Extracting HMW DNA using the Nanobind® HT 1 mL blood kit for human whole blood on the Hamilton NIMBUS Presto system

Procedure & checklist

PacBio

This procedure describes the workflow for high-throughput automated extraction of HMW (50–300 kb) DNA from 1 mL of human whole blood using the Hamilton NIMBUS Presto robotic instrument. This procedure requires the Nanobind HT 1 mL blood kit (102-762-800) and is recommended for HiFi sequencing.

The Nanobind HT 1 mL blood kit has enough reagents for 96 extractions to be run in 4 runs x 24 samples. We do not recommend running fewer than 24 samples per run as the kit is designed to accommodate dead volumes for a maximum of 4 runs (4 runs x 24 samples).

Required materials and equipment

Equipment/reagent	Manufacturer (part number)
Nanobind® HT 1 mL blood kit	PacBio® (102-762-800)
NIMBUS Presto assay ready workstation	Hamilton Company
KingFisher Presto 24 deep-well head with heating block	Thermo Fisher Scientific (24078841)
KingFisher 24 deep-well plates	Thermo Fisher Scientific (95040470)
KingFisher 24 deep-well tip comb & plates	Thermo Fisher Scientific (97002610)
60 mL Reagent Reservoir	Hamilton Company (56694-01)
200 mL Reagent Reservoir	Hamilton Company (56695-01)
1000 µL Conductive Filter Tips	Hamilton Company (235905)
300 µL Conductive Filter Tips	Hamilton Company (235903)
300 µL Wide Bore 0.71 mm Orifice Conductive Filter Tips	Hamilton Company (235452)
Screw cap micro tube, 2 mL	Sarstedt Inc (72.694.406)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA Quantification	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits

102-995-600 REV02 MAR2024

Prior to starting

Buffer CW1 and CW2 are supplied as concentrates. This kit uses CW1 with a 60% final ethanol concentration. This kit uses CW2 with a 60% final ethanol concentration. Before using, add the appropriate amount of ethanol (96–100%) to Buffer CW1 and Buffer CW2 as indicated on the bottles.

Kit storage

RNase A should be stored at 4°C upon arrival.

Nanobind disks and all other buffers should be stored at room temperature (15–30°C).

Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Product use

Nanobind kits are intended for research use only.

Headspace

The automation script for the following protocol includes “headspace” volume in each plate. The “headspace” volumes are virtual volumes added to the automation script to improve the retention of the Nanobind disks on the magnetic rod and do not interfere with the extraction efficiency or performance.

Procedure and checklist

Automated HMW (50–300 kb) DNA extraction protocol

Input requirements

Amount: 1 mL of human whole blood

- Yield for human whole blood will vary from 3–70 µg based on donor white blood cell concentration.
- Blood samples should be frozen as quickly as possible after being drawn.
- Storage at 4°C should be limited to 2 days or fewer to prevent sample degradation.
- Blood samples should be aliquoted to avoid repeated freeze-thaws.
- K2 EDTA is the recommended anticoagulant. Samples stored in sodium heparin (NaHep) and citrate (NaCit) also performed well in limited testing.
- No systematic difference has been observed in DNA QC or sequencing results between fresh and frozen blood samples.

Prior to beginning protocol

- If using frozen blood, incubate in a water bath or dry block heater at 37°C for 15 minutes to thoroughly thaw the sample. Mix the sample by inverting the tube 15–20 times immediately prior to use. **Improperly thawed and mixed samples may result in inconsistent DNA yield and purity.**
- Work with Hamilton to ensure the NIMBUS Presto assay ready workstation is properly configured and has the correct methods installed.
- Prepare an .xls worklist based on the example below. Column 1 should begin with “Sample_ID” and list one sample ID for each sample being processed, from “Sample1” up to “Sample24”. Column 2 should begin with “Sample_Position” and list out the corresponding well of the sample being processed, from “A1” up to “D6”. If processing fewer than 24 samples, leave all rows blank underneath the last sample ID and sample position.

	A	B	C	D
1	Sample_ID	Sample_Position		
2	Sample1	A1		
3	Sample2	B1		
4	Sample3	C1		
5	Sample4	D1		
6	Sample5	A2		
7	Sample6	B2		
8	Sample7	C2		
9	Sample8	D2		
10	Sample9	A3		
11	Sample10	B3		
12	Sample11	C3		
13	Sample12	D3		
14	Sample13	A4		
15	Sample14	B4		
16	Sample15	C4		
17	Sample16	D4		
18	Sample17	A5		
19	Sample18	B5		
20	Sample19	C5		
21	Sample20	D5		
22	Sample21	A6		
23	Sample22	B6		
24	Sample23	C6		
25	Sample24	D6		
26				
27				

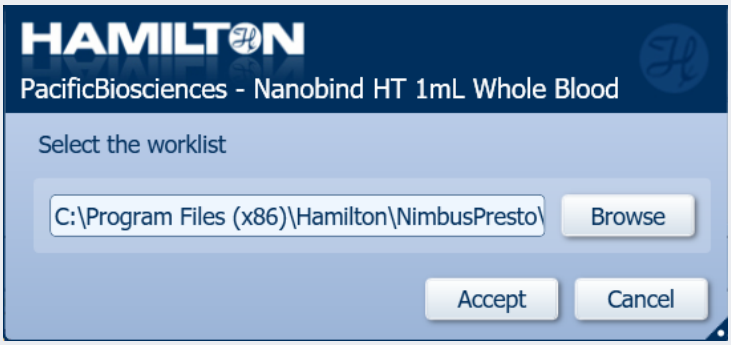
HMW DNA extraction – mammalian whole blood (1 mL)

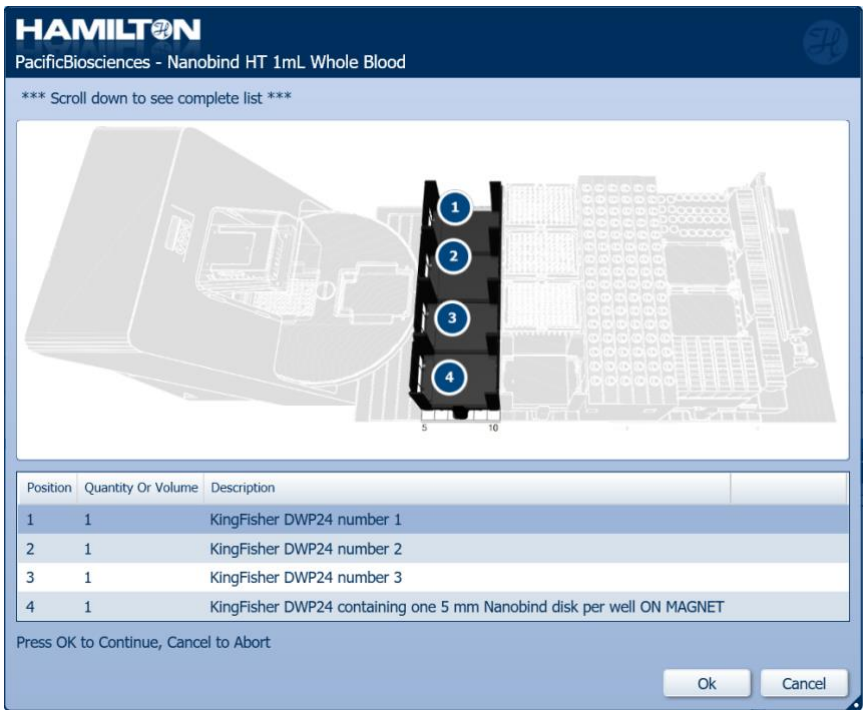
This procedure describes the workflow for automated HMW DNA extraction from 1 mL of human whole blood on the Hamilton NIMBUS Presto. This protocol uses the KingFisher 24 deep-well magnetic head, 24 deep-well plates, and 24 deep-well tip comb. This protocol cannot be run with the 96 deep-well head and 96 deep-well plates.

<table>
<tr><th>✔</th><th>Step</th><th>Instructions</th></tr>
<tr><td></td><td>1.1</td><td>Collect 3 KingFisher 24 deep-well plates and prepare as indicated in the following table. Add the components to Plate 1 (Sample) only after all other plates and reagents have been prepared.

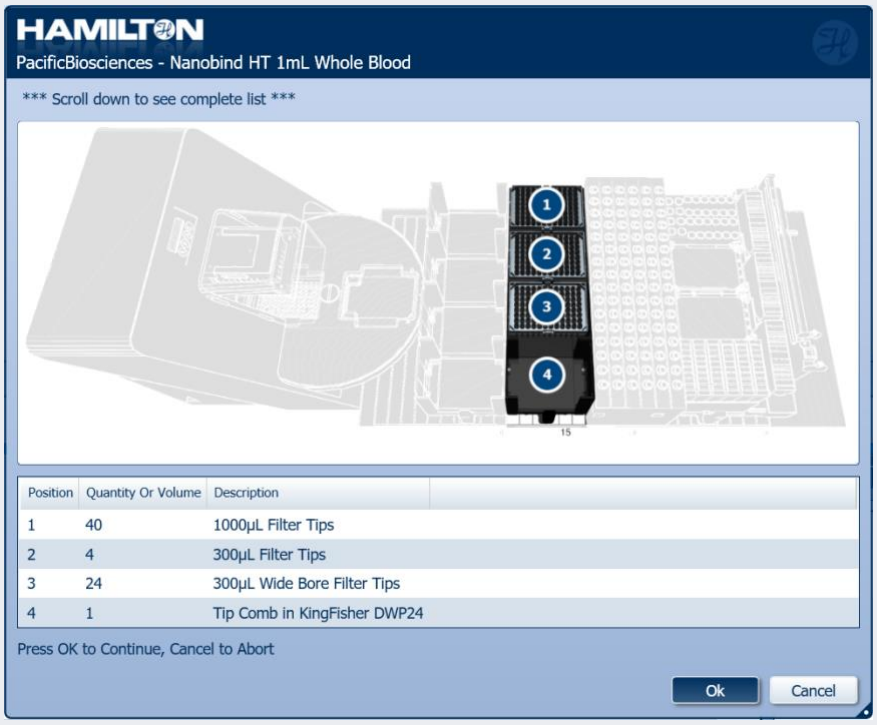
<table>
<tr><th>Plate number</th><th>Plate name</th><th>Reagent</th><th>Volume per well</th></tr>
<tr><td>1</td><td>Sample Plate</td><td colspan="2">Sample from Step 1.2</td></tr>
<tr><td>2</td><td>Nanobind Storage Plate</td><td colspan="2">One 5 mm Nanobind disk per well*</td></tr>
<tr><td>3</td><td>Tip Comb Plate</td><td colspan="2">KingFisher 24 deep-well tip comb</td></tr>
</table>

*Nanobind disks do not need to be perfectly centered in the wells, but ensure they are at the bottom of the well and not stuck to the sides.</td></tr>
<tr><td></td><td>1.2</td><td>Prepare sample in the Sample Plate:
• Add 1 mL of human whole blood to each well.
o Thaw frozen human whole blood at 37°C using a water bath or dry block heater for 15 minutes. Invert mix >15 times to thoroughly mix immediately prior to use.</td></tr>
<tr><td></td><td>1.3</td><td>Ensure the instrument is set up with the 24 deep-well magnetic head and 24 deep-well heating block.</td></tr>
<tr><td></td><td>1.4</td><td>Select the Nanobind HT 1mL Whole Blood script (102-999-000) on the Hamilton NIMBUS Presto instrument computer. Follow the prompts to select the run parameters.</td></tr>
<tr><td></td><td>1.5</td><td>An .xls worklist is used to specify the plate coordinates of the samples (see Prior to beginning protocol section). Click “Browse” and select the appropriate worklist, then click “Accept”.

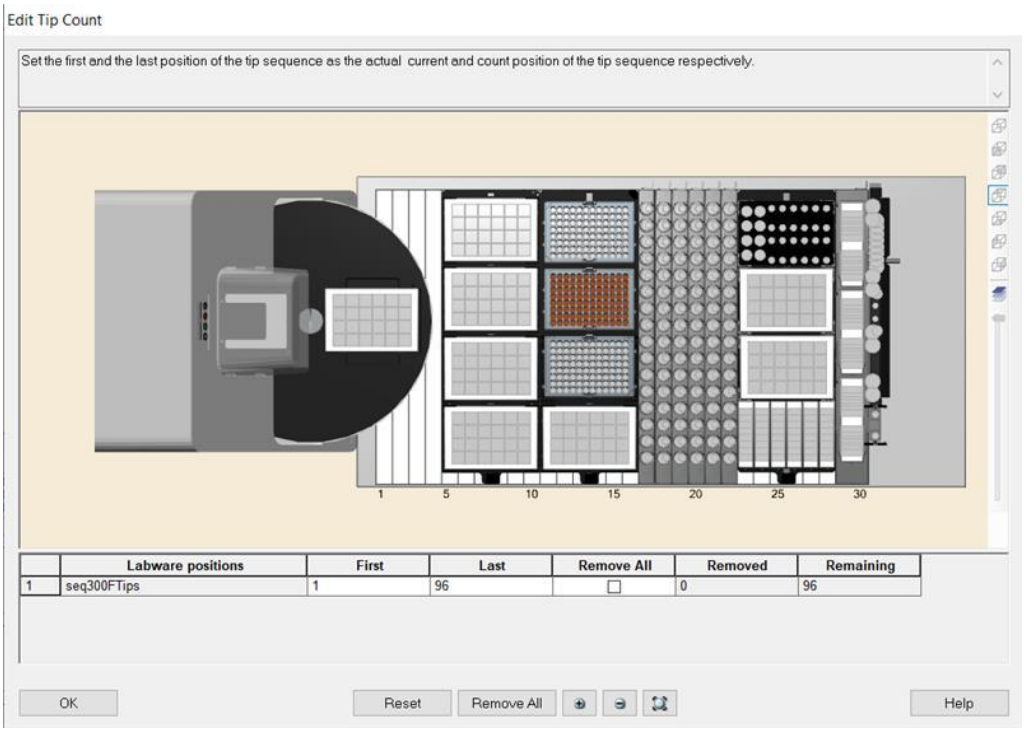
 
</td></tr>
<tr><td></td><td>1.6</td><td>Load 3 empty KingFisher 24 deep-well plates on the listed carrier positions. Load the Nanobind Storage Plate on top of the magnet on the listed carrier position, then click “OK”.</td></tr>
</table>



1.7 Load the listed number of 1000 µL conductive filter tips, 300 µL conductive filter tips, and 300 µL wide-bore conductive filter tips on the listed carrier positions. Load the Tip Comb plate on the listed carrier position, then click “OK”.

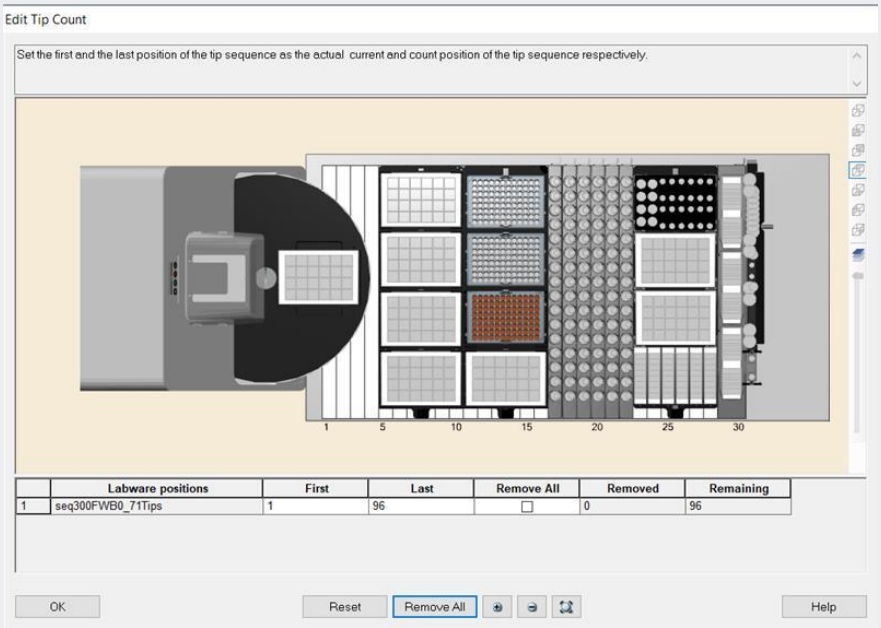


1.8 Use the cursor to set the first and last position of the 300 µL conductive filter tips, then click “OK”.



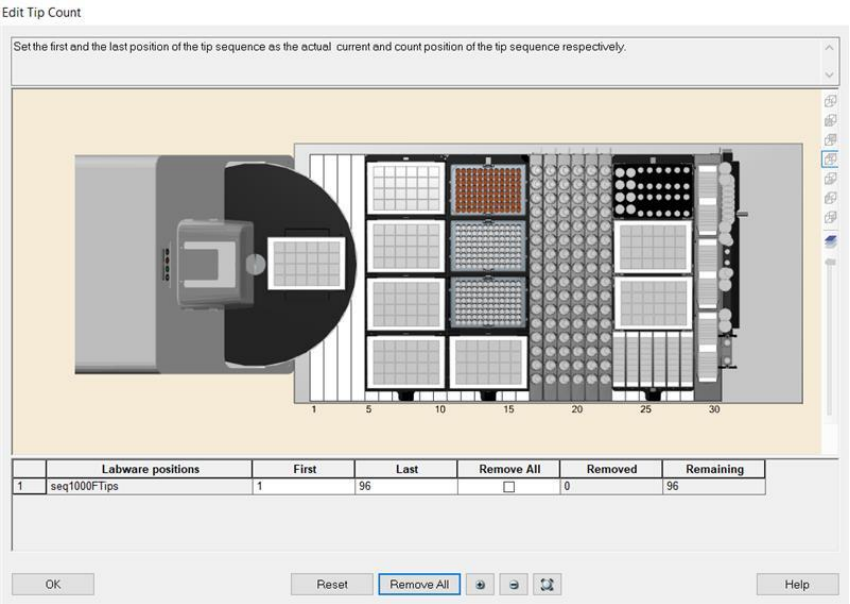
Use the cursor to set the first and last position of the 300 µL wide-bore conductive filter tips, then click “OK”.

1.9



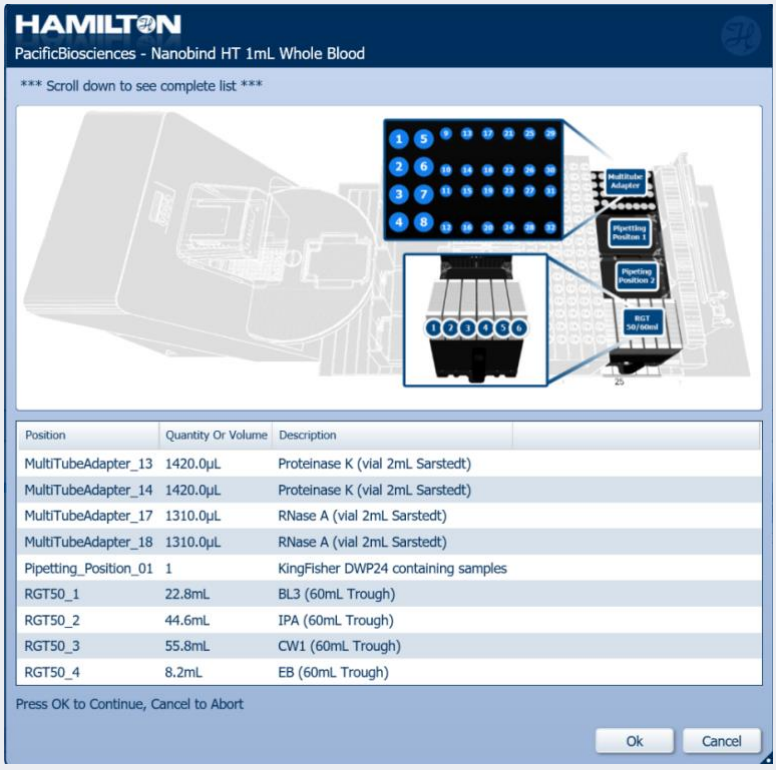
Use the cursor to set the first and last position of the 1000 µL conductive filter tips, then click “OK”.

1.10



1.11

Fill the listed number of 2 mL screw cap micro tubes with the listed volumes of Proteinase K and RNase A, then load them on the listed positions in the MultiTube Adapter. Load the Sample Plate on the listed carrier position. Using a serological pipette, fill 60 mL reagent reservoirs with the listed volume of Buffer BL3, isopropanol, Buffer CW1, and Buffer EB and load them on the listed positions of the reagent reservoir carrier. Then, click “OK”.



1.12

Fill one 200 mL reagent reservoir with the listed volume of Buffer CW2, then load it on the listed position of the reagent reservoir carrier. Then, click “OK”.

Close the front cover of the instrument, then click “OK” to start the method.

1.13

1.14	At the end of the run (~130 minutes after start), click “OK” to end the run. 
1.15	Remove plates from the instrument.
1.16	Transfer eluates from the Eluate Plate to a new storage plate or storage tubes if desired.
1.17	Pipette-mix the sample 10 times with a standard P200 pipette to homogenize and disrupt any unsolubilized “jellies” that may be present. • Take care to disrupt any regions that feel more viscous than other regions. • Limited pipette mixing will not noticeably reduce DNA size or sequencing read lengths but is important for accurate quantitation and consistent sequencing performance.
1.18	Let eluate rest overnight at room temperature to allow DNA to solubilize. • Visible “jellies” should disperse after resting.
1.19	Following overnight rest, pipette-mix 10 times with a standard P200 pipette and analyze the recovery and purity as described in the QC procedure section.

QC procedure

It is recommended that QC is performed after the DNA has been allowed to rest at room temperature overnight and appears homogeneous under visual examination and when pipetting.

1. Perform a NanoDrop UV/VIS measurement to determine total nucleic acid concentration as well as purity (A260/A280, A260/A230).
    - If the DNA is very heterogeneous or contains large amounts of unsolubilized "jellies", refer to the **Nanobind HT kit Guide & overview "Heterogeneity and viscosity"** section for more information.
2. Perform a Qubit dsDNA BR assay measurement to determine DNA concentration.
    - We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the dsDNA BR assay kit. We do not recommend the dsDNA HS assay kit as we have found the concentration measurements to be inconsistent.
3. Perform a Qubit RNA BR assay measurement to determine RNA concentration (optional).
    - We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the RNA BR assay kit.
4. Use Agilent Femto Pulse for HMW DNA size QC.
    - We recommend diluting the sample to 250 pg/µL. Finger tap to mix.
    - Avoid mixing with a standard pipette. This will shear the DNA. Always use a wide-bore pipette when making dilutions.
    - Use the Genomic DNA 165 kb Kit (Agilent Technologies) for unsheared gDNA.

Storage of DNA

DNA can be stored in Buffer EB at 4°C for several months. Long term storage at -20°C or -80°C can be used if necessary. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Results

See the example results table and Figure 1 reflecting typical parameters as listed below.

- DNA extracted from 1 mL human whole blood will yield ~3–70 µg depending on donor white blood cell count.
- 260/280 ratios should consistently be 1.8–2.0.
- 260/230 ratio can vary from 1.3–2.2.
- Samples with UV purities within the expected range should sequence well. UV purities outside of these ranges may indicate abnormalities in the extraction process.
- The mode of extracted human whole blood DNA measured on the Femto Pulse system (Agilent Technologies) is typically 100 kb+.

Sample	WBC count (10^9 cells/L)	260/280	260/230	Nanodrop (ng/µL)	Qubit DNA yield (µg)
Donor #1	4.6	1.86	2.05	98.0	15.5
Donor #2	6.3	1.86	1.93	171.3	26.4
Donor #3	9.6	1.90	2.18	334.4	51.8

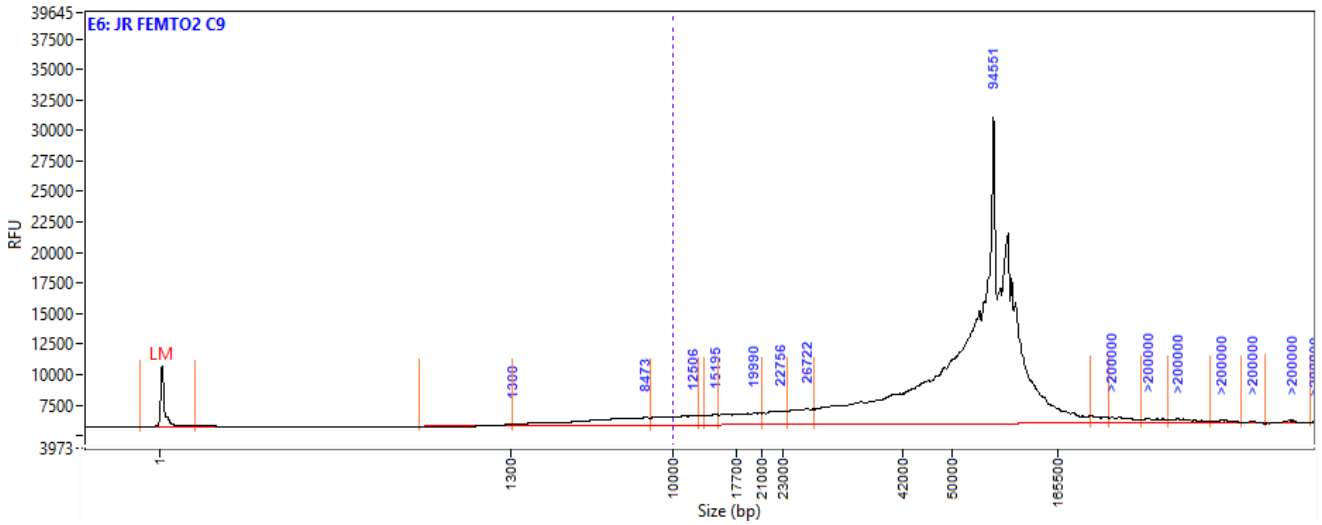


Figure 1. DNA size distribution of unsheared gDNA isolated from a 1 mL human whole blood sample using the Hamilton NIMBUS Presto on the Femto Pulse system (Agilent Technologies).

Troubleshooting FAQ

1. **What do I do if the DNA is heterogenous and/or contains visible insoluble "jellies"?**
   - HMW DNA is inherently difficult to work with. The longer the DNA, the more heterogeneous it will be.
   - Homogeneity can be improved by mixing 5–10 times with a standard P200 pipette. Take care to disrupt any particularly viscous regions. Overnight incubation at RT will then allow the HMW DNA to relax back into solution.
2. **Why is my DNA yield lower than expected?**
   - DNA yield can be affected by improper thawing and/or mixing of the blood immediately prior to beginning the Hamilton NIMBUS Presto protocol. Refer to the recommendations in the **Prior to beginning protocol** section to properly prepare the sample.
   - If the sample is heterogeneous, you may be sampling from an area of the eluate that is less concentrated. Take measurements from the top, middle, and bottom of the eluate to get an average concentration.
   - Occasionally, samples can yield lower DNA recoveries than expected. This is typically due to factors beyond the control of the protocol, such as inherent sample inhomogeneity from certain donors or low white blood cell count. If the DNA yield is insufficient for sequencing and additional blood sample remains, rerun the protocol and contact PacBio for further steps.
   - If there is very little to no DNA yield, the Nanobind disk may have been left in the Lysis/Binding plate. This is a rare occurrence but can happen. We recommend rerunning the program at a lower cell input if additional sample remains.
3. **Why are the purities lower than expected? Is this a problem?**
   - A correlation has NOT been seen between UV purity and sequencing performance and no particular attention to the UV purity is needed if it is within the expected range for that particular sample type. Generally, human whole blood DNA results in UV purities of 260/230 >1.3 and 260/280 >1.8. Samples with UV purity slightly outside of this range will likely still sequence well. Samples with UV purity far outside this range should be treated with caution.
   - DNA purity can be affected by improper thawing and/or mixing of the blood immediately prior to beginning the Hamilton NIMBUS Presto protocol. Refer to the recommendations in the **Prior to beginning protocol** section to properly prepare the sample.
4. **Why isn't the protocol running and/or why is there an error message?**
   - Check to ensure the correct script is installed. Contact Hamilton for assistance in installing the appropriate scripts.
   - Check to ensure the correct magnet head and heat block are installed in the KingFisher Presto.
   - Check to ensure all plates and reagent reservoirs are in the correct positions. The KingFisher Presto will give an error message if it does not detect the tip comb (i.e., the tip comb is not in the correct position). The Hamilton NIMBUS Presto will give an error if no liquid is detected or if there is insufficient volume.
   - For other error messages, please contact Hamilton.

Revision history (description)	Version	Date
Initial release	01	April 2023
Updated protocol name to distinguish from non-human mammalian blood	02	March 2024