Extracting HMW DNA from saliva collected in DNA Genotek™ Oragene™ devices using Nanobind® kits

Procedure & checklist

This protocol describes the extraction of HMW DNA (50–300+ kb) from saliva collected with a DNA Genotek Oragene•DNA saliva collection device using Nanobind kits. Applicable collection devices are the Oragene•Dx (OGD-500, OGD-510, OGD-600, OGD-610), Oragene•DISCOVER (OGR-500, OGR-600) and Oragene•DNA (OG-500, OG-510, OG-600, OG-610). This protocol is recommended for PacBio® HiFi sequencing and requires the Nanobind CBB kit (102-301-900) or the Nanobind PanDNA kit (103-260-000).

Required material and equipment

Equipment	Model
Nanobind® CBB kit or Nanobind® PanDNA kit	PacBio® (102-301-900 or 103-260-000)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
ThermoMixer	Eppendorf (5382000023)
Platform rocker or Mini-Tube Rotator	Thermo Scientific (M48725Q) or Fisher Scientific (05-450-127)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431081)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantitation	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits
Air or water incubator capable of maintaining 50°C	Any MLS

Before you begin

For all protocols

Eppendorf Protein LoBind tubes (Eppendorf #022431081) are highly recommended for all extractions to reduce protein contamination from tube carryover. Protein LoBind tubes are more effective in reducing carryover contamination than DNA LoBind tubes or other tubes and will result in improved UV purity.

Prior to starting

The PanDNA kit contains 3 wash buffers (CW1, CW2 and, PW1) to extract various sample types. The CBB kit only contains 2 wash buffers (CW1 and CW2). Buffers CW1, CW2, and PW1 are supplied as concentrates. CW1 and

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CW2 are used with a 60% final ethanol concentration. PW1 is used with a 70% final ethanol concentration. Before using, add the appropriate amount of ethanol (96–100%) to Buffers CW1, CW2, and PW1, as indicated on the bottles.

Note: Not all buffers are used in every procedure.

Kit storage

RNase A and Buffer CT (Nanobind PanDNA kit, 103-260-000, only) should be stored at 4°C upon arrival.

Nanobind disks and all other buffers should be stored at room temperature (15–30°C).

Buffer NPL (Nanobind PanDNA kit, 103-260-000, only) may form precipitates if stored cooler than room temperature. If this happens, precipitates will return to solution when stored at room temperature. Alternatively, the buffer can be warmed in a water bath to re-dissolve precipitates.

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 CBB and Nanobind Pan DNA kits are intended for research use only.

Considerations when working with DNA from saliva

Human DNA content

Saliva DNA is ~80–90% human, compared to buccal swabs which can be as low as 10%.

Source

Most isolated DNA in saliva comes from white blood cells, not buccal epithelial cells.

Yield

Improper saliva sample collection may lead to low DNA yields. Ensure the samples are collected according to the manufacturer's instructions for use included with the Oragene saliva collection devices (Saliva collection video). In particular, subjects should:

- Avoid eating, drinking, smoking, chewing gum, or brushing their teeth for 30 minutes prior to collecting a saliva sample.
- Ensure the proper volume of saliva is collected as indicated by the 'Fill To' line on the Oragene device.

Input requirement

- 500 µL of saliva collected and stabilized in an Oragen device.
- Yield for saliva will vary from ~1 to ~45 µg depending on the donor.
- Saliva collected and stabilized in the Oragene tube buffer must contain >2 µg of DNA in 500 uL for efficient Nanobind extraction. DNA content can be checked prior to extraction by Qubit BR measurement (as described in procedure step 1.2)
- Saliva collected in Oragene devices is stable at RT up to 5 years as describe in this white paper from DNA Genotek.
- Initial 50°C incubation needs to be performed once per sample (procedure step 1.1).

Procedure and checklist

1. HMW DNA extraction from Oragene collected saliva

✔	Step	Instructions
	1.1	Follow DNA Genotek directions for saliva collection. • Mix thoroughly by inversion to ensure saliva and stabilization solution are completely combined. • Prior to extraction, incubate the entire collected saliva 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 one time; repeat extractions do not require repeat incubations. • Following incubation, invert-mix to ensure sample homogeneity. **Note:** If a water bath is used, ensure the sample-containing portion of the tube remains immersed in water.
	1.2	Perform a Qubit BR premeasurement of the raw sample to determine if the DNA content of the tube is sufficient for efficient Nanobind extraction • Vortex the saliva sample for 5s prior to measurements. • Quantify a 10 µL aliquot using the Qubit dsDNA BR assay. **Important:** Ensure thorough homogenization of the saliva sample to avoid inaccurate measurements. • **If you have less than 4 ng/µL (less than 2 µg in 500 µL), the extraction recovery may be low and we recommend recollecting the saliva sample for extraction.**
	1.3	Vortex the Oragene sample tube to mix and add 500 µL of sample to a 1.5 mL Protein LoBind tube. **Note:** The narrow taper of the 1.5 mL tube is critical for proper removal of wash buffer in steps 1.15 & 1.16 and for thorough recovery of eluate in step 20.
	1.4	Add 20 µL of Proteinase K.
	1.5	Add 20 µL of RNaseA.
	1.6	Add 200 µL of Buffer BL3. Vortex for 1s to resuspend.
	1.7	Incubate on a ThermoMixer at 900 rpm and 55°C for 45 min.
	1.8	Add Nanobind disk to lysate and add 700 µL isopropanol. Inversion- mix 10X. • The Nanobind disk must be added before isopropanol.
	1.9	Mix on a platform rocker at 20 rpm or tube rotator at 9 rpm for 15 min at RT.
	1.10	Place the tube on the magnetic tube rack using the procedure described in the Nanobind PanDNA Kit Guide & Overview.
	1.11	Discard liquid with a pipette using the procedure described in the Nanobind PanDNA Kit Guide & Overview.
	1.12	Add 700 µL of Buffer CW1 and inversion-mix 4X. Replace the tube rack on the magnetic base and discard the supernatant.
	1.13	Add 500 µL of Buffer CW2 and inversion-mix 4X. Replace the tube rack on the magnetic base and discard the supernatant.
	1.14	Repeat Step 1.13.
	1.15	Remove any residual liquid from the cap of the tube.
	1.16	Spin the tube on a mini-centrifuge for 1s. With the tube rack already on the magnetic base and right side up, place the tube on the tube rack and remove the residual liquid. • If the Nanobind disk is blocking the bottom of the tube, gently push it aside with the tip of the pipette towards the magnet.
	1.17	Repeat step 1.16. If residual wash buffer remains on the sidewalls of the tube, repeat step 1.16 again.
	1.18	Remove the tube from the magnet.

1.19	Add 100 µL of Buffer LTE directly onto the Nanobind disk and incubate at RT for 10 min. Ensure the Nanobind disk is submerged in Buffer LTE.
1.20	Collect DNA by transferring eluate to a new 1.5 mL Protein LoBind microcentrifuge tube.
1.21	Spin the tube containing the Nanobind disk on a mini-centrifuge for 5s and combine any additional liquid that comes off of the disk with the previous eluate. Repeat if necessary. **Note:** This 5 s spin is critical for recovering the DNA. A 2nd elution will not be necessary if Step 1.21 is performed as described.
1.22	Pipette-mix the sample 5X with a standard P200 pipette to homogenize the sample and disrupt any unsolubilized "jellies" that may be present.
1.23	Let sample rest overnight at RT to allow DNA to solubilize further. **Note:** The DNA will solubilize after resting at RT or by coaxing it into solution using gentle mixing. For samples that need to be used immediately, we recommend pipette mixing or needle shearing.
1.24	Analyze the recovery and purity of the DNA by NanoDrop and Qubit as described in the QC Procedure. • After resting overnight, pipette-mix the sample 5X with a standard P200 pipette before performing QC procedure. • The extracted HMW DNA can be heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The bigger the DNA, the more this will be apparent.

PROTOCOL COMPLETE

QC procedures

It is recommended that QC is performed after the DNA has been allowed to rest at RT overnight and appears homogeneous under visual examination and when pipetting.

1. Perform triplicate NanoDrop UV/VIS measurements from the top, middle, and bottom of the tube to determine total nucleic acid concentration as well as purity (A260/A280, A260/230).
    - HMW DNA is inherently difficult to work with as viscosity and inhomogeneity are often issues. We recommend taking at least three measurements, sampling from the top, middle, and bottom of the tube, to get an accurate concentration reading. We typically see concentration %CV values of <20%. However, if the DNA is very large, the %CV can exceed 30–40%.
    - If the DNA is very heterogeneous or contains large amounts of unsolubilized "jellies", refer to the kit Guide & overview "Heterogeneity and viscosity" and "Troubleshooting FAQ" sections for more information.
2. Perform triplicate Qubit dsDNA BR assay measurements from the top, middle, and the bottom of the tube to determine DNA concentration.
    - We recommend taking the average of multiple measurements to ensure an accurate DNA concentration reading.
    - We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the dsDNA BR assay kit. We do not recommend the dsDNA HS assay kit, as we have found the concentration measurements to be unreliable.
3. Perform a single Qubit RNA BR assay measurement to determine the RNA concentration (optional).
    - We recommend taking a single measurement to get an approximate RNA concentration reading.
    - We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the RNA BR assay kit.
4. Use the Agilent Femto Pulse for HMW DNA size QC.

- We recommend diluting the sample to 250 pg/µL.
- Follow Agilent instructions for diluting the sample.
- Use the Genomic DNA 165 kb Kit (Agilent Technologies) for unsheared gDNA.

Storage of DNA

DNA can be stored in Buffer LTE (formerly Buffer EB) at 4°C for several months. Long term storage at −20°C or −80°C can be used if necessary. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Revision history (description)	Version	Date
Initial release	01	October 2024