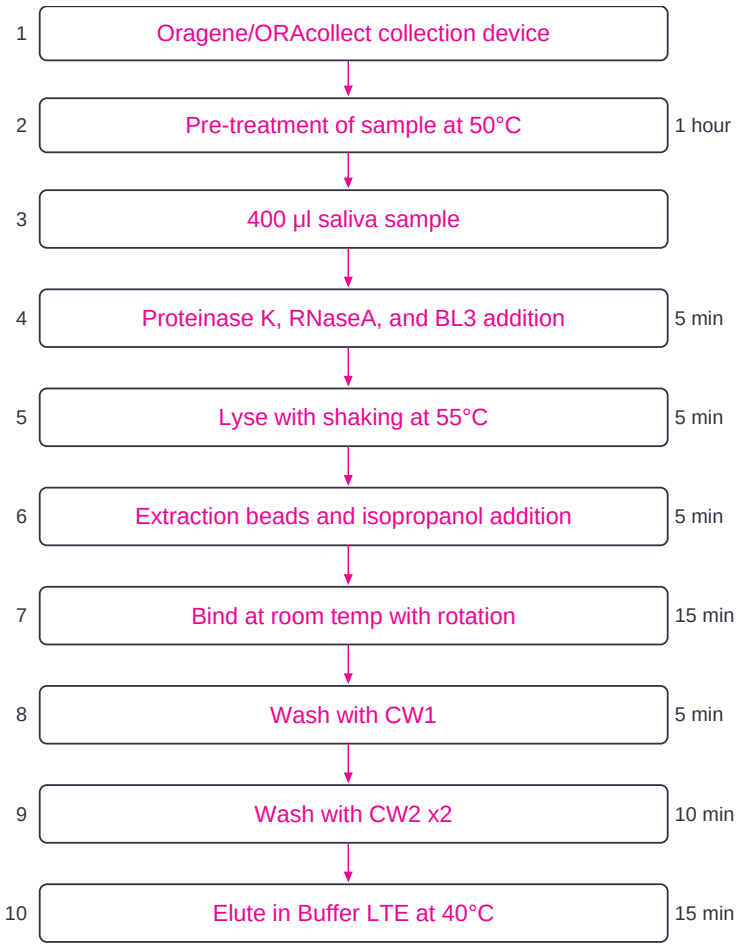
DNA extraction from saliva samples using the Nanobind® HT plus kit

Procedure & checklist

Overview

This protocol describes the extraction of genomic DNA from samples collected with DNA Genotek Oragene or ORAcollect collection devices using the PacBio® Nanobind HT plus kit. Applicable collection devices include the Oragene•Dx (OGD-600, OGD-610), Oragene•DISCOVER (OGR-500, OGR-600), or ORAcollect (OCD-100, OCD-100A). Genomic DNA extracted by this method is compatible with downstream PacBio HiFi applications and sequencing. Manual processing of 8 samples requires approximately 1 hour, not including sample pre-treatment.

Workflow



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Required materials and equipment

Reagents for sample collection	Part Number
DNA Genotek ORAcollect•Dx collection device *or*	OCD100
DNA Genotek Oragene•Dx collection device	OGD600
Reagents for extraction	**Part Number**
Nanobind® HT plus kit:	PacBio® 103-898-000 (bundle PN)
• Nanobind HT plus Kit RT	PacBio® 103-872-900
• Nanobind HT Kit 4C	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	ThermoFisher Scientific Q33265/Q33266
Qubit 1X dsDNA HS Assay kit	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: Saliva

- The required input volume is 400 µL of sample collected and stabilized in an Oragene or ORAcollect device.
- Improper sample collection may lead to low DNA yields. Ensure the samples are collected according to the manufacturer's instructions.
- Prior to extraction:
    - Mix collection device containing sample by inverting.
    - Incubate the entire collected sample in the original collection tube at 50°C (1h in a water incubator, 2h in air incubator). This incubation only needs to be done once; repeat extractions do not require additional incubations. After heating, mix again by inversion to ensure the sample is homogeneous.
- Non-human contamination in saliva samples will vary by donor.

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 from Oragene collection devices typically ranges from 2–30 µg, depending on the donor. The extracted DNA mass from ORAcollect collection devices typically ranges from 1–15 µg, depending on the donor.

- 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: GQN_{10kb} typically ranges between 6–9 for samples collected with Oragene devices, and 3–8 for samples collected with ORAcollect devices.

DNA storage

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

Reagent 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

Important: Ensure that the sample has undergone an initial 50°C incubation (see "Before you begin" section).

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 **400 µL of stabilized saliva.** Pulse vortex (1s max speed 10 times) and spin down **Note:** The addition of sample to Proteinase K rather than vice versa increases lysis efficiency and purity 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 **100 µL of BL3** slowly against the side of the tube or well. Pulse vortex 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 **540 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 **15 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.
1.10	Discard supernatant.
1.11	Add **700 µL of Buffer CW1**, remove the tube from the magnet, invert to mix 4 times and spin down. Place the tube back on the magnetic base and discard the supernatant.
1.12	Add **700 µL of Buffer CW2,** remove the tube from the magnet, invert to mix 4 times and spin down. Place the tube back 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 and vortex to mix. **Note:** If running the PureTarget application, elution in 50 µl is recommended.
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 sample on an appropriate magnet and allow beads to separate fully from the solution. Transfer the eluate to a new DNA LoBind tube.
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

Note: If running **Short read eliminator (SRE)**, Oragene saliva recoveries typically range between 50–80%; ORAcollect saliva recoveries from SRE typically range between 40–70%.

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

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