PacBio

DNA extraction from whole blood using the Nanobind® HT plus kit on the KingFisher Apex system

Guide & Overview

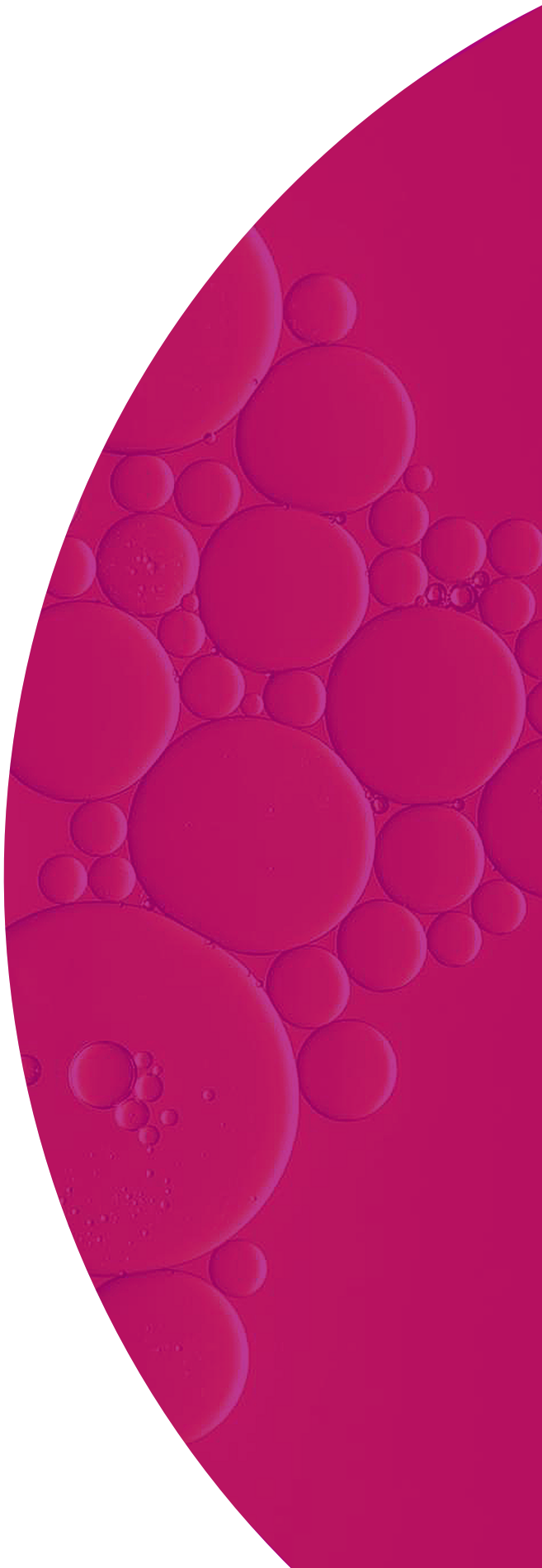


Table of contents

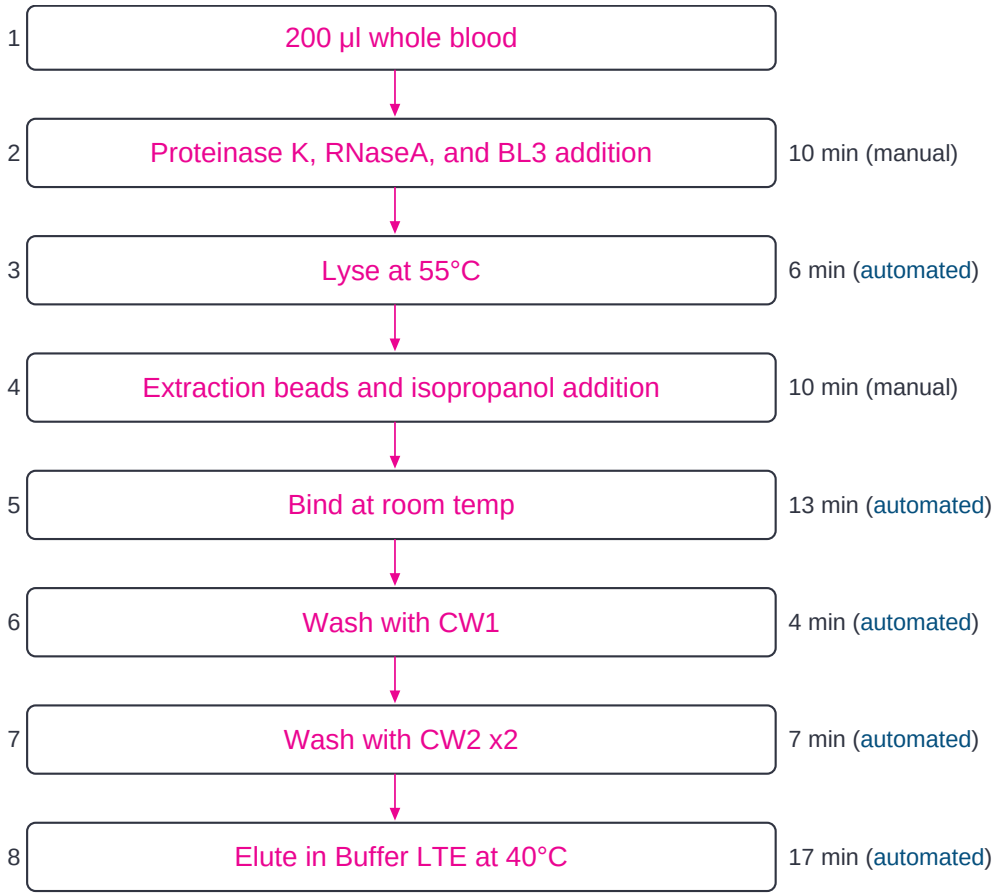
Overview **3**
Workflow **3**
Required materials and equipment **4**
Before you begin **5**
Sample input: Whole blood 5
Quality control 5
DNA storage 5
Reagent preparation and handling 5
Safety precautions 5
Product use 5
Workflow steps **6**

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Overview

This protocol describes the extraction of genomic DNA from 200 µL of whole blood using the PacBio® Nanobind HT plus kit and the KingFisher Apex system. Genomic DNA extracted by this method is compatible with downstream PacBio HiFi applications and sequencing. Processing of 96 samples requires approximately 1.5 hours (30 minute setup, 1 hour instrument run time).

Workflow



© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

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
Equipment for automated processing	**Part Number**
"Nanobind HT Plus Blood v1.kfx2" Kingfisher Apex script	103-941-200
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
TempAssure 0.2 mL PCR 8-Tube Strips (for aliquoting RNAseA and ProteinaseK)	USA Scientific 1402-4700
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

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

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.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

Workflow steps

1. Collect 6 deep well plates and aliquot reagent based on the number of samples to be processed.

Apex Position	Reagent Plate	Volume per well
6	Buffer LTE Plate	50–100 µL*
5	Buffer CW2 Plate 2	700 µL
4	Buffer CW2 Plate 1	700 µL
3	Buffer CW1 Plate	700 µL
2	Sample Plate	See step 2
1	KingFisher Apex 96 deep-well tip comb placed in plate	NA

Note: *5 – 10 µl volume loss is expected for the elution.

Important: the sample and reagents must be added to the well in the order listed below.

2. Prepare **Sample Plate**. Aliquot ProteinaseK and RNaseA into PCR strip tubes for ease of use with a multi-channel pipette.
    - Add **20 µL of Proteinase K** to a deep well plate.
    - Add **200 µL of whole blood** to the deep well plate.

Note: The addition of blood to Proteinase K rather than vice versa increases lysis efficiency and purity through improved mixing.

    - Add **20 µL of RNaseA**.
    - Add **200 µL of BL3** slowly against the side of the well.

Note: Do not add BL3 directly to the sample solution as this may impact extraction performance.

3. Ensure the KingFisher Apex is set up with the 96 deep well magnetic head and the 96 deep well heating block.
4. Select the "**Nanobind HT Plus Blood v1**" script on the KingFisher Apex instrument and select "Start."
5. Insert plates into the KingFisher Apex instrument as indicated on the display, with well A1 of the plate placed in the upper right corner.
6. Follow the Apex prompts for loading the plates. Select "Next" after each plate to confirm position. The protocol will begin when the final plate is loaded and the "Next" button is selected.
7. When prompted by the instrument (approximately 6 minutes after starting the run), remove the sample plate from the instrument. Add **25 µl of vortexed Extraction beads** and **440 µl of isopropanol** to each well. Re-insert the plate and select "Next" to resume the protocol.

Note: Add Isopropanol slowly against the side of the well into the Sample Plate.

© 2026 PacBio. All rights reserved. Research use only. Not for use in diagnostic procedures.

8. At the end of the run, remove plates as indicated on the instrument display. The protocol will end when the final plate is removed and the “Next” button is selected.
9. Quick spin the plate and transfer eluate to a new DNA LoBind plate or 1.5 mL DNA LoBind tube.
10. Pipette mix and vigorously vortex extracted genomic DNA to ensure it is homogeneous prior to quantification. Measure DNA concentration with a Qubit fluorometer.

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

Research use only. Not for use in diagnostic procedures. © 2026 Pacific Biosciences of California, Inc. (“PacBio”). All rights reserved. Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third-party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at pacb.com/license. Pacific Biosciences, the PacBio logo, PacBio, Circulomics, Omniome, SMRT, SMRTbell, Iso-Seq, Sequel, Nanobind, SBB, Revio, Revio SPRQ, Onso, Apton, Kinnex, PureTarget, SPRQ, Vega, and Vega SPRQ are trademarks of PacBio. Reference to third-party companies, products, or services are for informational purposes only and do not imply endorsement, sponsorship, or affiliation.