW gDNA from 5-10 mg of fresh frozen animal and human tissues. The results show (Table 1) the qualities of the DNA and single molecule DLS met and exceeded current Bionano Prep data collection standards.

Conclusions

Bionano Prep SP provides a rapid gDNA isolation protocol for fresh frozen animal and human tissues (~6 hours total time/6-8 samples), improving capability for research of genome finishing, structural variant comparative analysis and cancers. 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.

Reference

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