Application Note

Automated Extraction of Ultra High Molecular Weight (UHMW) DNA for Bionano Optical Genome Mapping on the Hamilton Long String STAR V

Long String Genomics

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Introduction

NGS methods are well established for detecting most sequence variants. However, these approaches are unable to accurately resolve complicated repetitive or high GC content regions of the genome. This makes the detection of structural variants especially challenging. With Bionano Optical Genome Mapping (OGM), structural variants can be detected in an unbiased, genome-wide fashion at high sensitivity. This revolutionary technology sheds light on previous "blind spots" for conventional sequencing and cytogenetic assays; however, it requires isolation of UHMW DNA. These molecules, typically ranging between 250 kbp up to > 1 Mbp in length, are more difficult to obtain, as compared to conventional isolation procedures.

In response to this technical challenge, Hamilton has partnered with Bionano Genomics to fully automate this process with specially designed hardware to handle paramagnetic disks.

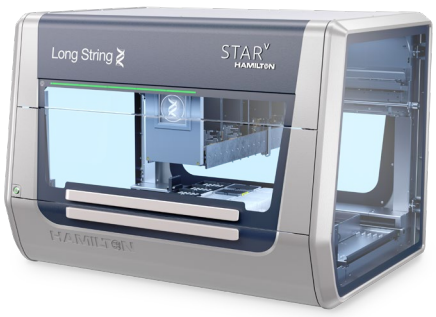


Figure 1: The Hamilton Long String STAR V.

This allows for the binding and release of genomic DNA with significantly less fragmentation. UHMW DNA extracted from this automated process is labeled and produces high-quality data for OGM analysis.

In the ever-evolving field of genomic research, technological innovation offers the opportunity to gain insight into previously unresolved questions about genome biology. The mapping of ultra-long DNA molecules transforms the way that we look at the genome, with unprecedented resolution on its structure.

Hamilton now provides the world's first automated solution for Ultra High Molecular Weight (UHMW) DNA extraction on the Long String STAR V. With this highly efficient and time-saving sample processing solution, Optical Genome Mapping (OGM) can be achieved at higher scale.

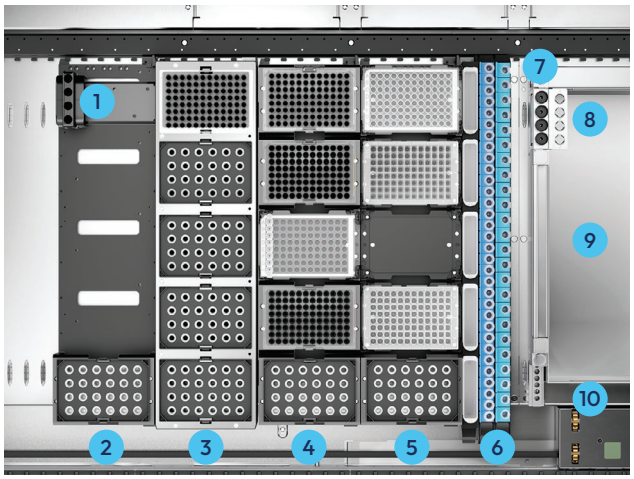
- Scalable preparation of UHMW DNA for Bionano OGM for increased number of samples
- Optimal magnetic disk handling through Hamilton MagRod technology avoids DNA shearing
- Automated walk-away workflow with instrumentation for reduced hands-on time

Method Description

The method is based on the Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol (30398), with adaptations optimized for the automated process. In brief, frozen HL-60 cell pellets containing 1.0 million cells are manually resuspended in DNA stabilizing buffer containing RNase A and provided to the system in a 96 deep well plate. In the current method, automated resuspension of cell pellets and RNase A pipetting have not been implemented. The following steps are then performed entirely on the Long String STAR V instrument.

Lysis and digestion are performed in specifically formulated buffer, together with Proteinase K. UHMW DNA is then bound to Bionano Prep SP Disks by adding isopropanol. Three subsequent washing steps are performed to remove contaminants from the DNA bound to the disks. For final elution, the disks are transferred to strips prefilled with elution buffer, placed on top of a microtiter plate.

In the end, the user can then perform an off-deck centrifugation step, which leaves the final eluate in the microtiter plate while the disks are retained in the Hamilton Elution Strips. This process results in UHMW DNA extracts suitable for Bionano Genomics' downstream Direct Label and Stain (DLS) process, that enzymatically applies genome-specific label patterns, for which data can be finally collected on the Bionano Genomics Saphyr® system.



1. Hamilton Quad CO-RE® Gripper
2. MFX Tip Module with MagRod Sleeve Rack
3. Carrier with Tips and MagRod Sleeve Racks
4. Carrier with Tips, Microtiter Plate and Sleeve Rack
5. Carrier with Microtiter Plate, Deep Well Plates and MagRod Sleeve Rack
6. Reagent Trough and Sample Tube Carriers
7. MagRods
8. Sleeve Eject Plate
9. Solid Waste
10. Barcode Reader

Figure 2: The Long String STAR V Deck Layout.

Discover the bigger picture more quickly

Deliver novel discovery in genetic testing with UHMW DNA extraction for Optical Genome Mapping

System Description

The Long String STAR V is an Assay Ready Workstation with a standardized hardware configuration for automated magnetic disk or magnetic bead handling workflows. The product is suitable for Research Use Only (RUO). It is based on the Microlab® STAR V platform and features four state-of-the-art 1 mL CO-RE® pipetting channels, as well as four 5 mL CO-RE® channels for disk handling steps.

Application Software

The Long String STAR V VENUS Framework has been designed and developed specifically for method developments on the Long String STAR V.

Kit Description

Bionano's SP Blood & Cell Culture DNA Isolation Kit (80042) was the basis for developing an automated workflow for UHMW DNA Extraction on the Long String STAR V. The established protocol provides UHMW DNA from a simple lyse, binding, washing and elution procedure. Instead of magnetic beads or silica spin columns, the kit uses paramagnetic disks, which are covered by a nanostructured silica surface. This provides a high DNA binding capacity and helps to protect the bound DNA from shearing forces. The recovered UHMW DNA is typically between 250 kbp up to > 1 Mbp in size and ranges in concentration from 50–120 ng/µL. The automated protocol was tested, using human leukemia cell line HL-60 derived cell pellets.

Visual Workflow

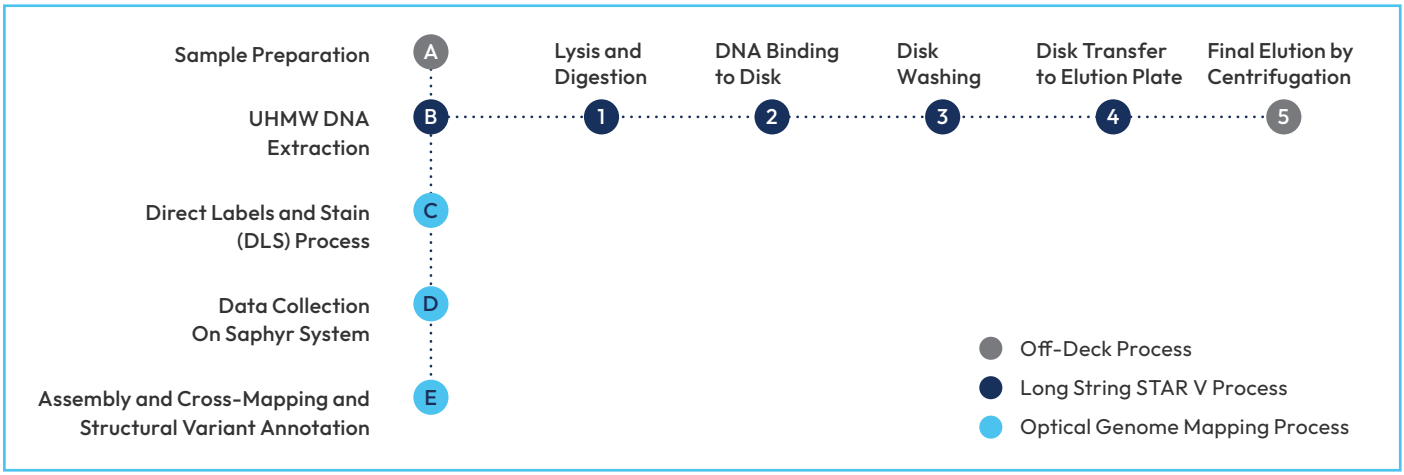


Figure 3: Graphical overview of the Bionano workflow for Optical Genome Mapping, including the automated UHMW DNA Extraction process on Hamilton's Long String STAR V.

Qualification Setup

HL-60 cells (DMSZ, ACC3) were cultured in DMEM + 10% FBS and 1x Pen/Step at 37°C in a humidified atmosphere containing 5% CO_2. Cells were harvested by centrifugation and stored as cell pellets of 1.5 M HL-60 cells frozen at -80°C prior to use. On the day of the experiment, cell pellets were thawed at 37°C for 30 seconds and resuspended in 40 µL DSB/RNase Cocktail mastermix by manual pipetting. The resulting cell suspensions were pooled and a volume corresponding to 1.0 million cells was transferred to individual wells of a 96 deep well plate and provided to the Long String STAR V. After performing the on-deck lysis and digestion, binding, washing and disk transfer to strips stacked on top of an elution plate, centrifugation of the elution plate assembly was performed at room temperature for 1 min at 2200 rcf. The method was qualified for processing of 12 samples per run. In total, 4 biological qualification runs were performed with identical parameters (3 runs with 4 samples, 1 run with 12 samples).

After elution of UHMW DNA on the Long String STAR V, samples were sheared 4x using a standard bore 200 µL tip, followed by 1 hour of end-over-end mixing on a Hula mixer, and incubation o/n at room temperature. UHMW DNA was quantified, using the Qubit dsDNA BR Assay Kit (Cat. No. Q32853) according to manufacturer's instructions. DNAs were then labeled, using a modified version of the Bionano Prep Direct Label and Stain (DLS) Protocol (30206, Rev G9). Labeled DNAs were quantified, using Qubit 1X dsDNA HS Assay Kit (Q33230) and loaded on Bionano Chips and run on a Saphyr instrument according to the System User Guide (PN 60325). 400 Gbp of optical data for molecules > 150 kbp was collected for all samples.

Technology

Optimal magnetic disk handling is ensured by Hamilton's proprietary MagRod technology. Paramagnetic disks can be manipulated by 5 mL pipetting channels directly without the need for additional on-deck equipment. Adjustable parameter settings ensure optimal workflow results. Hamilton MagRod Sleeves are manufactured with the highest precision and shaped for the best possible magnetic disk positioning. The perfect fit protects the MagRods from surrounding liquids and reagents. Hamilton Elution Strips are used to separate the magnetic disks from the final UHMW DNA sample by centrifugation. The disk remains in the strip, while the eluate is drawn into a microtiter plate.

Results

The results obtained by the automated process indicate that sample yields are reproducible and match the expected values from manual extractions, as specified in the Bionano Prep SP Frozen Cell Pellet DNA Isolation Protocol (30268 Rev D) (Figure 4A, blue bars).

Additional sample concentration measurements were taken after DLS labeling to ensure sufficient yields for further processing (Figure 4A, gray dots). The data indicated that suitable amounts of DNA were recovered and present for all samples isolated on the Long String STAR V for collection on the Saphyr.

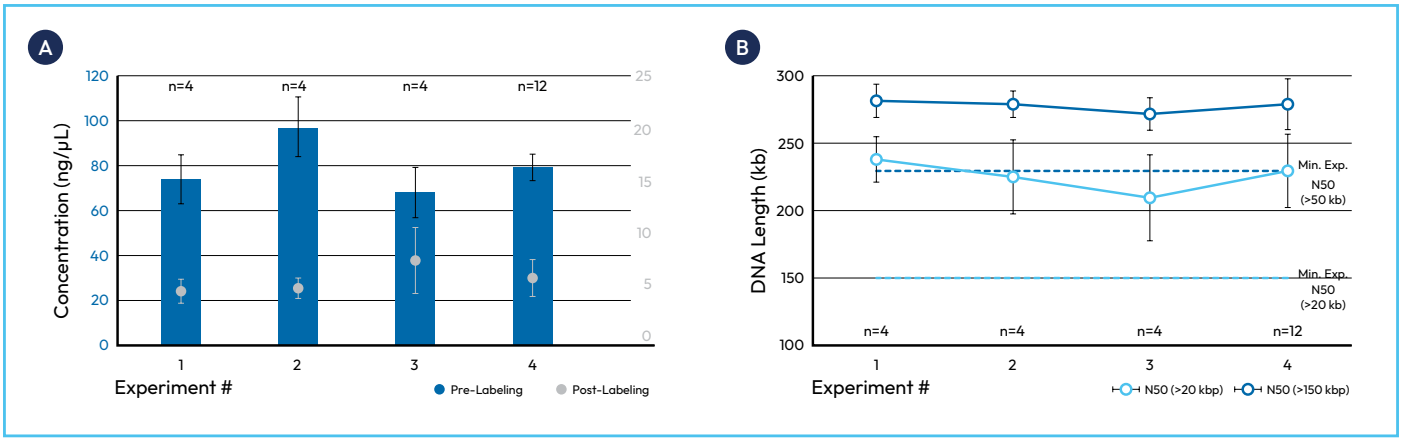


Figure 4: Concentrations and molecule sizes of UHMW DNA extracted with the Long String STAR V. A) Concentrations of samples measured by Qubit assay after automated extraction (blue bars, plotted on the left y-axis) and manual downstream labeling (gray dots, plotted on the right y-axis). B) DNA lengths estimated by data analysis obtained from individual Saphyr runs. N50 of molecules that are 20 kbp and longer or 150 kbp and longer are indicated. Minimal expected threshold levels are indicated by dashed lines. Sample numbers for all experiments are indicated. Data points represent mean values, and error bars represent SD values within single experiments.

Data analysis of samples run on the Saphyr instrument revealed that molecule lengths exceed the thresholds provided by Bionano Genomics and demonstrate the presence of UHMW DNA larger than 230 kbp (Figure 4B). The DNA isolated from the Hamilton Long String STAR V contains Mbp length molecules which overall exceed the OGM quality threshold of 230 kbp post extraction, labeling, linearization, and imaging.

Further QC metrics were derived from the data collected on the Saphyr instrument (Figure 5). All samples showed consistent labeling performance within the ranges of specificity and sensitivity (Figures 5A, B). Map Rates surpassed the thresholds for quality metrics provided by Bionano Genomics (Figure 5C).

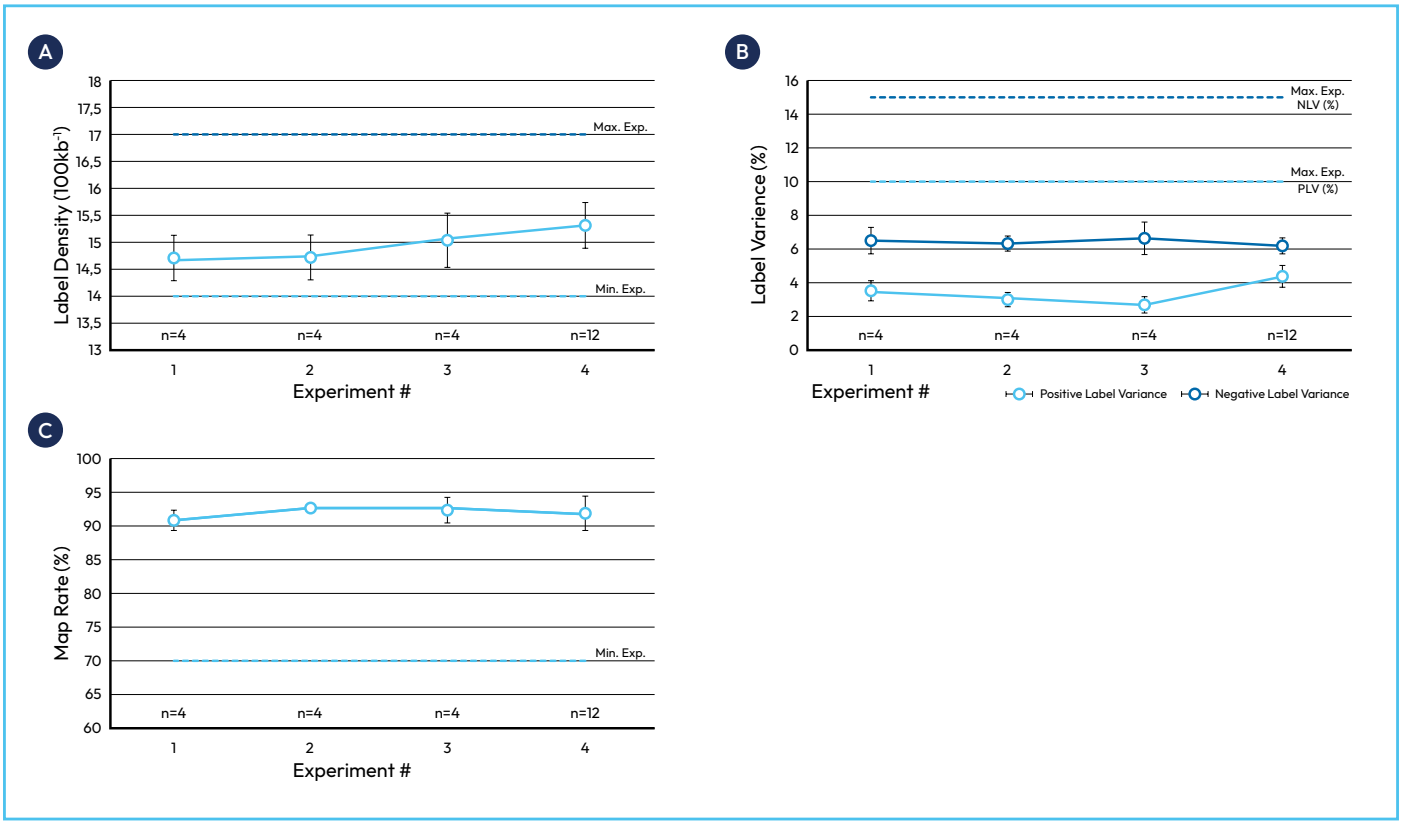


Figure 5: Key performance target metrics of optical data collected from UHMW DNA samples extracted with the Long String STAR V. A) Enzymatically labeled sites per 100 kbp. B) Positive Label Variance (PLV; percentage of labels absent in reference) and Negative Label Variance (NLV; percentage of reference labels absent in molecules). C) Percentage of molecules that are 150 kbp or longer that were successfully mapped to the human reference genome. Minimal and maximal expected threshold levels are indicated by dashed lines. Sample numbers for all experiments are indicated. Data points represent mean values and error bars represent SD values within single experiments.

Throughput and Capacity

The Long String STAR V can process up to 12 samples per run and 24 samples in a single working day (8 hours). This represents a significant increase over the manual method, for which 6 samples per run are recommended.

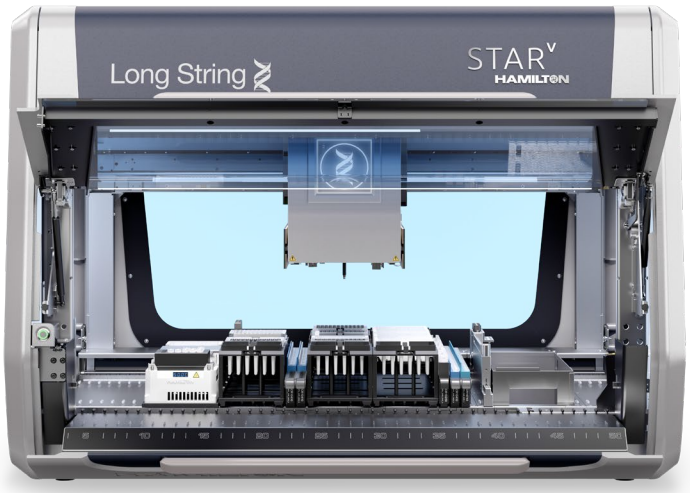
Summary

As experts in precision automated liquid handling and a focus on genomic workflows, Hamilton is stepping into the most advanced application areas. We have partnered with Bionano Genomics to establish the world's first automation solution for UHMW DNA extraction. Through this collaboration with the leading industry kit and technology provider, we seek to empower the scientific community. In conjunction with Optical Genome Mapping, the Long String STAR V will enable consistent and reproducible extraction of UHMW DNA from samples of interest at an unprecedented scale and will provide another layer of structural variant data to your genetic research.

Samples per run	Duration (h:min)
4	1:37
12	3:48

Table 1: Execution times of runs for UHMW DNA Extraction on the Long String STAR V, dependent upon sample number.

Others	
System Requirements	**Part Number / Provider**
Long String STAR V	806699 / Hamilton
System Dimensions	
Width	149 cm
Depth	95.9 cm
Height with closed door	96.3 cm
Height with open door	100.5 cm
Weight approx.	250 kg
Labware and Reagents	**Part Number / Provider**
50 µL CO-RE Tips Filter	235984 / Hamilton
300 µL CO-RE Tips Filter	235903 / Hamilton
1000 µL CO-RE Tips Filter	235905 / Hamilton
Deep Well Plates (2.2 mL) PP	235656 / Hamilton
MagRod Sleeves	10149424 / Hamilton
Elution Strips	10149425 / Hamilton
20 mL Reagent Reservoirs	96424-02 / Hamilton
Microplate 96/U, Wells Clear, RecoverMax	0030601203 / Eppendorf
2.0 mL Screw Cap Microtubes	72694.406 / Sarstedt



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