PacBio

DNA extraction from saliva samples using the Nanobind® HT plus kit on the KingFisher Apex system

Guide & Overview

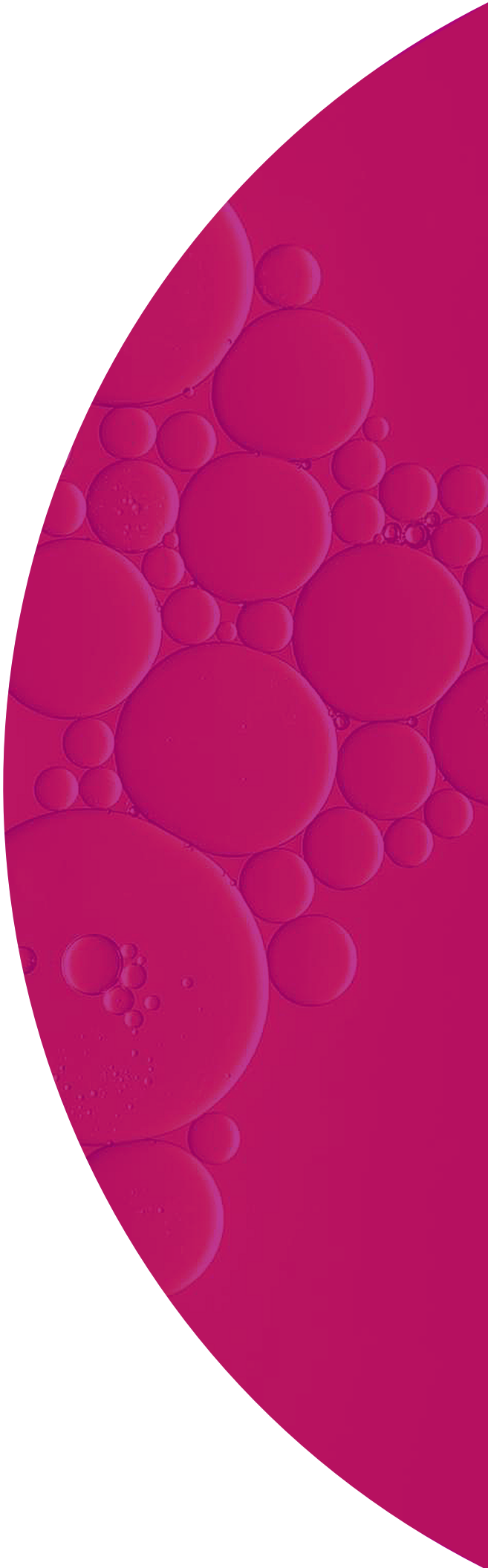


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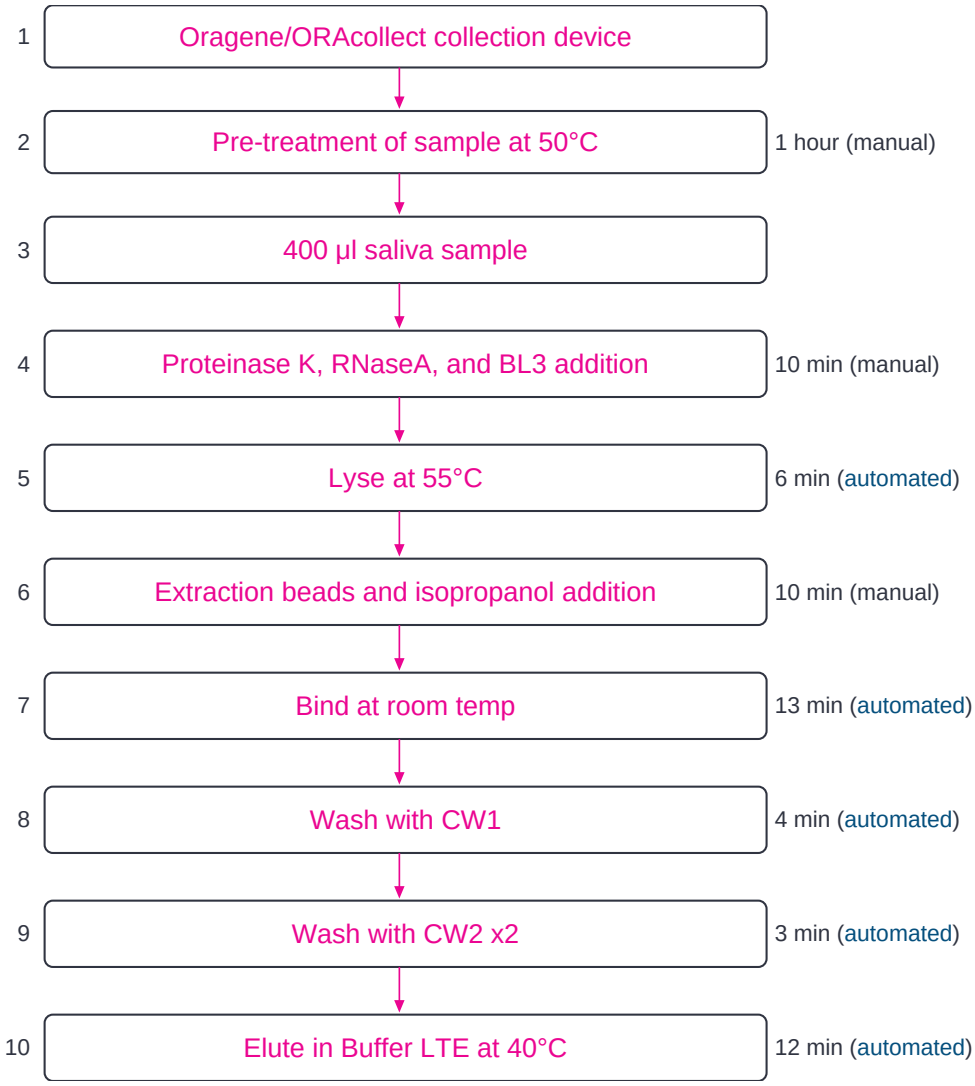
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Overview

This Guide & overview describes the extraction of genomic DNA from samples collected with DNA Genotek Oragene or ORAcollect collection devices using the PacBio® Nanobind HT plus kit and the KingFisher Apex system. 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. Processing of 96 samples requires approximately 1.5 hours (30 min setup, 1 hour instrument run time), 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
Equipment for automated processing	**Part Number**
"Nanobind HT Plus Saliva v1.kfx2" Kingfisher Apex script	103-941-300
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
Recommended 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

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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.

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Product use

Nanobind HT plus kits are intended for research use only.

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: *If running the PureTarget™ application, 50 µL is the recommended elution volume.

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 the **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 **400 µL of stabilized saliva** to the deep well plate.

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

    - Add **20 µL of RNaseA**.
    - Add **100 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 instrument is set up with the 96 deep well magnetic head and the 96 deep well heating block.
4. Select the "**Nanobind HT Plus Saliva 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.

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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 **540 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 Lysis/Binding solution.

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.

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 13, 20262026

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