HAMILTON

APPLICATION NOTE

Automated extraction of High Molecular Weight (HMW) DNA with PacBio® Nanobind® technology on the Hamilton NIMBUS® Presto Assay Ready Workstation

Authors: Jeffrey M. Burke[1], Kim Neufeld[2], Lucas Durandi[2], Enrique Bayo Iglesias[2], Jörg Breitling[2], Dominik Laubscher[2], Nadine Thaler[2], Kelvin Liu[1], Birgit Ottenwälder[2]

[1]Pacific Biosciences, 1305 O'Brien Dr, Menlo Park, CA 94025 USA
[2]Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland

Introduction

Rapidly emerging long-read sequencing technologies, including PacBio Single Molecule, Real-Time (SMRT®) sequencing, provide substantial advantages for *(de novo)* genome assembly, detecting structural variants, and higher accuracy in repetitive or high GC content regions. A key requirement for long-read, third-generation sequencing approaches is the extraction of HMW DNA (50 kb – 250 kb). As the field matures and sample numbers increase, robust, high-throughput sample preparation methods are needed to support large-scale studies in pan-genomics and clinical research applications.

- Reliable isolation of HMW DNA from bacterial and human cell culture samples
- Complete walk-away solution without the need for user interaction
- Higher capacity of sample numbers to be processed

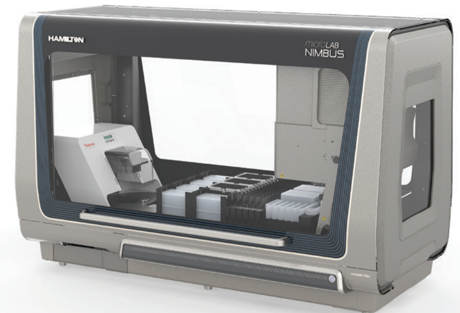


Figure 1: The Hamilton NIMBUS Presto Assay Ready Workstation

Method Description

We have successfully automated the PacBio Nanobind HT CBB kit on the Hamilton NIMBUS Presto instrument for the extraction of HMW DNA from Gram-negative bacteria and human cells.
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.

In brief, $5x10^8$ *E. coli* cells were pelleted by centrifugation at 16000 x g for 1 min at 4°C. Alternatively, frozen pellets of $1x10^6$ HL-60 cells (human leukemia cell line) were used. Samples were resuspended in 50 µL of 1X PBS and provided to the NIMBUS Presto in a 96 deep-well plate. The workflow involves Proteinase K and RNase A digestion prior to Nanobind disk binding. Washing and elution steps are all performed in 96 deep-well plates.

Quality control procedures are performed after eluted DNA is allowed to rest overnight at room temperature. The Qubit 3.0 system measurements with dsDNA BR Assay Kit (Thermo Fisher Scientific) were performed to determine sample concentrations. Pulse-Field Gel Electrophoresis (PFGE) runs were performed on a Bio-Rad CHEF DR II system, using SYBR Gold staining to determine DNA size. Cross-contamination checks were performed by Qubit system measurements and qPCR assays.

PacBio

Moving into the long-read, third-generation sequencing era with automation from Hamilton

System Description

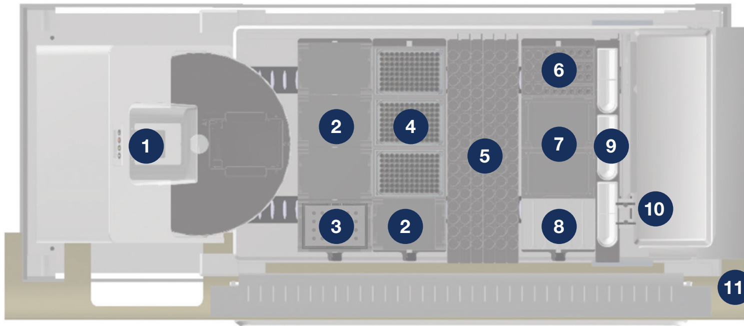


Figure 2: Layout of the NIMBUS Presto for HMW DNA extraction

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

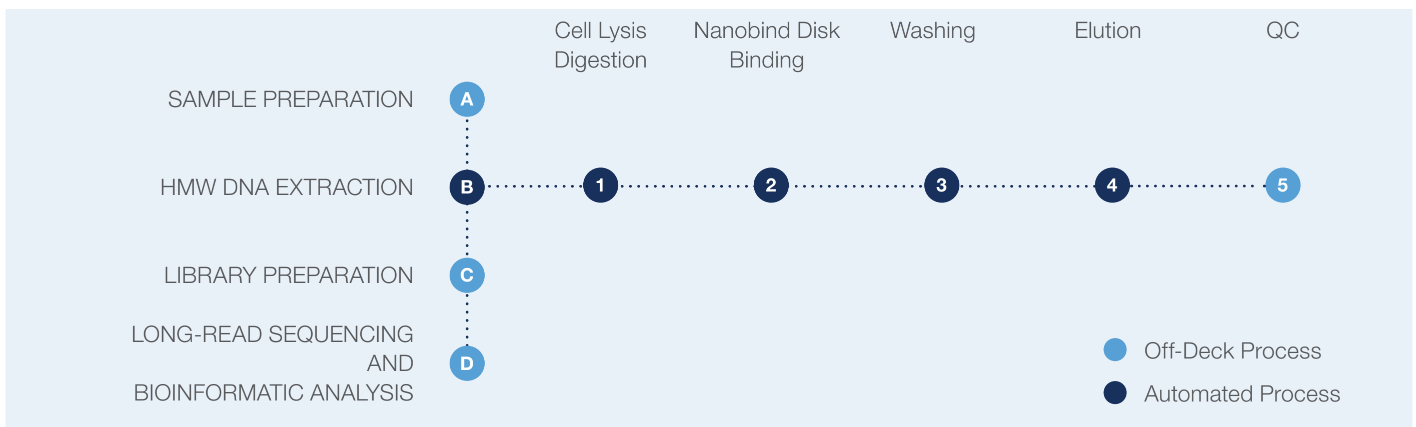


Figure 3: Graphical overview of the PacBio Nanobind HT CBB kit workflow for long-read sequencing

Technology

The HMW DNA extraction process benefits from the parallel processing capability of Hamilton's NIMBUS Presto Assay Ready Workstation solution. While Microlab® NIMBUS® is handling sample and plate preparation steps, the fully integrated KingFisher™ Presto instrument is handling the high-throughput extraction workflow portion.

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 concentrations of fi ve biological verifi cation runs with different sample sizes were determined for both E. coli and HL-60 cells. The average sample yield was 62.8 ± 14.7 ng/µL for E. coli and 61.1 ± 3.64 ng/µL for HL-60 cells (Fig. 4A). Elution volumes were 100 µL per sample, resulting in an average HMW DNA yield of 6.3 µg and 6.1 µg for E. coli and HL-60, respectively. A comparison of automated and manual preparation revealed similar yields for E. coli cells. For human HL-60 cells, no direct comparison between automated and manual procedure was made. However, compared to semi-automated extraction from GM12878 cells on a KingFisher™ Duo Prime instrument, NIMBUS Presto extraction was of similar efficiency (Fig. 4B). Considering the input cell numbers, all metrics matched the expected target range values specified by the kit handbook.

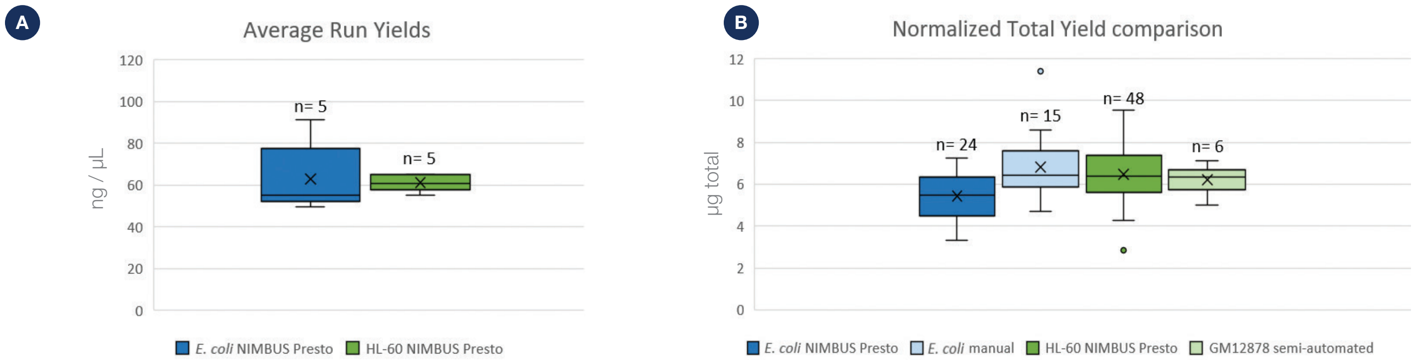
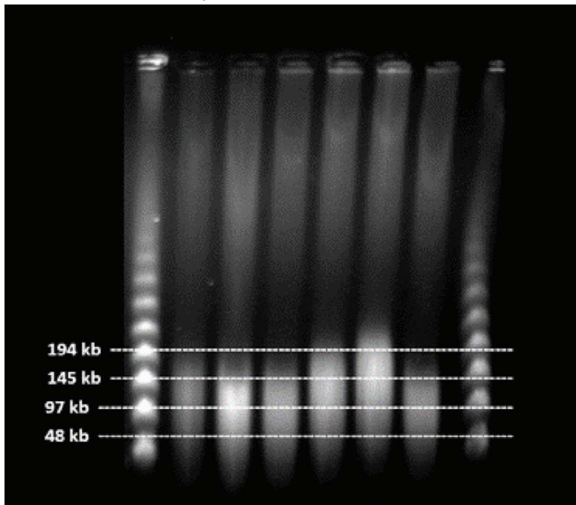


Figure 4: HMW DNA yields obtained from manual and (semi-) automated workflows
A) Comparison of mean HMW DNA concentrations across different biological verification runs obtained with NIMBUS Presto. Run numbers are indicated and were performed with different sample sizes (blue: *E. coli* with 24, 24, 48, 48 and 96 sample runs / green: HL-60 with 8, 16, 32, 48 and 48 sample runs). Mean sample concentrations were calculated for each experiment. The boxplot depicts the distribution of these mean concentrations. B) Total HMW DNA yields of individual verification runs compared to manual extraction. Sample sizes are indicated. Yields are normalized to $1x10^8$ *E. coli* cells (blue) and $1x10^6$ human cells (green). The boxplots depict the distribution of single eluates within each run. Minimum and maximum (whisker borders) as well as mean value line with quartile ranges are indicated. Outliers are depicted as single dots

To estimate HMW DNA molecule length, Pulsed-Field Gel Electrophoresis (PFGE) was performed. Loading dye was added to 7 µL of eluate and loaded on a 1% agarose gel. The gel was run on a BIORAD CHEF-DR II, with the following parameters: 5.5 V/cm, 14 °C, pump speed 70, start switch time 35 min, end switch time 90 min, in 0.5% TBE buffer, for 22 h. The gel was then imaged using a DNR MiniBIS Pro imaging system. Results revealed HMW DNA with the expected molecule lengths as specified by PacBio (50 – 250 kb) (Figure 5).

E. coli HMW DNA samples



HL-60 HMW DNA samples

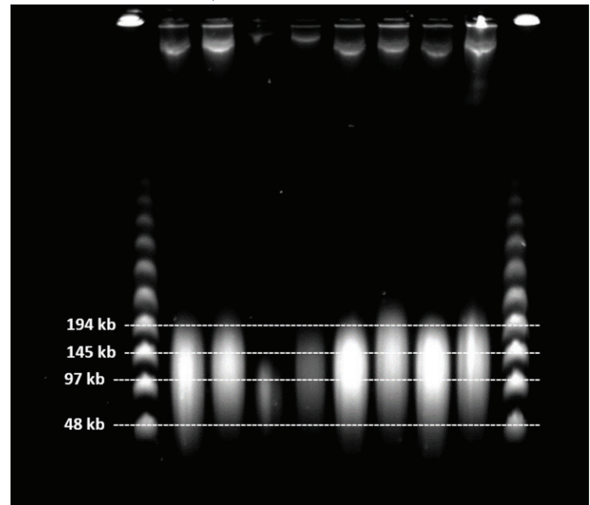


Figure 5: Pulsed-Field Gel Electrophoresis gel image of extracted HMW DNA samples using NIMBUS Presto
Estimated average DNA size was 120 kb, with fragments ranging from ~50 – 250 kb. For *E. coli*, selected samples of a 24-sample run are displayed. For HL-60, 2 low concentration (lane 3 and 4) and 4 normal concentration samples from a 16-sample run are displayed.

Extraction results obtained with the NIMBUS Presto are comparable to standard manual processing (regarding both concentration and size distribution) and suitable for downstream long-read sequencing approaches.

Carryover

Checkerboard assays were performed to detect possible cross-contamination. Parallel processing of negative control samples (using PBS instead of cell pellets) and *E. coli* or HL-60 cells confirmed that no cross-contamination was detectable. Potential carry-over was excluded by the Qubit system concentration measurements (Fig. 6A, 6B). Additionally, qPCR was performed for individual runs using the TaqMan Fast Advanced Master Mix (REF 4444556) with GAPDH (Hs02786624_g1) or *zntR* (Ba04646242_S1)-specific TaqMan Gene Expression Assays for HL-60 or *E. coli* samples, respectively (Fig. 6C, 6D).

A Qubit
Sample Conc. (ng/µL) — *E. coli*

	1	2	3	4	5	6	7	8	9	10	11	12
A	47.3	0	64.3	0	82.5	0	50.3	0	52.2	0	67.3	0
B	0	61.9	0	35.2	0	69.4	0	49.4	0	68.2	0	66.5
C	46.5	0	49.1	0	57.1	0	56.1	0	56.9	0	60.8	0
D	0	48.8	0	47.7	0	91.5	0	60.9	0	61.4	0	54.5
E	41.2	0	44.3	0	40.1	0	39.2	0	49.7	0	70.6	0
F	0	43.7	0	50.4	0	53.2	0	64.1	0	51.6	0	46.9
G	63.3	0	52.8	0	51.3	0	55.2	0	47.4	0	50.3	0
H	0	47.9	0	44.2	0	85.4	0	56.1	0	73.4	0	61.6

B Qubit
Sample Conc. (ng/µL) — HL-60

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	71.2	0	57.4	0	49.3	0	68.9	0	60.8	0	42.7
B	74.7	0	46.3	0	60.9	0	69.1	0	78.2	0	81.7	0
C	0	61.3	0	55.8	0	42.7	0	63.2	0	76.5	0	52.2
D	51.6	0	61.4	0	58.5	0	55.1	0	60.9	0	83.3	0
E	0	64.4	0	67.7	0	57.6	0	50.7	0	69.9	0	59.4
F	94.9	0	48.2	0	85.5	0	28.4	0	76.9	0	58.9	0
G	0	77.1	0	64.5	0	65	0	64.1	0	66.2	0	53.8
H	65.6	0	93.9	0	95.4	0	62.3	0	69.4	0	83.4	0

C qPCR *(zntR)*
Ct Values — *E. coli*

	1	2	3	4	5	6	7	8	9	10	11	12
A		N.D.		N.D.		N.D.		N.D.		N.D.		N.D.
B	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
C		N.D.		N.D.		N.D.		N.D.		N.D.		N.D.
D	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
E		N.D.		N.D.		38		N.D.		N.D.		N.D.
F	N.D.	22	N.D.	22	N.D.	22	N.D.		N.D.		N.D.	
G		38		N.D.		N.D.		N.D.		N.D.		N.D.
H	N.D.		37		N.D.		N.D.		N.D.		N.D.	

D qPCR (GAPDH)
Ct Values — HL-60

	1	2	3	4	5	6	7	8	9	10	11	12
A	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
B		N.D.		N.D.		N.D.		N.D.		N.D.		N.D.
C	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
D		N.D.		N.D.		N.D.		38		N.D.		N.D.
E	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
F		N.D.	21	N.D.		N.D.	22	N.D.		N.D.	21	N.D.
G	N.D.		N.D.		N.D.		N.D.		N.D.		N.D.	
H		N.D.		N.D.		N.D.		N.D.		N.D.		N.D.

Figure 6: Qubit and qPCR assay of eluates to check for cross-contamination
A&B); cross-contamination check by concentration measurements. White wells were filled with PBS. A) Blue fields were filled with bacteria and B) green fields were filled with human HL-60 cells resuspended in PBS. After the run, quantification was performed by using a Qubit system. Numbers indicate measured concentrations in ng/µl. C&D) To further increase the resolution of the cross-contamination check, three positive samples and all negative samples from the checkerboard runs were quantified by qPCR. Samples with cycle threshold values of ≥ 35 or undetermined Ct value (N.D.) were considered negative.

Discussion / Summary

As the evolution in genomics continues, novel innovations and technologies continue to drive new discoveries. Third-generation sequencing of long DNA fragments is continuing to improve and has already surpassed conventional NGS methods in some genomic analysis areas. Long-read sequencing belongs to the gold standard tools in *de novo* genome assembly. For other applications, long reads are superior in resolving complex genomic structures.

Together with PacBio, Hamilton has addressed the need for reliable automated HMW DNA extraction. The NIMBUS Presto provides a walk-away automation solution for PacBio Nanobind kits that delivers results that meet current standards, with the ability to expand to even higher throughputs.

Others

System Requirements	Part Number	Provider
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	10102396	Hamilton Bonaduz AG
Ambion Magnetic Stand 96	282661	Hamilton Bonaduz AG
300 µL CO-RE Tips (0.71 mm, wide-bore)	235452	Hamilton Bonaduz AG

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