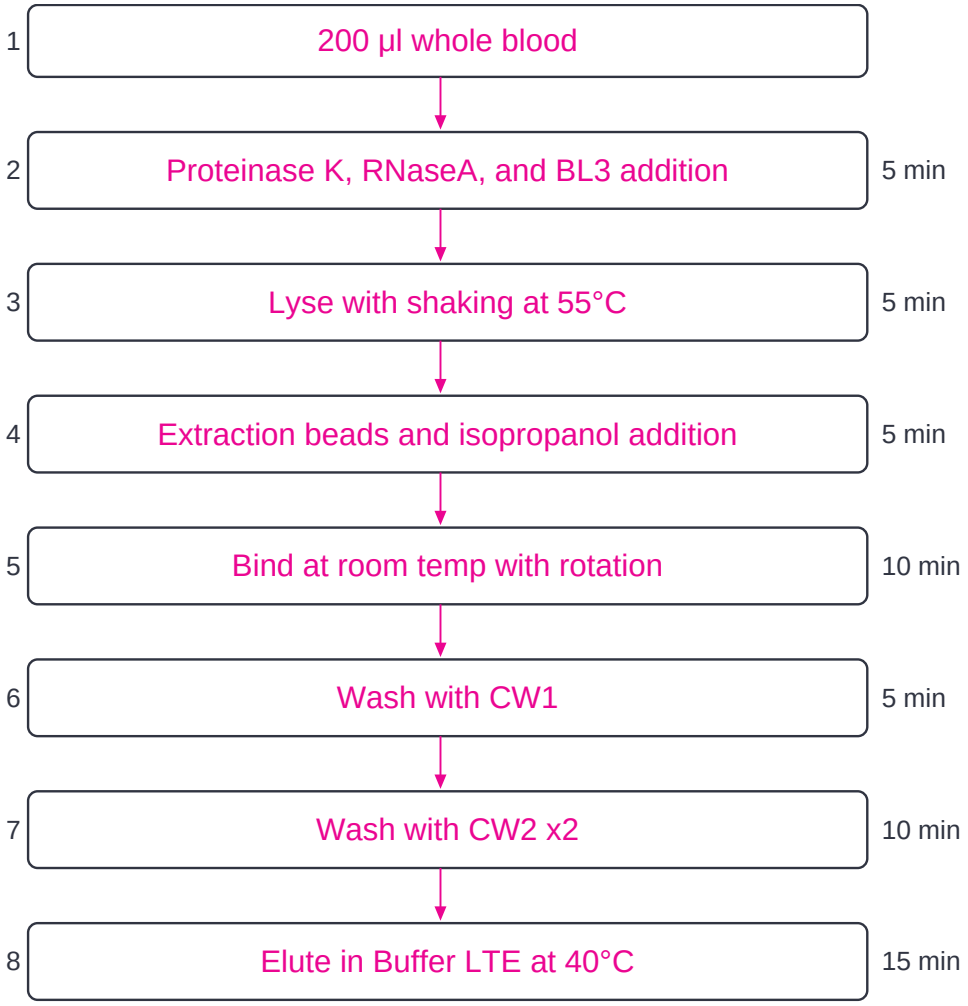
DNA extraction from whole blood using the Nanobind® HT plus kit

Procedure & checklist

Overview

This protocol describes the extraction of genomic DNA from 200 µL of whole blood using the PacBio® Nanobind HT plus kit. Genomic DNA extracted by this method is compatible with downstream PacBio HiFi applications and sequencing. Processing of 8 samples requires approximately 1 hour.

Workflow



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Required materials and equipment

Reagents for extraction	Part Number
Nanobind® HT plus kit: • Nanobind HT plus Kit RT • Nanobind HT Kit 4C	PacBio® 103-898-000 (bundle PN) PacBio® 103-872-900 PacBio® 102-763-000
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
DNA sizing and quantification	**Part Number**
Femto Pulse system	Agilent Technologies, Inc. M5330AA
Femto Pulse gDNA 165kb analysis kit	Agilent Technologies, Inc. FP-1002-0275
Qubit 4 Fluorometer or Qubit Flex Fluorometer	ThermoFisher Scientific Q33238 or Q33327
Qubit 1X dsDNA BR Assay Kit or Qubit 1X dsDNA HS Assay kit	ThermoFisher Scientific Q33265/Q33266 ThermoFisher Scientific Q33230/Q33231
Equipment for manual processing	**Part Number**
Magnetic stand compatible with 1.5 ml	Any MLS
Platform rocker or mini-tube rotator	Thermo Scientific M48725Q or Fisher Scientific 88-861-051
ThermoMixer	Eppendorf 5382000023
1.5 mL Protein LoBind microcentrifuge tubes	Eppendorf 022431081
1.5 mL DNA LoBind microcentrifuge tubes	Eppendorf 022431021

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Before you begin

Sample input: whole blood

- 200 µL of whole blood is the recommended input volume.
- K2 EDTA is the recommended anticoagulant. Samples stored in sodium heparin (NaHep) and citrate (NaCit) also performed well in limited testing.
- Blood samples should be aliquoted to avoid repeated freeze-thaws.
- Storage at 4°C should be limited to 2 days or less to prevent sample degradation.
- No systematic difference has been observed in DNA QC or sequencing results between fresh and frozen blood samples
- 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 insufficiently mixed samples may result in inconsistent DNA yield and purity.

Quality control

- Vortex genomic DNA prior to quantification with the Qubit 1X dsDNA BR/HS assay. If using the Qubit 1X dsDNA HS assay, dilution of the sample may be necessary.

Note: The extracted DNA mass typically ranges from 3–15 µg and depends on the donor white blood cell count.

- The Agilent Femto Pulse (Genomic DNA 165 kb kit) is recommended for accurately sizing genomic DNA. Dilute the sample to 250 pg/µL according to Agilent SOP.

Note: GQN10kb of extracted DNA is typically greater than 8; GQN30kb is typically greater than 7.

DNA storage

DNA can be stored at 4°C for 1 month. Keep at −20°C or −80°C for long term storage.

Reagent preparation and handling

- Buffer CW1 and CW2 are supplied as concentrates. The final ethanol concentration required is 60%. Before use, add the volume indicated on the bottle of ethanol to Buffer CW1 and Buffer CW2.
- RNase A should be stored at 4°C upon arrival.
- Store Extraction beads at room temperature. Vortex prior to use.

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 HT plus kits are intended for research use only.

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Procedure and checklist

Before you begin: Set 1.5 mL ThermoMixers to 55°C and 40°C. If only one ThermoMixer is available, set to 55°C and lower to 40°C after step 1.5.

Step	Instructions for extraction from saliva and whole blood
1.1	**Important**: The sample and reagents must be added to the tube or well in the order specified below. Add **20 µL of Proteinase K** to a 1.5 mL Protein LoBind tube.
1.2	Add **200 µL of whole blood.** Pulse vortex (1s max speed 10 times) and spin down. **Note:** The addition of blood to Proteinase K rather than vice versa increases lysis efficiency through improved mixing.
1.3	Add **20 µL of RNaseA**. Pulse vortex (1s max speed 10 times) and spin down. Incubate at **room temperature for 3 minutes.**
1.4	Add **200 µL of BL3** slowly against the side of the tube. Pulse vortex (1s max speed 10 times) and spin down. **Note:** Do not add BL3 directly to the sample solution as this may impact extraction performance.
1.5	Incubate on a ThermoMixer at **900 rpm and 55°C for 5 min**. After incubation, pulse vortex (1s max speed 10 times) and spin down.
1.6	Add **25 µL of vortexed Extraction beads** to lysate.
1.7	Add **440 µL of 100% isopropanol** slowly against the side of the tube to lysate containing beads and invert to mix 4 times. **Note:** Do not add isopropanol directly to the sample solution as this may impact extraction performance.
1.8	Mix on a platform rocker at 20 rpm or tube rotator at 9 rpm for **10 min at room temperature**.
1.9	Quick spin the tube and place sample on an appropriate magnet and allow beads to separate fully from the solution. Magnetize for at least 4 minutes. **Note:** The bead pellet will be obscured by blood.
1.10	Discard supernatant.
1.11	Add **700 µL of Buffer CW1**, remove the tube from the magnet, and invert to mix 4 times. Replace the tube on the magnetic base and discard the supernatant.
1.12	Add **700 µL of Buffer CW2**, remove the tube from the magnet, and invert to mix 4 times. Replace the tube on the magnetic base and discard the supernatant.
1.13	**Repeat step 1.12** for a total of two CW2 washes.

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1.14	Remove residual wash buffer: • Remove samples from the magnet • Quick-spin • Place the tube back on the magnetic separation rack • Remove residual wash buffer and discard.
1.15	Air dry the beads for 1 min. Remove the tube from the magnet.
1.16	Add **50-100 µL of Buffer LTE** directly onto the beads.
1.17	Incubate at **40°C for 10 min**.
1.18	Pulse vortex until beads are completely resuspended and not adhering to the sides of the walls. Then place sample back at **40°C for 5 min**. Important: Incomplete resuspension of beads may lead to low recovery.
1.19	Quick spin the tube and place the sample on an appropriate magnet and allow beads to separate fully from the solution. Transfer the eluate to a new DNA LoBind tube. To avoid bead carryover, aspirate slowly and leave behind 5 µl of eluate.
1.20	Pipette mix and vigorously vortex extracted genomic DNA to ensure it is homogeneous prior to quantification. Measure DNA concentration with a Qubit fluorometer.

PROTOCOL COMPLETE

Revision history (description)	Version	Date
Initial release	01	November 2026

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