

Automated Isolation of High Molecular Weight (HMW) DNA from Human Blood Samples with PacBio® Nanobind® Technology on the Hamilton NIMBUS® Presto – Next Level Preparation of Extracts for Long-Read Sequencing

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Introduction

Long-read sequencing technology provides great potential in several clinically-relevant areas of human genomics. Recent advancements allowing increased sequencing throughput and accuracy at a more affordable cost have already been made. However, the applied workflows still contain bottlenecks, especially in the extraction process steps that increase variability in HMW DNA (50 kbp –250 kbp) yield and quality, while hindering the processing of higher sample numbers. Overcoming these hurdles by robust high-throughput, automated solutions will support the growing number of large-scale population genomics studies and clinical research applications.

Method Description

We have successfully automated the PacBio Nanobind HT 1 mL whole blood kit and Nanobind HT CBB kit on the Hamilton NIMBUS Presto instrument for the extraction of HMW DNA from fresh human blood samples. Two separate methods have been developed, supporting the processing of either 1 mL or 200 µL of whole blood. PacBio Nanobind magnetic disks (i.e. Nanobind disks) are covered with nanostructured silica that prevent DNA shearing and allow for the purification of high-quality, HMW DNA using traditional bind, wash, and elution workflows.

Blood samples were collected in K2-EDTA tubes and shipped overnight at 4 °C, frozen at –80 °C upon arrival and thawed at 37 °C for 15 minutes prior to extraction. White blood cell counts (defined as 10⁹ cells/Liter) were determined using a HemoCue® system. 1 mL or 200 µL of fresh human blood were provided to the NIMBUS Presto in a 24- or 96 Deep-Well Plate respectively. The workflow involves Proteinase K and RNase A digestion prior to Nanobind disk binding. Washing and elution steps are all

performed in Deep-Well Plates.

Quality control procedures were performed after eluted DNA was allowed to rest overnight at room temperature. The concentration was measured with the Qubit 3.0 system with dsDNA BR Assay Kit (Thermo Fisher Scientific). Femto Pulse (Agilent) runs were performed to estimate the HMW DNA size. Samples were sheared on the Diagenode Megaruptor 3 system (concentration = 50 ng/µL, volume = 100 µL, shear speed = 31). Libraries were prepared for sequencing using PacBio SMRTbell prep kit 3.0 and Sequel II binding kit 3.2. Final libraries were sequenced on a PacBio Sequel IIe system.

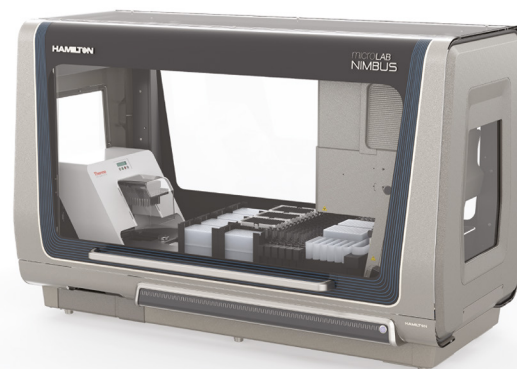


Figure 1: The Hamilton NIMBUS Presto Assay Ready Workstation

- Consistent high quality and quantity of HMW DNA
- Higher throughput isolations from clinically-relevant samples
- Safe blood sample processing and minimal hands-on time by users needed

Moving into the Long-Read, Third-Generation Sequencing Era with Automation from Hamilton

System Description

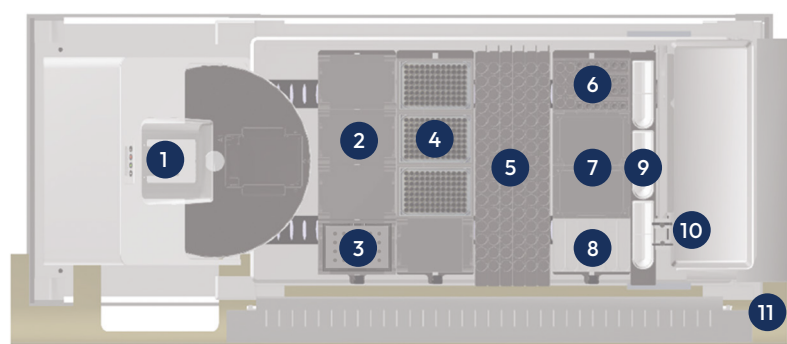


Figure 2: Layout of the NIMBUS Presto for HMW DNA extraction

- 1 KingFisher™ Presto & Turntable
- 2 DWP Stack (Storage)
- 3 Ambion Magnet
(PN 282661, must be purchased separately)
- 4 Tips
- 5 Sample Loading Area
- 6 Multi-tube Adapter for Reagents
- 7 DWP Position (Working Area)
- 8 60 mL Troughs for Buffers
- 9 200 mL Troughs for Wash Solutions
- 10 CO-RE® Gripper
- 11 Barcode Reader

Visual Workflow

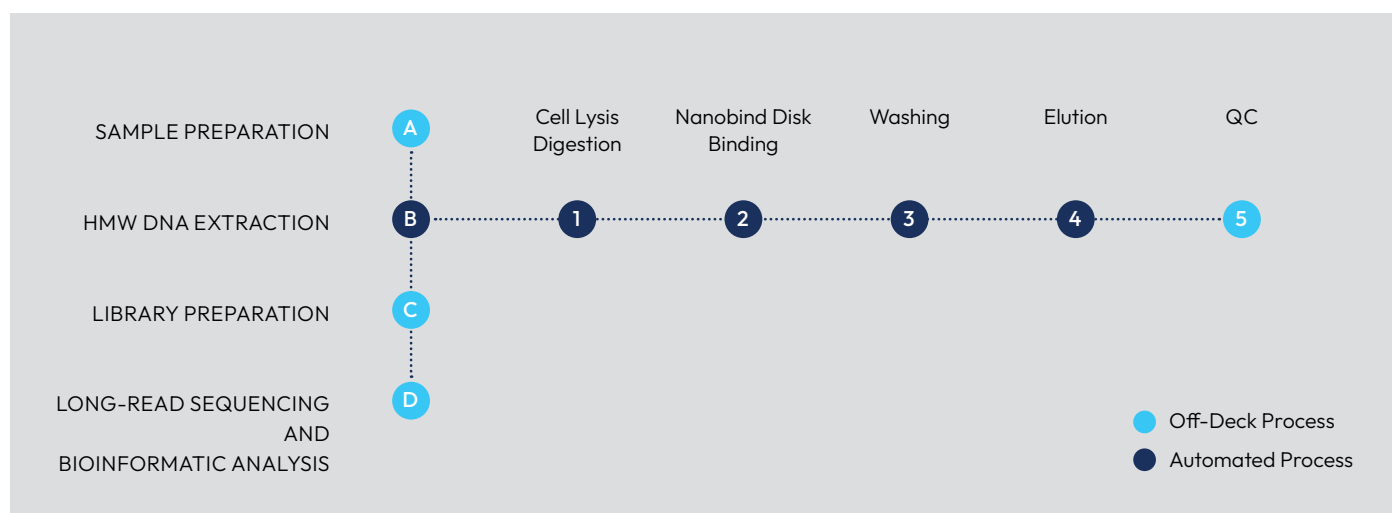


Figure 3: Graphical Overview of the PacBio Nanobind Workflow for Long-Read Sequencing NIMBUS Presto for HMW DNA extraction

Technology

The HMW DNA extraction process benefits from the parallel-processing capability of Hamilton's NIMBUS Presto Assay Ready Workstation solution. While the Microlab NIMBUS is handling sample and plate preparation steps, the fully-integrated KingFisher™ Presto instrument is handling the high-throughput extraction workflow steps. Proven for magnetic particle-based extraction processes, the KingFisher™ Presto application spectrum can be expanded to include PacBio Nanobind disk-based extraction.

Yield and Quality

DNA concentration measurements indicated the expected correlation between total yield and White Blood Cell count (WBC). Within the normal physiological range of white blood cell counts, the NIMBUS Presto reliably delivered sufficient DNA for further long-read sequencing library preparation (Figure 4).

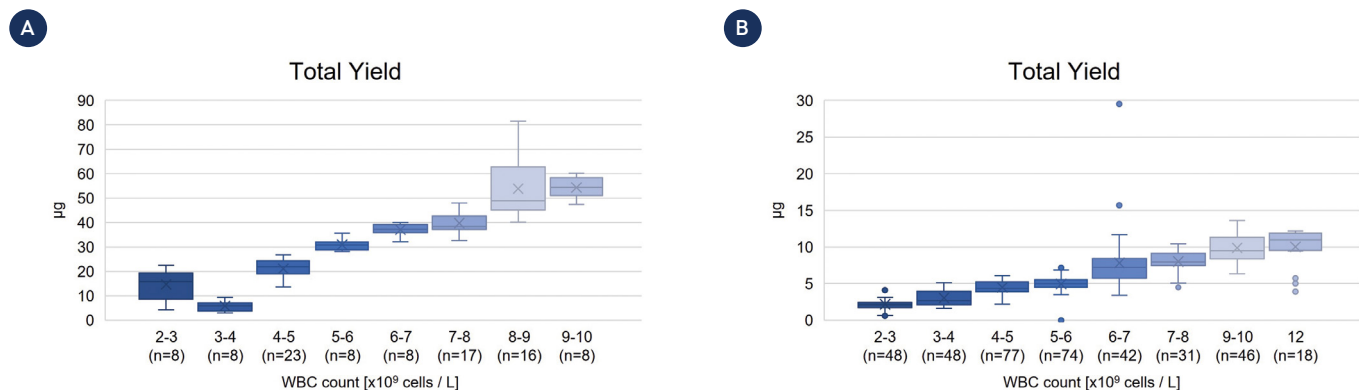


Figure 4: HMW DNA yields obtained from whole blood samples from donors grouped according to White Blood Cell count (WBC). Total high-quality, HMW DNA per sample extracted from either A) 1 mL or B) 200 µL of human blood, as estimated by Qubit assays. The data represents the results collected across 4 full size runs. HMW DNA eluate volumes are 200 µL. Please note the strong relationship between yield and WBC. The boxplots depict the distribution of single eluates. Minimum and maximum (whisker borders) as well as mean value line with quartile ranges are indicated. Outliers are depicted as single dots. Sample numbers for each WBC group (number of white blood cells per Liter of whole blood) are indicated in brackets.

Additionally, the NIMBUS Presto reliably delivered DNA samples of high purity in the physiological WBC range (Figure 5). Also, samples with slightly lower purity estimates (as observed in the lower WBC groups) have

performed well in downstream library preparation processes. Overall, the NIMBUS Presto delivers reproducibly-consistent DNA recovery and purity across all tested donors.

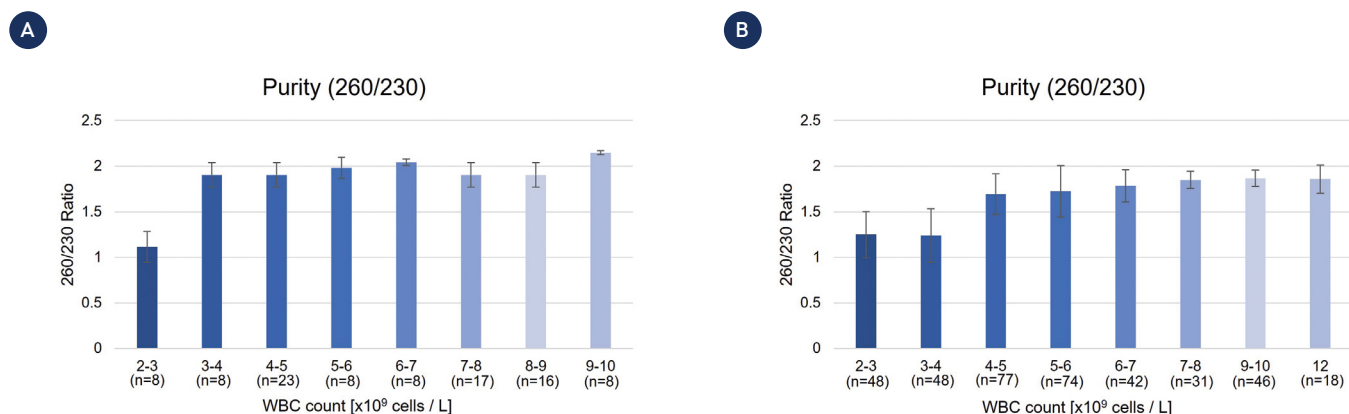


Figure 5: HMW DNA purities obtained from whole blood samples per White Blood Cell count (WBC) group. DNA purity of samples extracted from either 1 mL A) or 200 µL B) of human blood, as spectrophotometrically assessed by the 260/230 nm absorbance ratio, determined by Nanodrop. Optimal purity results were obtained from samples with white blood cell counts in the normal range of 4-12 x10⁹ cells / L. The data is depicted as mean values ± standard deviation. Sample numbers for each WBC group (number of white blood cells per Liter of whole blood) are indicated in brackets.

DNA size estimation confirmed the presence of HMW DNA that can be accurately sheared for further HiFi library preparation (Table 1). Long-read sequencing

data showed that read numbers as well as read length distributions are within the expected ranges (Table 2) and are comparable to manual DNA extractions.

Quality Metric		200 µL blood	1 mL blood
unsheared	DNA length (kbp)	126.8 (±10.7)	128 (±18.4)
	GQN at 10 kbp	9.5 (±0.1)	9.4 (±0.05)
sheared	DNA length (kbp)	15.1 (±2.2)	17.1 (±1.5)
	GQN at 10 kbp	8.5 (±0.5)	9 (±0.2)

Table 1: DNA molecule lengths of HMW DNA eluates generated on the NIMBUS Presto estimated by Femto Pulse analyses. Samples were measured in unsheared and sheared mode for both the 200 µL and the 1 mL protocols. Data are represented as mean DNA molecule length values ± standard deviations indicated in brackets. GQN at 10 kbp represents a genomic DNA quality number defined by Agilent and indicates the fraction of DNA molecules above 10 kbp (with a value range between 0 and 10).

Quality Metric		200 µL blood	1 mL blood
HiFi Yield (Gbp)		37.3	36.7
# HiFi reads		2'063'841	2'142'750
HiFi mean read length (kbp)		18.1	17.2

Table 2: HiFi long read sequencing quality of libraries generated from HMW DNA eluates originating from the NIMBUS Presto. Total HiFi Yield, the number of HiFi reads as well as mean read length distribution were estimated for both the 200 µL and the 1 mL protocols.

Throughput and Capacity

For the 1 mL whole blood protocol, processing a full-sized run with 24 samples (including hands-on time) requires 2h 35 min. For the 200 µL whole blood protocol, processing a full-sized run with 96 samples (including hands-on time) requires 2h 40 min.

Discussion / Summary

Driven by continuous innovations, the third-generation sequencing of long DNA molecules provides accurate detection of single nucleotide and structural variants. Furthermore, it goes beyond the capability of conventional NGS methods and enables coverage of hard-to-sequence regions of the genome, haplotype phasing, and epigenetic profiling.

However, the advantages for the scientific community are somewhat dampened by challenging manual extraction protocols requiring a high amount of user experience. Therefore, achieving stable yields and quality remains a challenge. This is especially important in the clinical setting. Automated extraction of HMW DNA powered by the Hamilton NIMBUS Presto ensures efficient sample processing, while guaranteeing high quality and yield. Our automation solutions bridge existing gaps in sample preparation, thus bringing benefits from the rapidly evolving third-generation sequencing area to the field. Ultimately, such integrated solutions will enhance the detection of pathogenic variants, especially in rare diseases, and they enable pharmacogenomic discoveries to drive personalized medicine.

Others

System Requirements	Part Number	Provider
General		
NIMBUS Extended HD Presto Base + Deck Components	49000-38	Hamilton Bonaduz AG
Std. PC Win 10, US + USB PCI Upgrade Kit	10113952	Hamilton Bonaduz AG
KingFisher Presto 96 Instrument (including Head and Heating Block)	10102396	Hamilton Bonaduz AG
Ambion Magnetic Stand 96	282661	Hamilton Bonaduz AG
For 1 mL whole blood processing		
KingFisher Presto 24-Well Head (including Heating Block)	10102427	Hamilton Bonaduz AG

System Requirements	Part Number	Provider
General		
60 mL Reagent Reservoir	56694-01	Hamilton Bonaduz AG
200 mL Reagent Reservoir	56695-01	Hamilton Bonaduz AG
1000 µL Conductive Filter Tips	235905	Hamilton Bonaduz AG
300 µL Conductive Filter Tips	235903	Hamilton Bonaduz AG
300 µL Wide Bore 0.71 mm Orifice Conductive Filter Tips	235452	Hamilton Bonaduz AG
For 1 mL whole blood processing		
Nanobind HT 1 mL whole blood kit	102-762-800	PacBio
KingFisher 24 Deep-Well Plate, sterile	95040480	Thermo Fisher Scientific
KingFisher 24 Deep-Well Tip Comb and Plate, sterile	97002620	Thermo Fisher Scientific
For 200 µL whole blood processing		
Nanobind HT CBB kit	102-762-700	PacBio
KingFisher 96 Deep-Well Plate, sterile	95040460	Thermo Fisher Scientific
KingFisher 96 Deep-Well Tip Comb and Plate, sterile	97002820	Thermo Fisher Scientific

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