

The PacBio logo is displayed in a bold, pink, sans-serif font. A single, large pink droplet is positioned at the end of the word 'Bio', appearing to fall from the top right corner of the frame. The background is a blurred laboratory setting with a rack of microcentrifuge tubes containing pink liquid.

PacBio

Technical overview – Nanobind HT kits for automated HMW DNA extraction

High-throughput HMW DNA extraction solutions for PacBio long-read sequencing

Nanobind HT kits for automated HMW DNA extraction

Technical Overview

1. Nanobind HT method overview
2. Nanobind HT workflow details
3. Nanobind HT example performance data
4. Technical documentation & applications support resources

Nanobind HT HMW DNA extraction for SMRT sequencing: Getting started

Nanobind HT literature

Nanobind HT protocols

Nanobind HT technical overview

DNA sample extraction, library prep & sequencing

NANOBIND HIGH-THROUGHPUT HMW DNA EXTRACTION

Automate DNA extraction and optimize your sample prep workflow for HiFi sequencing

PacBio® Nanobind® HT kits enable automation of DNA extraction, the first step of an end-to-end workflow that harnesses the power of HiFi sequencing. Nanobind HT kits are designed for whole human blood and mammalian cell samples and are compatible with four robotic platforms.

HAMILTON Automated sample prep

Nanobind HT kits

Nanobind HT CBB kit for 200 µL human blood and mammalian cell samples (96 reactions)

- Expected HMW DNA yield: 3–12 µg for human blood (for donor WBC count $\geq 4 \times 10^6$ cells/mL) and 5–15 µg for mammalian cells (for 10^6 cells)

Nanobind HT 1 mL blood kit for 1 mL human blood (96 reactions)

- Expected HMW DNA yield: 3–70 µg

An automated solution

Nanobind HT kits use magnetic disk processing to automate lysis, binding, washing and elution steps and are compatible with instruments from Hamilton and Thermo Fisher. Hamilton NIMBUS Presto is a walk away solution with automated plate filling. Thermo Fisher KingFisher instruments are semi-automated with manual plate filling and limited user interaction during the run.

Sample	Robotic Instrument	Input sample	DNA yield (µg)	A260/A280 ratio	A260/A230 ratio	DNA mean size (kb)	QON at 10 kb	HF1 mean read length (bp)	HF1 yield (Gb)
Mammalian cell (HG001)	NIMBUS Presto	10 ⁶ cells	6.4	1.92	2.04	167	9.4	16,563	41.9
Mammalian cell (HG001)	Apex	10 ⁶ cells	7.0	1.88	2.08	113	9.6	16,544	35.4
Whole human blood	NIMBUS Presto	200 µL	7.0	1.83	1.96	98	9.4	18,067	37.3
Whole human blood	Apex	200 µL	6.1	1.83	1.80	124	9.7	13,026	34.8
Whole human blood	NIMBUS Presto	1 mL	32.6	1.86	1.86	94	9.3	17,163	36.8
Whole human blood	Apex	1 mL	36.4	1.89	2.18	115	9.4	15,863	36.1

The table above shows automated extracted DNA from mammalian cells and human whole blood sequenced on the Sequel II system. Mean DNA size is approximately 100 kb and over 93% of the DNA molecules are longer than 10 kb (QON score). Sequencing results show HF1 yield > 35 Gb, mean read length at 13–18 kb and limited fragments of < 8 kb.

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Brochure— Nanobind high-throughput HMW DNA extraction (102-326-565)

Summary overview of Nanobind HT HMW DNA sample preparation workflow recommendations.

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

Procedure & checklist

This procedure describes the workflow for high-throughput automated extraction of HMW (50–300 kb) DNA from 1 mL of whole mammalian 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 one of the following formats: 1 run x 96 samples, 2 runs x 48 samples, or 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 (09040478)
KingFisher 24 deep-well tip comb & plates	Thermo Fisher Scientific (97002610)
40 mL Reagent Reservoir	Hamilton Company (56644-01)
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 (235432)
Screw cap micro tube, 2 mL	Sarstedt Inc (72.694.406)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
UV/Vs	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA Quantification	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits

Nanobind HT Procedures & checklists and Guide & overview – Nanobind HT kits (103-028-100)

Refer to PacBio's [Documentation](#) website for a full list of Nanobind HT HMW DNA sample preparation protocols for use with different automated sample purification systems.

Technical overview – Nanobind HT kits for automated HMW DNA extraction

High-throughput HMW DNA extraction solutions for PacBio long-read sequencing

February 2, 2022 | PacBio FAS / Technical Support / Distributor Support training
For internal use only.

Nanobind HT HMW DNA extraction performance on KingFisher Apex and Hamilton NIMBUS Presto

Example HMW DNA extraction QC results

	Human blood	Mammalian cells
• 200 µL or 1 mL fresh or frozen whole blood (max. 2 days storage at 4°C prior to extraction)		• Pippet of 1 x 10 ⁶ animal cells (fresh or frozen)
• For donor WBC count $\geq 4 \times 10^6$ cells/mL, expected HMW DNA yield is 3 – 12 µg for 200 µL blood sample and 3 – 70 µg for 1 mL blood sample		• Expected HMW DNA yield is 5 – 10 µg

QC results

- Mean DNA Fragment size > 90 kb
- > 93% of DNA fragments longer than 10 kb (QON at 10 kb)

QC quality

- Human blood and animal samples: 90% QON at 10 kb
- Human blood and animal samples: 90% QON at 10 kb

QC quantity

- Human blood and animal samples: 90% QON at 10 kb

Technical overview – Nanobind HT kits for automated HMW DNA extraction (103-020-800)

Technical overview presentation describes protocol details for using Nanobind HT kits for HMW DNA extraction and shows example sequencing performance data.

Automated DNA sample extraction (Nanobind HT kit + Automation system)

Use [Nanobind HT kits](#) with an automated sample purification system for high-throughput HMW DNA extraction



SMRTbell library preparation (SMRTbell prep kit 3.0)

Follow [Procedure & checklist](#) to construct a SMRTbell library using purified HMW DNA



SMRT sequencing (Sequel II/Ie & Revio systems)


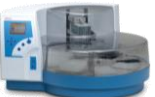


Prepare samples for long-read sequencing by following [SMRT Link](#) sample setup instructions



Nanobind HT HMW DNA extraction workflow overview

Available Nanobind HT HMW DNA extraction protocols

Select the appropriate Nanobind HT Procedure & checklist based on the type of automation system, sample type, desired sample throughput and available input sample amount

Automation system	Procedure & checklist	Sample type	Input Sample amount	Max # samples per run	Nanobind HT kit (96-RXN)	
					Kit	Part no.
KingFisher Duo 	Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Duo [102-996-200]	Mammalian cells	1x10 ⁶ cells*	12	Nanobind HT CBB kit	102-762-700
	Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Duo [102-995-800]	Whole blood	200 µL	12		
	Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Duo [102-995-400]	Whole blood	1 mL	6	Nanobind HT 1 mL whole blood kit	102-762-800
KingFisher Flex 	Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Flex [102-996-300]	Mammalian cells	1x10 ⁶ cells*	96	Nanobind HT CBB kit	102-762-700
	Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Flex [102-995-900]	Whole blood	200 µL	96		
	Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Flex [102-995-500]	Whole blood	1 mL	24	Nanobind HT 1 mL whole blood kit	102-762-800
KingFisher Apex 	Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Apex [102-996-100]	Mammalian cells	1x10 ⁶ cells*	96	Nanobind HT CBB kit	102-762-700
	Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Apex [102-995-700]	Whole blood	200 µL	96		
	Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Apex [102-995-300]	Whole blood	1 mL	24	Nanobind HT 1 mL whole blood kit	102-762-800
Hamilton NIMBUS Presto 	Nanobind HT HMW DNA extraction – Cultured cells – Hamilton NIMBUS Presto [102-996-400]	Mammalian cells	1x10 ⁶ cells*	96	Nanobind HT CBB kit	102-762-700
	Nanobind HT HMW DNA extraction – 200 µL whole blood – Hamilton NIMBUS Presto [102-996-000]	Whole blood	200 µL	96		
	Nanobind HT HMW DNA extraction – 1 mL whole blood – Hamilton NIMBUS Presto [102-995-600]	Whole blood	1 mL	24	Nanobind HT 1 mL whole blood kit	102-762-800

* Cell samples are typically resuspended in 50 µL of 1X PBS for Nanobind HT processing.

Supported automation platforms for high-throughput HMW DNA extraction using Nanobind HT kits

Nanobind HT HMW DNA extraction kits are optimized for use with Thermo Fisher Scientific KingFisher sample purification systems and Hamilton Microlab NIMBUS automated liquid handlers



	Thermo Fisher Scientific KingFisher Duo Prime			Thermo Fisher Scientific KingFisher Flex			Thermo Fisher Scientific KingFisher Apex			Hamilton Microlab NIMBUS Presto		
Instrument design	• Compact benchtop sample purification system			• Benchtop sample purification system			• Benchtop sample purification system with touchscreen			• Hamilton liquid handler with integrated KingFisher Presto sample purification system		
Nanobind HT throughput	• 6 or 12 samples / run			• 24 or 96 samples / run			• 24 or 96 samples / run			• 24 or 96 samples / run		
Nanobind HT workflow automation level	• Semi-automated • Manual plate filling + requires limited user interaction after run start			• Semi-automated • Manual plate filling + requires limited user interaction after run start			• Semi-automated • Manual plate filling + requires limited user interaction after run start			• Fully automated • Automated plate filling + fully walk-away after run start		
Nanobind HT processing time	12 (1x10 ⁶ cells)	12 (200 µL)	6 (1 mL)	96 (1x10 ⁶ cells)	96 (200 µL)	24 (1 mL)	96 (1x10 ⁶ cells)	96 (200 µL)	24 (1 mL)	96 (1x10 ⁶ cells)	96 (200 µL)	24 (1 mL)
Total time	1 hr 20 min	1.5 hrs	1 hr 55 min	1 hr 40 min	1 hr 50 min	2 hrs 15 min	1 hr 40 min	1 hr 50 min	2 hrs 15 min	2 hrs 25 min	2 hrs 35 min	2 hrs 30 min
Automation run time	1 hr 5 min	1 hr 15 min	1 hr 45 min	1 hr 5 min	1 hr 15 min	1 hr 45 min	1 hr 5 min	1 hr 15 min	1 hr 45 min	2 hrs	2 hrs 10 min	2 hrs 10 min
Hands-on time	15 min	15 min	10 min	35 min	35 min	30 min	35 min	35 min	30 min	25 min	25 min	20 min

Nanobind HT HMW DNA extraction workflow overview

Automation system configuration*

ThermoFisher
SCIENTIFIC

HAMILTON



KingFisher Duo/Flex/Apex



Microlab NIMBUS Presto

Verify installation of correct automation script



Verify installation of correct magnet head & heat block

Use either a 24-well or 96-well magnet head, tips & plate for Nanobind HT workflows



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Extracting HMW DNA using the Nanobind® HT 1 mL blood kit for mammalian whole blood on the Hamilton NIMBUS Presto system
Procedure & checklist

Required materials and equipment

Equipment/Reagent	Manufacturer (part number)
Automation System	Hamilton (KingFisher Duo/Flex/Apex)
Nanobind HT 1 mL Blood Kit	Hamilton Company (102-762-700)
KingFisher Duo/Flex/Apex	ThermoFisher Scientific (DS2427C)
KingFisher Duo/Flex/Apex	ThermoFisher Scientific (DS2427C)
24-Well Magnet Head	Hamilton Company (MAG-24)
96-Well Magnet Head	Hamilton Company (MAG-96)
24-Well Disposable Filter Tips	Hamilton Company (24000)
96-Well Disposable Filter Tips	Hamilton Company (24000)
24-Well Plate	Corning (3603)
96-Well Plate	Corning (3603)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)
Hamilton NIMBUS Presto	Hamilton (102-762-800)

Automation system-specific Nanobind HT Procedure & checklist

Nanobind HT HMW DNA extraction

PacBio

Nanobind HT CBB kit (102-762-700) [200 µL] /
Nanobind HT 1 mL blood kit (102-762-800) [1 mL]



Prepare sample, storage & reagent plates

Select appropriate automation script to run

Load prepared plates and other consumables onto work deck

Run Nanobind HT DNA extraction automation program

Store extracted DNA samples and perform QC



Qubit (DNA conc.)



Nanodrop (DNA purity)



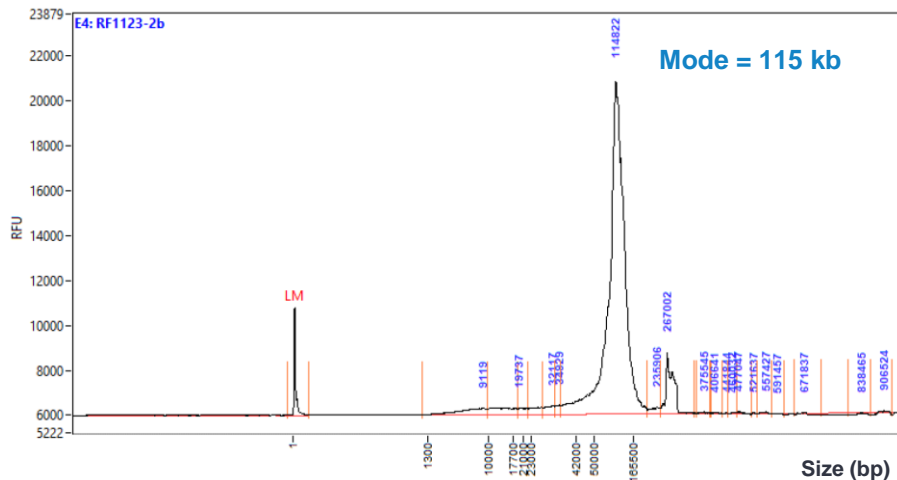
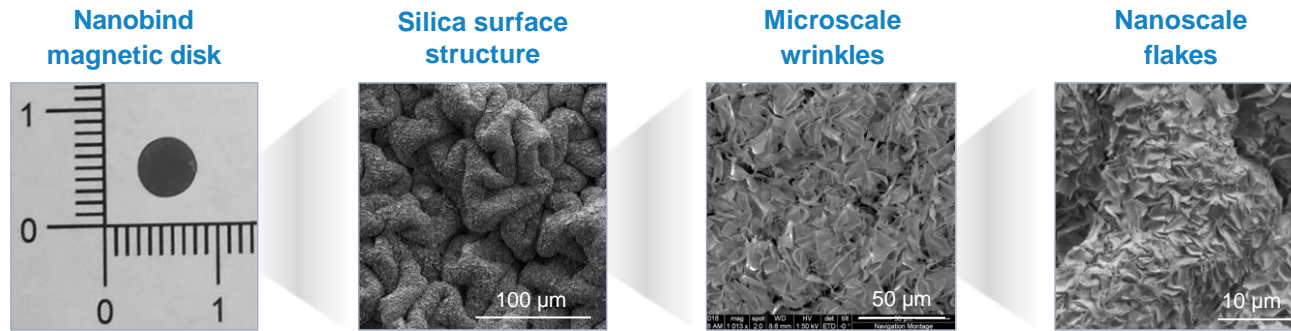
Femto Pulse (DNA sizing)

* Refer to appropriate vendor websites to download user guides and other documentation for specific automation systems.

How Nanobind HMW DNA extraction kits work with KingFisher systems

Nanobind kits enable extraction of high-molecular weight DNA from common samples as well as more challenging samples such as animal tissue, insects, fungi, and plants

Nanobind is a novel magnetic disk (3 – 5 mm) covered with a high density of micro- and nanostructured silica

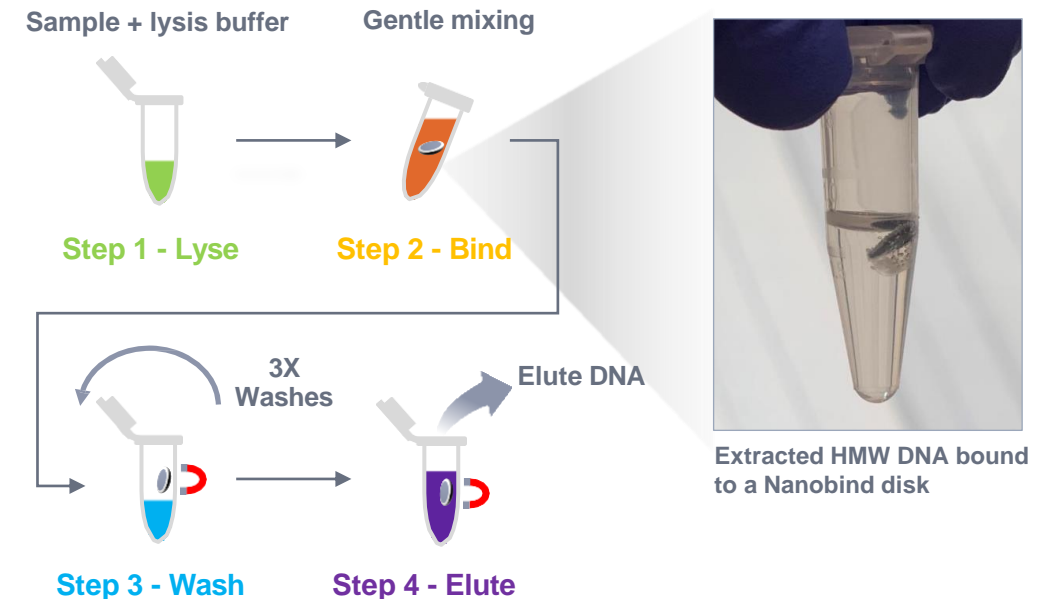


Nanobind disks bind and release DNA **without fragmentation**, yielding HMW DNA 50–300+ kb

Femto Pulse DNA sizing QC analysis of unsheared gDNA isolated from 5×10^6 GM12878 cells using Nanobind CBB kit

Rapid magnetic purification

- Rapid and simple bind, wash, and elute protocol
- Can perform manual processing using a magnetic separation rack
- Nanobind disks are automation compatible for high-throughput applications



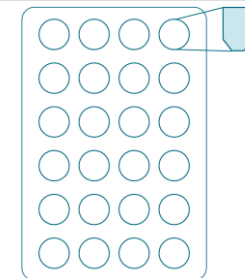
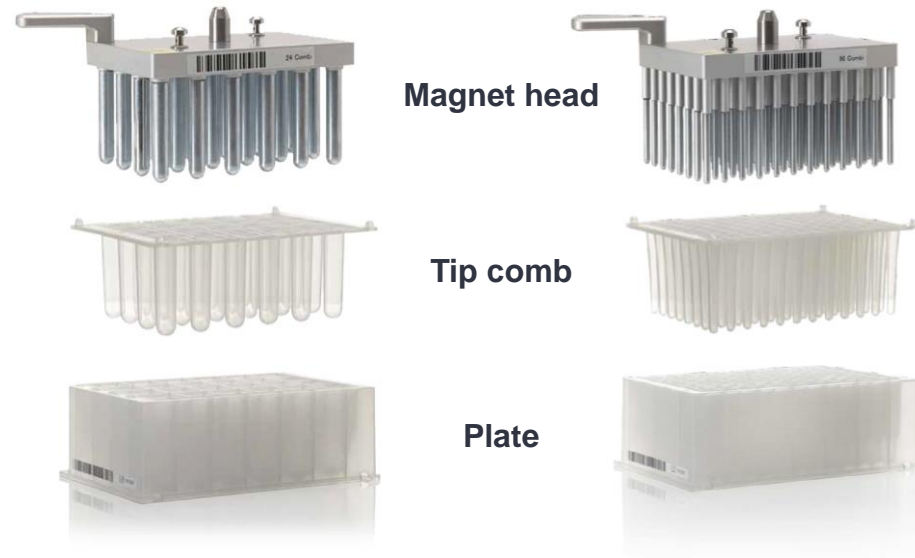
How Nanobind HMW DNA extraction kits work with KingFisher systems (cont.)

Kingfisher sample purification systems employ inverse magnetic particle processing (MPP) technology to enable automated transfer and processing of magnetic Nanobind disks in a microplate format.

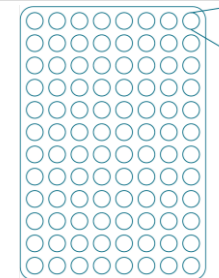
Kingfisher instruments



- Automate sample extraction by **moving magnetic particles or objects (not liquids)**
- Inverse MPP uses **magnetic rods** covered with a disposable **tip comb** and PCR or deep-well plates
- Function **without any liquid dispensing or aspiration parts or devices**
- Enable **quicker reaction times and a more efficient wash process**



24 deep-well plates
(200 – 5,000 μL)



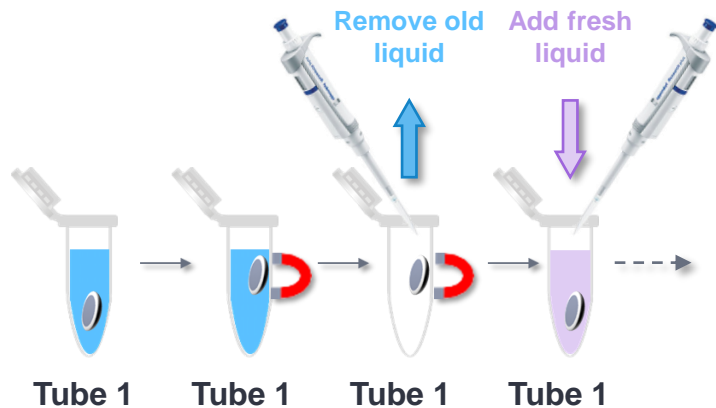
96 deep-well plates
(200 – 1,000 μL)



How Nanobind HMW DNA extraction kits work with KingFisher systems (cont.)

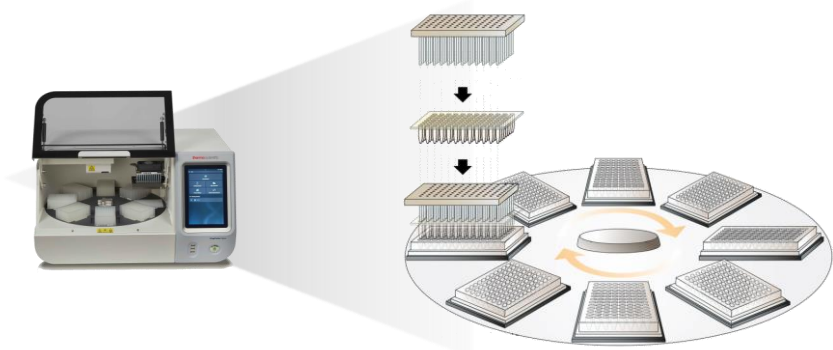
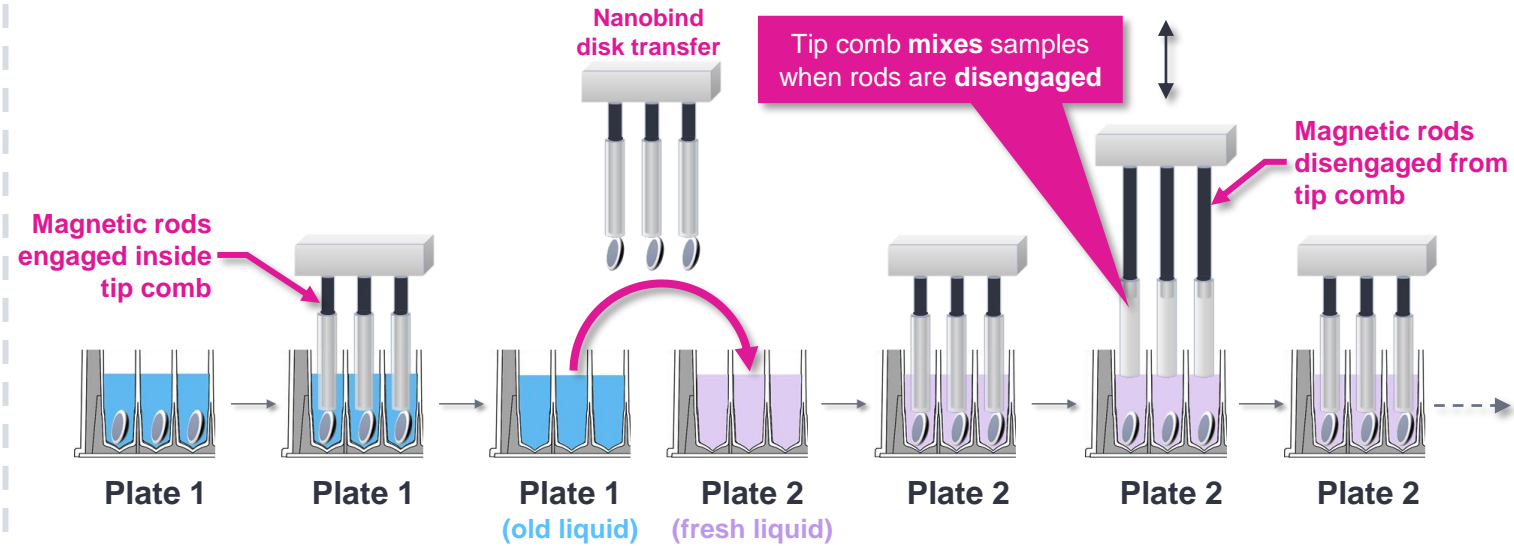
Standard manual processing with Nanobind disks

Liquid is moved into and out of a tube using a pipette while the magnetic Nanobind disk remains immobilized inside the tube



Automated inverse magnetic particle processing (MPP) with Nanobind disks

Rather than moving liquids, magnetic Nanobind disks are moved from plate to plate using magnetic rods covered with a disposable plastic tip comb

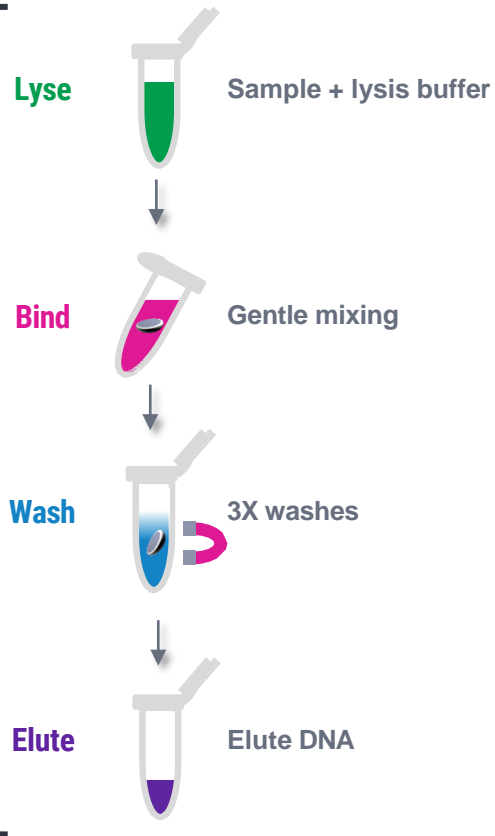


PacBio scripts support high-throughput HMW DNA extraction using Nanobind HT kits with semi-automated and fully-automated workflows



Manual

~1 – 1.5 hrs for 8 samples



Semi-automated (KingFisher Duo/Flex/Apex)

~2 hrs for 96 samples



Prepare DW plates containing tip comb, Nanobind disks & Nanobind kit reagents

Add samples to lysis / binding DW plate

Load prepared DW plates onto instrument

Lyse

Add isopropanol to lysis / binding DW plate

Bind

Wash 1 / 2 / 3

Elute



Fully-automated (Hamilton NIMBUS Presto)

~3.5 hrs for 96 samples



Prepare DW plates containing samples, tip comb and Nanobind disks

Load prepared DW plates & filter tip consumables onto instrument

Fill instrument work deck bulk reagent reservoirs with Nanobind kit reagents

Lyse

Bind

Wash 1 / 2 / 3

Elute

Manual user intervention required

[= Manual step

[= Automated step



Nanobind HT HMW DNA extraction workflow details

Nanobind HT Procedure & checklist documentation overview

Twelve Procedures & checklists to support 3 high-throughput HMW DNA extraction workflows on 4 automation systems

Nanobind HT Procedure & checklist protocols describe procedures for automated HMW DNA extraction from mammalian cells and 200 μ L/1 mL of blood using a Kingfisher Duo/Flex/Apex system or Hamilton NIMBUS Presto system

Procedure & checklist contents

1. Equipment and reagent list
2. Sample input requirements
3. Automated HMW (50 kb – 300+ Mb) DNA extraction protocol
4. QC procedure
5. Expected QC results
6. Troubleshooting FAQ

Nanobind HT Procedures & checklists have been validated using human whole blood samples and mammalian cells.



Extracting HMW DNA using the Nanobind® HT CBB kit for mammalian cultured cells on the KingFisher Apex system



Procedure & checklist

This procedure describes the workflow for high-throughput automated extraction of HMW (50–300 kb) DNA from cultured mammalian cells using the Thermo Fisher KingFisher Apex robotic instrument. This procedure requires the Nanobind HT CBB kit (102-762-700) and is recommended for HiFi sequencing.

The Nanobind HT CBB kit has enough reagents for 96 extractions to be run in one of the following formats: 1 run x 96 samples, 2 runs x 48 samples, or 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 equipment and materials

Equipment/reagent	Manufacturer (part number)
Nanobind HT CBB kit	PacBio® (102-762-700)
KingFisher Apex System	Thermo Fisher Scientific (5400930, includes Apex 96 deep well magnet head)
KingFisher Apex 96 deep-well magnet head	Thermo Fisher Scientific (24079930)
KingFisher Apex 96 deep-well heating block	Thermo Fisher Scientific (24075920)
KingFisher 96 deep-well plates, barcoded	Thermo Fisher Scientific (95040450B)
KingFisher 96 deep-well tip combs, barcoded	Thermo Fisher Scientific (97002534B)
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

Before you begin

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.

See PacBio sample preparation [Documentation](#)




Equipment & materials required for Nanobind HT HMW DNA extraction

For each type of sample purification system, use the correct matching magnet heads, heating blocks, plates and other consumables as listed in the appropriate Nanobind HT procedure

	KingFisher Duo		KingFisher Flex		KingFisher Apex ¹		Hamilton NIMBUS Presto	
Part	12 samples	6 samples	96 samples	24 samples	96 samples	24 samples	96 samples	24 samples
Magnet head	KingFisher Duo 12-pin head for 96 DW plates N12459 	KingFisher Duo 6-pin head for 24 DW plates N12460 	KingFisher Flex 96 DW head 24074430 	KingFisher Flex 24 DW head 24074440 	KingFisher Apex 96 DW head 24079930 	KingFisher Apex 24 DW Combi head 24079940 	KingFisher Presto 24 DW head 24078830 	KingFisher Presto 24 DW head 24078840* 
Tip comb	KingFisher Duo 12-tip comb for 96 DW plate 97003500 	KingFisher Duo 6-tip comb for 24 DW plate 97003510 	KingFisher 96 tip comb for DW magnets 97002534 	KingFisher 24 DW tip comb & plate 97002610 	KingFisher Apex 96 DW tip combs, barcoded 97002534B 	KingFisher Apex 24 DW tip combs, barcoded 97002610B 	KingFisher 96 tip comb for DW magnets 97002534 	KingFisher 24 DW tip comb & plate 97002610 
Plate	KingFisher 96 DW plate 95040450 	KingFisher 24 DW plate 95040470 	KingFisher 96 DW plate 95040450 	KingFisher 24 DW plate 95040470 	KingFisher 96 DW plates, barcoded 95040450B 	KingFisher 24 DW plates, barcoded 95040470B 	KingFisher DW 96 plate 95040450 	KingFisher 24 DW plate 95040470 
Heating block	KingFisher Duo heating block for 96 DW plate N12461 	KingFisher Duo heating block for 24 DW plate N12462 	96 DW heating block for KingFisher Flex/Presto 24075430 	24 DW heating block for KingFisher Flex/Presto 24075440 	KingFisher Apex 96 DW heating block 24075920 	KingFisher Apex 24 DW heating block 24075940 	96 DW heating block for KingFisher Flex/Presto 24075430 	24 DW heating block for KingFisher Flex/Presto 24075440* 

Apex is designed to use **barcoded** KingFisher plastics & hardware

Sample input requirements for Nanobind HT HMW DNA extraction

	Human whole blood	Mammalian cells
Nanobind HT kit 	 <p>Nanobind HT CBB kit (96 Rx) (102-762-700)</p> <p>Nanobind HT 1 mL whole blood kit (96 Rx) (102-762-800)</p>	 <p>Nanobind HT CBB kit (96 Rx) (102-762-700)</p>
Storage conditions	<ul style="list-style-type: none"> Use Potassium EDTA (K₂EDTA) anticoagulant <ul style="list-style-type: none"> Sodium heparin (NaHep) and citrate (NaCit) also performed well in limited testing Store at 4°C for ≤2 days to prevent sample degradation 	<ul style="list-style-type: none"> N/A
Sample input amount	<ul style="list-style-type: none"> Cells: White blood cell (WBC) count should be $\geq 4 \times 10^6$ cells/mL* to achieve ≥ 3 ug DNA yield for input into SMRTbell library prep Blood: Use either 200 µL or 1 mL of input human whole blood <ul style="list-style-type: none"> 200 µL: DNA yield is 1 – 12 µg depending on donor WBC conc. 1 mL : DNA yield is 3 – 70 µg depending on donor WBC conc. 	<ul style="list-style-type: none"> Use 1x10⁶ diploid human cells* or equivalent Determine cell counts using a hemocytometer or cell counter For non-diploid/non-human cells: Scale cell input to contain 5–25 µg of DNA <ul style="list-style-type: none"> Warning: >25 µg inputs can cause Nanobind disks to be “dropped” in Lysis/Binding solution and/or cause well-to-well contamination
Sample freezing / thawing conditions	<ul style="list-style-type: none"> Freeze blood samples as quickly as possible after being drawn <ul style="list-style-type: none"> Aliquot blood samples to avoid repeated freeze-thaws Thaw blood samples in water bath/dry block heater at 37 °C (15 min) <ul style="list-style-type: none"> Mix by inverting tube >15 times immediately prior to use Note: Improperly thawed and mixed samples may result in inconsistent DNA yield and purity 	<ul style="list-style-type: none"> Cell pellets should be frozen dry with as much liquid removed as possible. <ul style="list-style-type: none"> No cryoprotectant needed
No systematic difference observed in DNA QC or sequencing results between fresh and frozen samples		

Nanobind HT HMW DNA extraction workflow step details

Before you begin

End-to-end overall Nanobind HT workflow

Prepare sample, storage & reagent plates



Select appropriate automation script to run



Load prepared plates and other consumables onto work deck



Run selected Nanobind HT DNA extraction protocol



Store extracted DNA samples and perform QC

Semi-automated workflow (KingFisher Duo/Flex/Apex)



Fully-automated workflow (Hamilton NIMBUS Presto)



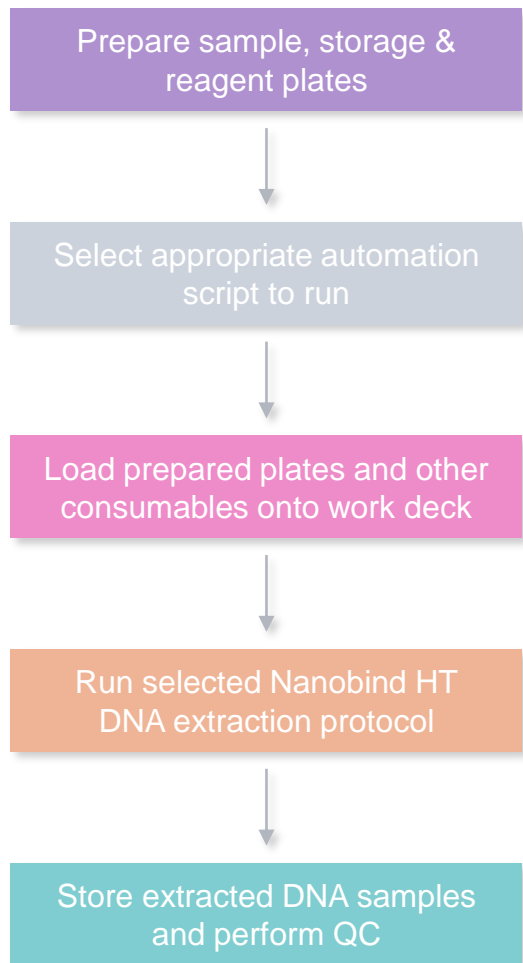
Prior to beginning Nanobind HT protocol

- Ensure that the **proper automation script** has been installed (see [Nanobind HT kit Guide & overview](#) “Programs” section)
 - To obtain **KingFisher automation scripts**, contact [PacBio Technical Support](#) at support@pacb.com
 - To obtain **Hamilton NIMBUS Presto automation scripts**, contact [Hamilton Technical Support](#) at roboticservice@hamiltoncompany.com
- Ensure that instrument is set up with the **correct magnet head and heating block**.

Nanobind HT HMW DNA extraction workflow step details

Before you begin (cont.)

End-to-end overall Nanobind HT workflow



Semi-automated workflow (KingFisher Duo/Flex/Apex)



Fully-automated workflow (Hamilton NIMBUS Presto)



Prior to beginning Nanobind HT protocol

For Hamilton NIMBUS Presto only:

- Prepare a *.xls **sample worklist**
- Column 1 = “Sample_ID”
 - List one sample ID for each sample to be processed
- Column 2 = “Sample_Position”
 - List the corresponding well location for each sample

Sample worklist enables NIMBUS liquid handler to **automatically** prepare lysis reactions on instrument work deck using a loaded sample plate

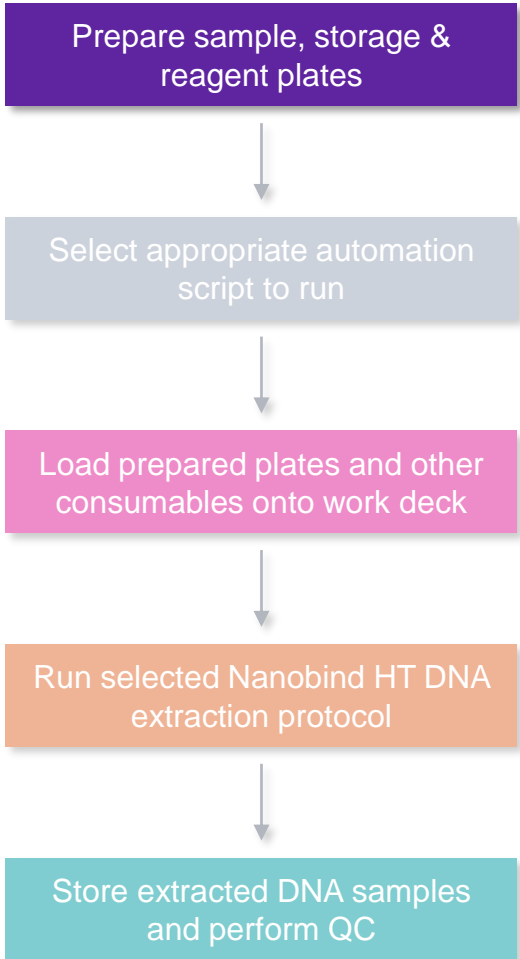
	A	B	C	D
1	Sample_ID	Sample_Position		
2	Sample1	A1		
3	Sample2	B1		
4	Sample3	C1		
5	Sample4	D1		
6	Sample5	A2		
7	Sample6	B2		
8	Sample7	C2		
9	Sample8	D2		
10	Sample9	A3		
11	Sample10	B3		
12	Sample11	C3		
13	Sample12	D3		
14	Sample13	A4		
15	Sample14	B4		

Nanobind HT HMW DNA extraction workflow step details

Sample, storage & reagent plate preparations

NIMBUS Presto workflow requires upfront preparation of **fewer plates** compared to Duo/Flex/Apex workflows

End-to-end overall Nanobind HT workflow



Semi-automated workflow (KingFisher Duo/Flex/Apex)

Manually prepare deep-well plates with required quantities of Nanobind reagents & consumables

Example list of **7 DW plates** to be prepared for Kingfisher Apex.

Plate	Plate name	Reagent	Notes
1	Lysis / binding	Sample / lysis / binding solution	Prepare only after all other plates have been prepared (see below)
2	Nanobind storage	1 Nanobind disk per well*	200 µL / cells → 3 mm disk 1 mL → 5 mm disk
3	Wash 1	Buffer CW1	200 µL sample / cells → 700 µL 1 mL sample → 2 mL
4	Wash 2	Buffer CW2	200 µL sample / cells → 700 µL 1 mL sample → 2 mL
5	Wash 3	Buffer CW3	200 µL sample / cells → 700 µL 1 mL sample → 2 mL
6	Elution	Buffer EB	200 µL sample / cells → 100 µL 1 mL sample → 200 µL
7	Tip comb storage	For 24- or 96-tip comb	Insert tip comb into a DW plate for loading onto instrument

Plate 1 (lysis / binding plate) preparation procedure :

1. Add Proteinase K
2. Add whole blood sample
3. Add Buffer BL3
4. Add RNase A

Note: Sample and reagents **MUST** be added to each well in the order described in the Procedure

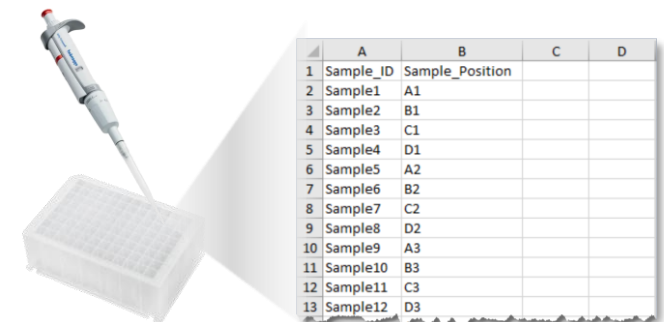
Fully-automated workflow (Hamilton NIMBUS Presto)

Example list of **3 DW plates** to be prepared for NIMBUS Presto.

Plate	Plate name	Reagent	Notes
1	Sample plate	Samples for analysis	Prepare only after all other plates have been prepared
2	Nanobind storage	1 Nanobind disk per well*	200 µL sample / cells → 3 mm disk 1 mL sample → 5 mm disk
3	Tip comb storage	For 24- or 96-tip comb	Insert tip comb into a DW plate for loading onto instrument

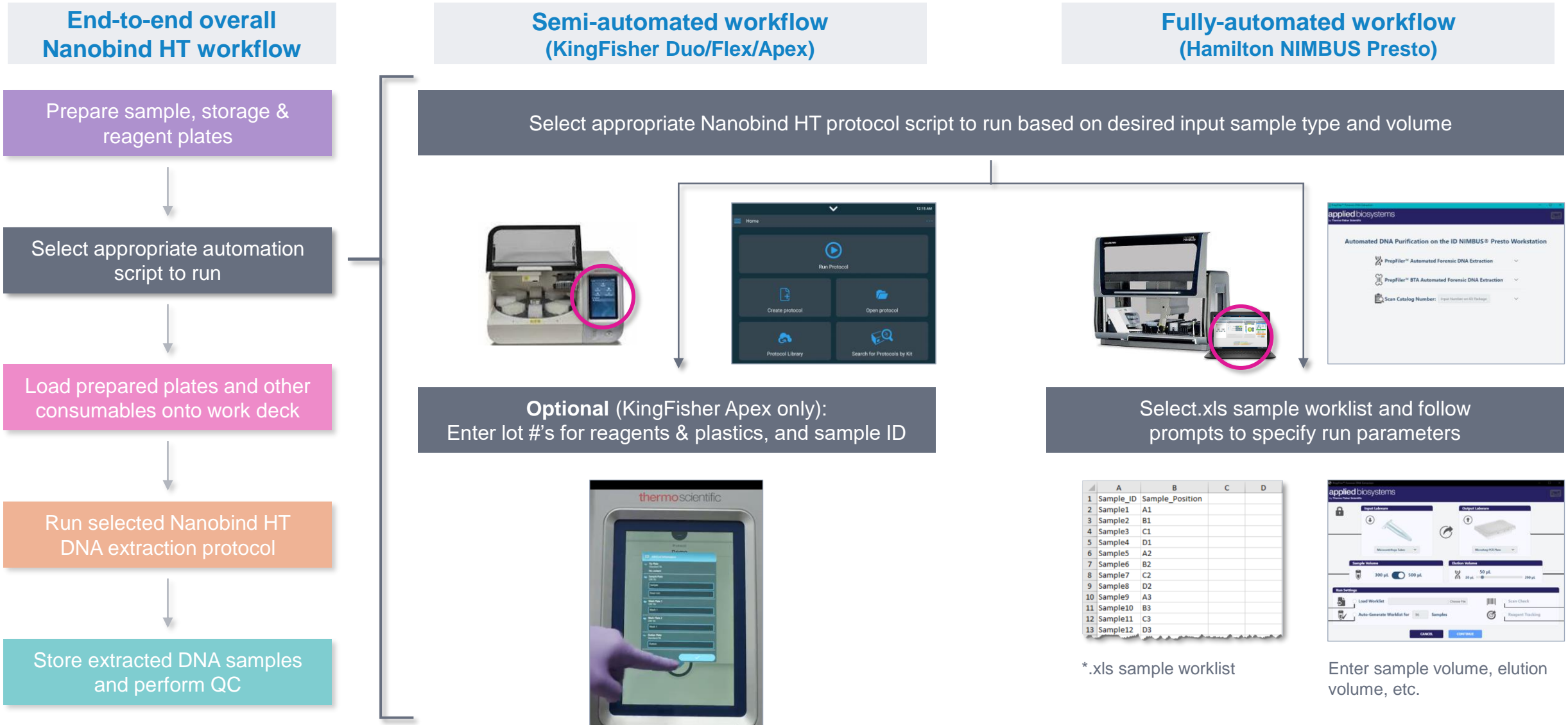
Plate 1 (sample plate) preparation procedure:

1. Add whole blood sample to appropriate well positions as specified in *.xls sample worklist.



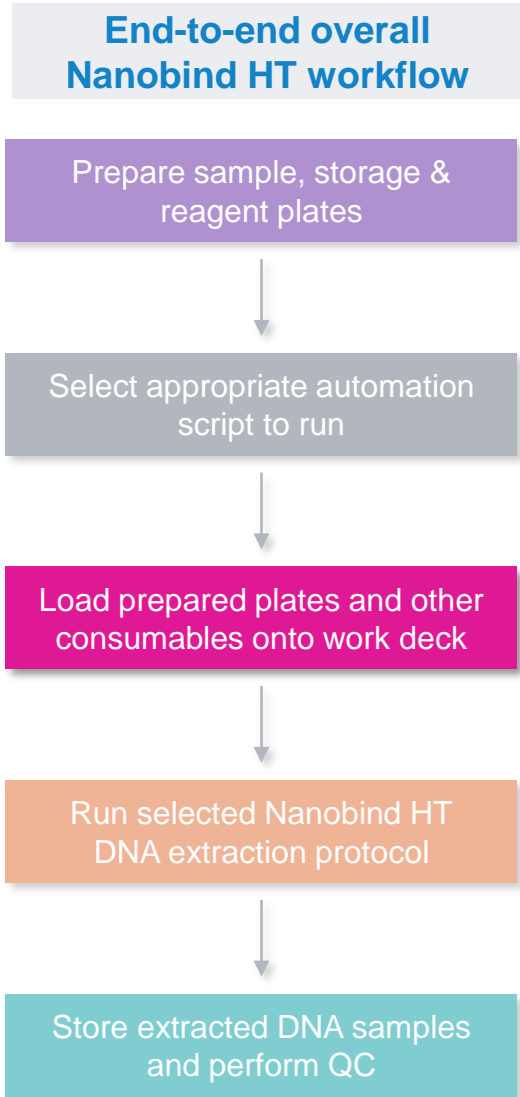
Nanobind HT HMW DNA extraction workflow step details

Automation script selection



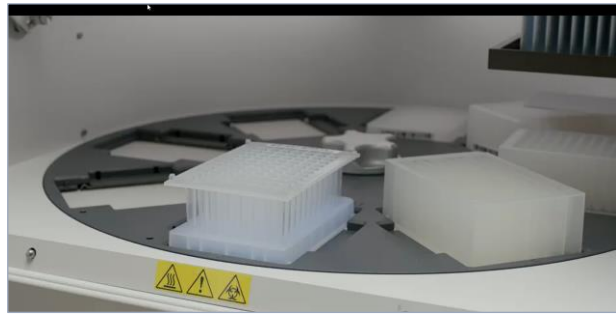
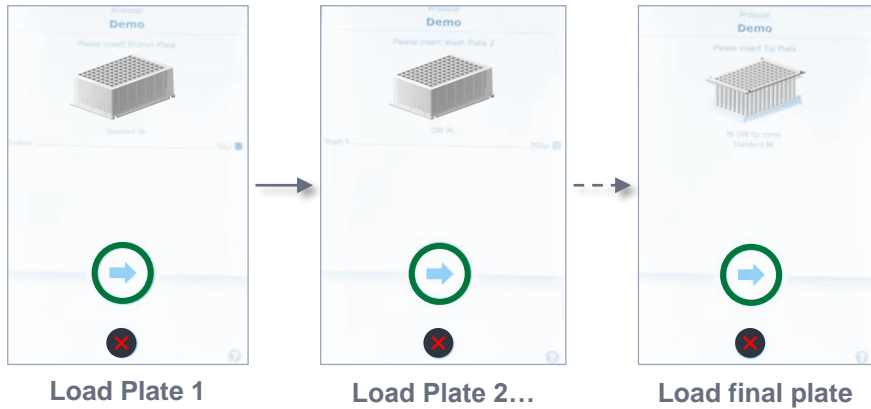
Nanobind HT HMW DNA extraction workflow step details

Work deck preparation



Semi-automated workflow (KingFisher Duo/Flex/Apex)

Sequentially load all prepared plates onto instrument work deck



Fully-automated workflow (Hamilton NIMBUS Presto)

Load 3 empty DW plates and Nanobind storage plate

Position	Quantity	Volume	Description
1	1		Kingfisher DWP24 number 1
2	1		Kingfisher DWP24 number 2
3	1		Kingfisher DWP24 number 3
4	1		Kingfisher DWP24 containing one 5 min Nanobind disk per well ON MAGNET

Load conductive filter tip boxes, wide-bore conductive tip boxes and tip comb plate*

Position	Quantity	Volume	Description
1	40		100µL Filter Tips
2	4		300µL Filter Tips
3	24		300µL Wide Bore Filter Tips
4	1		Tip Comb in Kingfisher DWP24

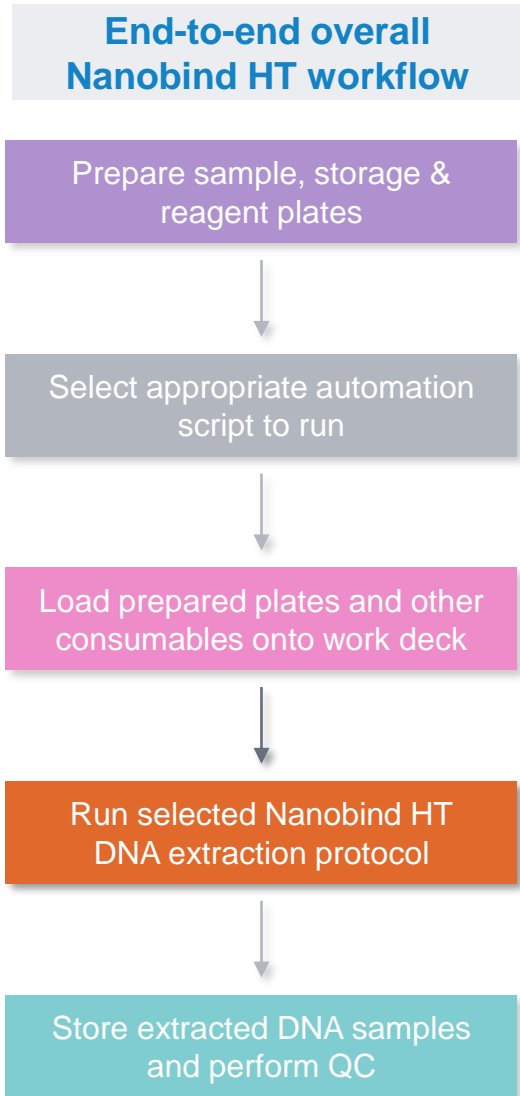
Load sample plate and fill reagent tubes & reservoirs

Position	Quantity	Volume	Description
MULTIblockadapter_13	1400.0µL		Protease K (vial 2mL, Sarstedt)
MULTIblockadapter_14	1400.0µL		Protease K (vial 2mL, Sarstedt)
MULTIblockadapter_17	1310.0µL		RNase A (vial 2mL, Sarstedt)
MULTIblockadapter_18	1310.0µL		RNase A (vial 2mL, Sarstedt)
Pipetting_Position_01	1		Kingfisher DWP24 containing samples
RCT50_1	22.8mL		B.L3 (60mL, Trough)
RCT50_2	44.6mL		IPA (60mL, Trough)
RCT50_3	55.8mL		CWI (60mL, Trough)
RCT50_4	8.2mL		EB (60mL, Trough)

* Note: If you do not load enough conductive filter tips to complete the run, you will be warned to load additional tips or start the run at your own risk.

Nanobind HT HMW DNA extraction workflow step details

Automation script execution



Semi-automated workflow (KingFisher Duo/Flex/Apex)

The run will start automatically when final plate is loaded and **Next** button is pressed.



User action required: When prompted, remove Lysis/Binding plate (**Plate 1**) from instrument and manually add IPA to each well. After adding IPA, return plate to instrument and resume program.



Note: Add isopropanol (IPA) gently against the **side** of the well into the Lysis / Binding solution.*

* Adding IPA directly to the Lysis / Binding solution may affect extraction purity.

Fully-automated workflow (Hamilton NIMBUS Presto)

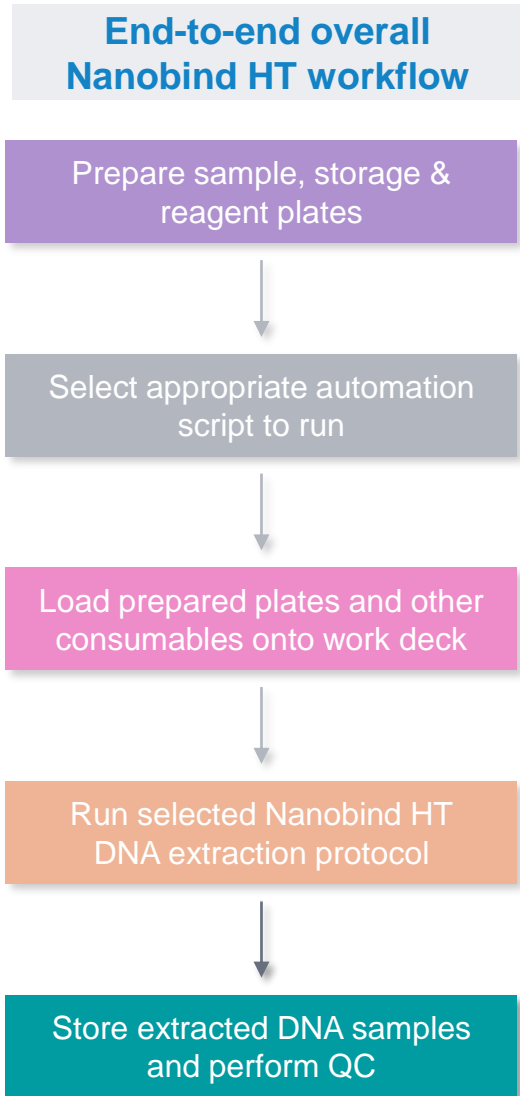
Close front cover of instrument to protect against environmental contamination and click **Ok** to start run



No user action required until run completes

Nanobind HT HMW DNA extraction workflow step details

Sample storage and QC



Semi-automated workflow (KingFisher Duo/Flex/Apex)

Fully-automated workflow (Hamilton NIMBUS Presto)

When run ends, remove each plate from instrument as prompted.



Manually transfer eluates from Elution Plate to a new storage plate or storage tubes



KingFisher Duo/Flex/Apex automation scripts are designed to **leave Nanobind disks in elution plate** after program completes

NIMBUS Presto automation scripts are designed to **place Nanobind disks back into the disk storage plate**, so the elution plate should not contain any disks inside

Pipette mix extracted DNA 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.



Note: Limited pipette mixing will not noticeably reduce DNA size or sequencing read lengths but is important for accurate quantitation and consistent sequencing performance

Let eluate rest overnight at room temp. to allow DNA to solubilize. Following overnight rest, pipette mix 10 times with a standard P200 pipette. Visible “jellies” should disperse after resting.

Recommended QC procedure for HMW DNA samples extracted using Nanobind HT kits

Use recommended tools to perform DNA QC evaluation of high-molecular weight DNA



Qubit
fluorometer

DNA concentration QC

Use Qubit dsDNA broad range (BR) assay kit for DNA concentration QC

- We recommend using a Qubit fluorometer (Thermo Fisher Scientific) with the dsDNA BR assay kit since it provides more reliable concentration measurements of (unsheared) HMW DNA

Optional: Determine RNA concentration using the Qubit RNA BR assay kit (Thermo Fisher Scientific)

- We recommend taking a single measurement to obtain an approximate RNA concentration reading



Nanodrop
spectrophotometer

DNA purity QC

Perform UV/VIS assay measurements with a Nanodrop to determine total nucleic acid conc. as well as purity (A260/A280, A260/230)

- **Note:** Inconsistent Nanodrop concentration or spectrophotometric readings may be obtained if the DNA is very heterogeneous or contains large amounts of unsolubilized “jellies”
- For information on how to disrupt particularly viscous regions, refer to the kit Guide & overview “Heterogeneity and viscosity” section or the “Troubleshooting FAQ” section in the Procedures & checklists



Femto Pulse
system

DNA sizing QC

Use a Femto Pulse system with Genomic DNA 165 kb Kit (Agilent Technologies) for HMW genomic DNA sizing QC

- We recommend diluting samples to 250 pg/μL (use wide-bore pipette for making dilutions and gentle mixing)
- Avoid mixing with a standard pipette! This will shear dilute solutions of DNA.

Expected QC results for HMW DNA samples extracted using Nanobind HT kits

Example ranges typically observed for DNA concentration, DNA purity and DNA sizing QC metrics



Qubit
fluorometer

DNA concentration QC

- 200 μ L human whole blood typically yields **~3 – 12 μ g** of DNA depending on donor white blood cell (WBC) count
- 1 mL of human whole blood typically yields **~3 – 70 μ g** depending on donor WBC count
- 1×10^6 GM24385 cells typically yields **~4 – 10 μ g**
- 1×10^6 MCF-7 cells typically yields **~12 – 18 μ g**



Nanodrop
spectrophotometer

DNA purity QC

- 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 expected ranges should sequence well
- UV purities outside of these ranges may indicate abnormalities in extraction process



Femto Pulse
system

DNA sizing QC

- Mode of extracted human cell and whole blood DNA measured on Femto Pulse system (Agilent Technologies) is typically **100 kb+**

Refer to Nanobind HT Procedure & checklist [documentation](#) for guidance on troubleshooting specific types of DNA extraction quality issues



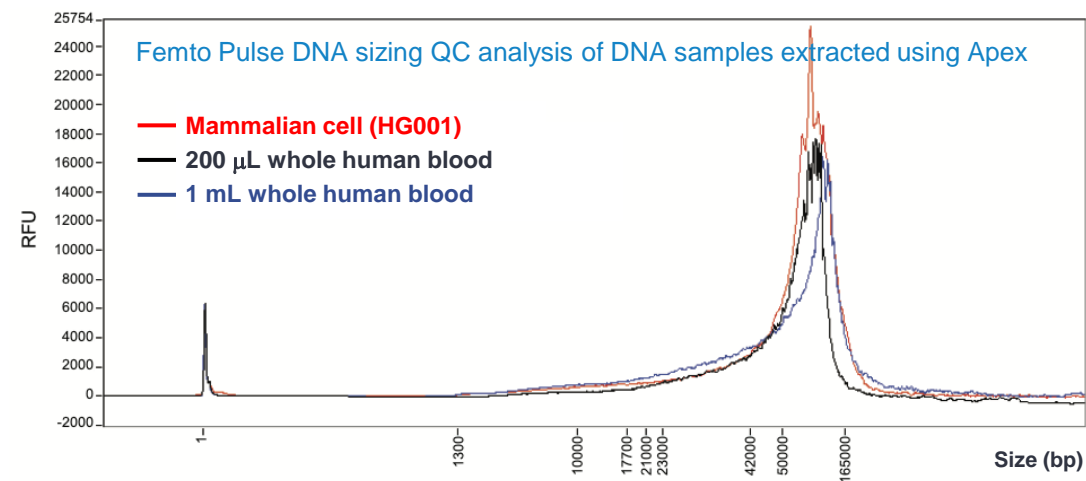
Nanobind HT HMW DNA extraction example performance data

Nanobind HT HMW DNA extraction performance on KingFisher Apex and Hamilton NIMBUS Presto

Example HMW DNA extraction QC results

Human whole blood	Mammalian cells
<ul style="list-style-type: none"> 200 μL or 1 mL fresh whole blood (max. 2 days storage at 4°C) or frozen For donor WBC count $\geq 4 \times 10^6$ cells/mL, expected HMW DNA yield is 3 – 12 μg for 200 μL blood sample and 3 – 70 μg for 1 mL blood sample 	<ul style="list-style-type: none"> 1 x 10⁶ diploid cells (fresh culture or frozen cell pellet) Expected HMW DNA yield is 5 – 15 μg

Sample	Robot	Input sample	DNA yield (μ g)	A260/A280 ratio	A260/A230 ratio	DNA mean size (kb)	GQN at 10 kb
Mammalian cell (HG001)	Nimbus	10 ⁶ cells	6.4	1.92	2.04	167	9.4
Mammalian cell (HG001)	Apex	10 ⁶ cells	7.0	1.88	2.08	113	9.6
Whole human blood	Nimbus	200 μ L	7.0	1.83	1.96	98	9.4
Whole human blood	Apex	200 μ L	6.1	1.83	1.80	124	9.7
Whole human blood	Nimbus	1 mL	32.6	1.86	1.86	94	9.3
Whole human blood	Apex	1 mL	36.4	1.89	2.18	115	9.4



Sizing QC results exceed SPK3 WGS library preparation input DNA quality requirements

- Mean DNA fragment size >90 kb
- >93% of DNA fragments longer than 10 kb (GQN at 10 kb)

Input DNA quality requirements for Procedure & checklist – Preparing whole genome and metagenome sequencing libraries using SMRTbell prep kit 3.0 ([102-166-600](#))

DNA Quality	Human, plant, and animal samples	Microbial and Metagenomic samples
DNA size distribution (Femto Pulse system)	50% ≥ 30 kb & 90% ≥ 10 kb	90% ≥ 7 kb

Nanobind HT HMW DNA extraction performance on KingFisher Apex and Hamilton NIMBUS Presto

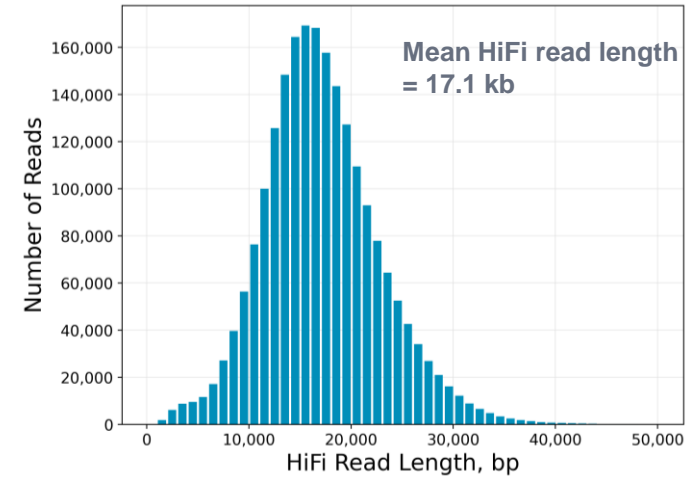
Example HiFi sequencing performance results

Sample	Robot	Input sample	HiFi mean read length (bp)	HiFi yield (Gb)
Mammalian cell (HG001)	Nimbus	10 ⁶ cells	16,563	41.9
Mammalian cell (HG001)	Apex	10 ⁶ cells	16,544	35.4
Whole human blood	Nimbus	200 µL	18,067	37.3
Whole human blood	Apex	200 µL	13,026	34.8
Whole human blood	Nimbus	1 mL	17,163	36.8
Whole human blood	Apex	1 mL	15,863	36.1

SPK3 WGS libraries constructed with Nanobind HT extracted DNA show excellent HiFi sequencing performance

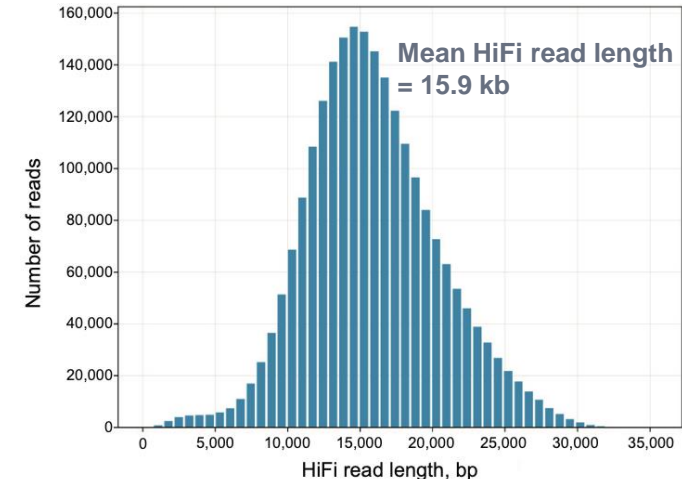
- HiFi yield >35 Gb
- Mean HiFi read length 13 – 18 kb
- Limited number of reads <8 kb

HiFi read length distribution



1 mL blood – NIMBUS Presto

HiFi read length distribution



1 mL blood – KingFisher Apex



Technical documentation & applications support resources

Technical resources for Nanobind HT HMW DNA extraction

PacBio Nanobind HT HMW DNA sample preparation literature

- Brochure – Nanobind HT HMW DNA extraction ([102-326-565](#))
- Guide & overview – Nanobind HT kits for automated HMW DNA extraction ([103-028-100](#))
- Overview – Nanobind HT HMW DNA extraction-robotic procedures ([103-032-000](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Apex ([102-995-300](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Duo ([102-995-400](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 1 mL whole blood – KingFisher Flex ([102-995-500](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 1 mL whole blood – Hamilton NIMBUS Presto ([102-995-600](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Apex ([102-995-700](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Duo ([102-995-800](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 200 µL whole blood – KingFisher Flex ([102-995-900](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – 200 µL whole blood – Hamilton NIMBUS Presto ([102-996-000](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Apex ([102-996-100](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Duo ([102-996-200](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – Cultured cells – KingFisher Flex ([102-996-300](#))
- Procedure & Checklist : Nanobind HT HMW DNA extraction – Cultured cells – Hamilton NIMBUS Presto ([102-996-400](#))
- Technical overview - Nanobind HT kits for automated HMW DNA extraction ([103-020-800](#))

Technical resources for Nanobind HT HMW DNA extraction (cont.)

Third-party automation instrumentation literature

- Hamilton Technical Note – Automated extraction of High Molecular Weight (HMW) DNA with PacBio Nanobind technology on the Hamilton NIMBUS Presto Assay Ready Workstation ([AN-2205-05](#))
- Hamilton Technical Note – 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 ([AN-2212-03](#))

Posters

- PacBio poster (2022) – High-throughput workflow for human whole genome sequencing using PacBio HiFi [[Link](#)]

Nanobind HT consumable product literature

- Nanobind HT 1 mL blood kit (PN [102-762-800](#))
 - Product insert: Nanobind HT 1 mL blood kit 4C
 - Product insert: Nanobind HT 1 mL blood kit RT
- Nanobind HT CBB kit (PN [102-762-700](#))
 - Product insert – Nanobind HT CBB kit 4C
 - Product insert – Nanobind HT CBB kit RT
- PacBio sample prep consumables website [[Link](#)]



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