Extracting HMW DNA from insects using the Nanobind® PanDNA kit

Procedure & checklist

This protocol describes the extraction of HMW (50–300+ kb) DNA from insects and other arthropods. It is recommended for PacBio® HiFi sequencing. This protocol requires the Nanobind PanDNA kit (103-260-000).

Required materials and equipment

Equipment/reagent	Manufacturer (part number)
Nanobind® PanDNA kit	PacBio® (103-260-000)
Magnetic tube rack	Thermo Fisher Scientific DynaMag-2 (12321D)
RNase-Free Disposable Pellet Pestles	Thermo Fisher Scientific (12-141-364)
Surgical Scalpel	Thermo Fisher Scientific (22-079-712)
ThermoMixer	Eppendorf (5382000023)
Platform rocker or Mini-Tube Rotator	Thermo Fisher Scientific (M48725Q) or Thermo Fisher Scientific (05-450-127)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431081)
2.0 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431102)
70 µm strainer	Thermo Fisher Scientific (NC1444112)
Wide bore 200 µL pipette tips (optional)	USA Scientific (1011-8410)
Wide bore 1000 µL pipette tips (optional)	Thermo Fisher Scientific (2079G)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantitation	Thermo Fisher Scientific Qubit 3.0, dsDNA BR and RNA BR Assay Kits

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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 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

Buffer CT, Buffer RBC 10X, and RNase A should be stored at 4°C upon arrival.

Nanobind disks and all other buffers should be stored at room temperature (18–25°C).

Buffer NPL may form precipitates if stored below 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 PanDNA kits are intended for research use only.

Input requirements

- The appropriate input mass will differ from insect to insect but should be an amount that yields 3–30 µg of DNA.
- A consideration in determining the appropriate mass is that the exoskeleton (which is largely composed of chitin, wax, and protein) may contribute significantly to the mass, but does not contain any DNA.
- Using too high of an input will overload the lysis chemistries and negatively impact the DNA yield and quality.
- If working with an insect with a body mass lower than 20 mg, several individuals must be pooled together to reach 20–30 mg input to start extraction
- When possible, use the thorax. Remove any wings or legs.

Note: An exception is insects such as crickets or grasshoppers where the hindlegs contain a substantial amount of muscle. In these cases, the hindlegs are recommended for DNA isolation.

- The head may be used for DNA isolation but be aware that some insects' eyes may contain high amounts of pigments. Enhancing the washes may mitigate the carryover of these pigments, but the presence of some pigment in DNA eluate has not negatively impacted library prep or HiFi yield in limited testing.
- When possible, avoid using the abdomen due to the comparatively higher concentration of gut microbes relative to the thorax or head.
- When possible, use pupa(e) or larva(e) since they will have less chitin and typically less pigment than adults.
- Some examples of tested input masses are:
    - *Drosophila melanogaster* bulk fruit flies: 25–50 mg
    - *Anopheles stephensi* bulk mosquitos: 20–35 mg
    - *Acheta domestica* house individual cricket, 1 head, 1 thorax, or 1–2 legs: 30–60 mg
    - *Hippodamia convergens* ladybug individual beetle: 18–35 mg
    - *Reticulitermes flavipes* bulk termite larvae: 20–45 mg
    - Dragonfly nymph, head or thorax: 40–80 mg
    - Wax moth larvae heads: 50–60 mg
    - Millipede head: 100–130 mg
- For optimal results, insects should be fresh or flash frozen. Avoid storage in ethanol which may hinder DNA isolation.

Procedure and checklist

1. HMW DNA extraction – insects

✔	Step	Instructions
	1.1	Place a 1.5 mL Protein LoBind microcentrifuge tube on ice and chill the centrifuge to 4°C
	1.2	Add insect(s) to a chilled 1.5 mL Protein LoBind microcentrifuge tube.
	1.3	Add 200 µL cold Buffer CT and crush insect(s) with pellet pestle 10–20X. • Push the insect with the pestle firmly into the bottom of the tube each time. • Try to keep the insect between the tip of the pestle and the bottom of the tube. • One key goal is to "break" open the exoskeleton so that the inner tissue (which contains the DNA) will be exposed to lysis and digestion reagents in steps 8–12. Thorough exoskeletal and tissue disruption is key to efficient lysis. It is also important to keep the sample cold during the entire disruption process. • Keep Buffer CT cold on ice while performing extraction and return to 4°C after use.
	1.4	Add 500 µL more of cold Buffer CT and inversion mix 5X.
	1.5	Centrifuge at 16,000 x *g* and 4°C for 2 min. Discard supernatant. • Some insects may have a waxy or opaque residue that rests atop the supernatant after this spin; be sure to remove as much of this residue as possible. It may be easier to pipette the residue using 200 µL wide-bore pipette tips.
	1.6	Add 1 mL cold Buffer CT and pipette resuspend. Transfer the homogenate to a 2.0 mL Protein LoBind microcentrifuge tube by decanting and/or pipetting. • A 1 mL wide-bore pipette tip may facilitate resuspension and transfer if there are large chitinous pieces in the homogenate. • The 2.0 mL tube is **critical** for efficient lysis because of the shape of the tube. • Using a 1.5 mL tube will result in poor lysis, lower yield, and lower quality because the taper of the 1.5 mL tube does not allow adequate fluid mixing in steps 9 and 11.
	1.7	Centrifuge at 16,000 x *g* and 4°C for 2 min. Discard supernatant. • Some insects may have a waxy or opaque residue that rests atop the supernatant after this spin; be sure to remove as much of this residue as possible. It may be easier to pipette the residue using 200 µL wide-bore pipette tips.
	1.8	Add 20 µL of Proteinase K.
	1.9	Add 200 µL Buffer NPL. Vortex 1 s to resuspend.
	1.10	Incubate on a ThermoMixer at 900 rpm and 55°C for 1 h.
	1.11	Add 20 µL of RNase A.
	1.12	Incubate on a ThermoMixer at 900 rpm and 55°C for 15 min.
	1.13	Centrifuge at 16,000 x *g* and 4°C for 5 min to pellet debris and undigested material.

1.14	Place a 70 µm filter into a 1.5 mL Protein LoBind microcentrifuge tube and transfer supernatant to the filter. Spin the tube with the filter on a mini-centrifuge for <1 s. Discard the filter and the 2.0 mL tube containing the precipitated pellet and remnants of sample. • **Optional:** Supernatant can be transferred without using a filter, but be careful to avoid pipetting small, exoskeletal bits or any undigested tissue.
1.15	Add 50 µL of Buffer BL3 and inversion mix 5X.
1.16	Incubate on a ThermoMixer at 900 rpm and 55°C for 15 min.
1.17	Add Nanobind disk to lysate and add 400 µL isopropanol. Inversion mix 10X. • The Nanobind disk must be added before isopropanol. • A large, cloudy mass may appear upon addition of IPA and inversion mixing.
1.18	Mix on a platform rocker at 20 rpm for 15 min at RT.
1.19	Place the tube on a magnetic tube rack using the procedure described in the Nanobind PanDNA kit Guide & overview.
1.20	Discard liquid with a pipette using the procedure described in the Nanobind PanDNA kit Guide & overview.
1.21	Add 500 µL of Buffer CW1, inversion mix 4X, replace tube rack on the magnetic base, and discard the supernatant.
1.22	Repeat Step 21.
1.23	Add 500 µL of Buffer CW2, inversion mix 4X, replace tube rack on the magnetic base, and discard the supernatant.
1.24	Repeat Step 23.
1.25	Remove any residual liquid from cap of the tube.
1.26	Spin tube on a mini-centrifuge for 1 s. 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.
1.27	Repeat step 26. If residual wash buffer remains on the sidewalls of tube, repeat step 26 again.
1.28	Remove the tube from magnet.
1.29	Add 100 µL of Buffer LTE (formerly Buffer EB) directly onto the Nanobind disk and incubate at RT for 10 min.
1.30	Collect DNA by transferring eluate to a new 1.5 mL Protein LoBind microcentrifuge tube.
1.31	Spin the tube containing the Nanobind disk on a mini-centrifuge for 5 s and combine any additional liquid that comes off of the disk with the previous eluate. Repeat if necessary. • This **5 s spin is CRITICAL** for recovering the DNA. A 2nd elution **will not be necessary** if Step 31 is performed as described. • A standard P200 pipette can be used in this step to transfer the last of the eluate. • Some tissue types with high DNA content per mg input may result in a dense, clear gel that adheres strongly to the Nanobind disk. **This clear gel is DNA!** For these tissue types, this

	spin step is critical for recovering all the DNA. Repeat until all the clear gel has spun off the Nanobind disk.
1.32	Pipette-mix the sample 5X with a standard P200 pipette to homogenize the sample and disrupt any unsolubilized “jellies” that may be present. • 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.
1.33	Let the sample rest overnight at RT to allow DNA to solubilize further.
1.34	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.

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 top, middle, and bottom of 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.

1. Perform triplicate Qubit dsDNA BR assay measurements from the top, middle, and bottom of 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 the concentration measurements have been found to be unreliable.
2. Perform a single Qubit RNA BR assay measurement to determine 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.
3. Use 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.

Results

- DNA was extracted from fruit flies, mosquitos, crickets, and wax moths.
- 260/280 ratios are typically in the 1.7–2.0 range.
- 260/230 ratios are typically in the 1.0–2.2 range. This metric may be lower for insects due to the high amounts of pigments in this sample class but does not necessarily impact library prep or HiFi yield.

Table 1. Example QC measurements for extractions from various insect species.

Sample	260/280	260/230	Nanodrop Top (ng/µL)	Nanodrop Middle (ng/µL)	Nanodrop Bottom (ng/µL)	Qubit dsDNA (ng/µL)	Qubit RNA (ng/µL)
47 mg fruit flies	1.83	1.37	285	285	275	97.6	65.4
33 mg mosquitos	1.72	0.96	396.4	409	431.3	167	21.6
59 mg cricket legs (2 legs)	1.86	2.04	188.6	209.7	151.3	163	22.8
50 mg wax moth larvae heads	1.81	1.50	100	95.5	95.7	57.8	N/A

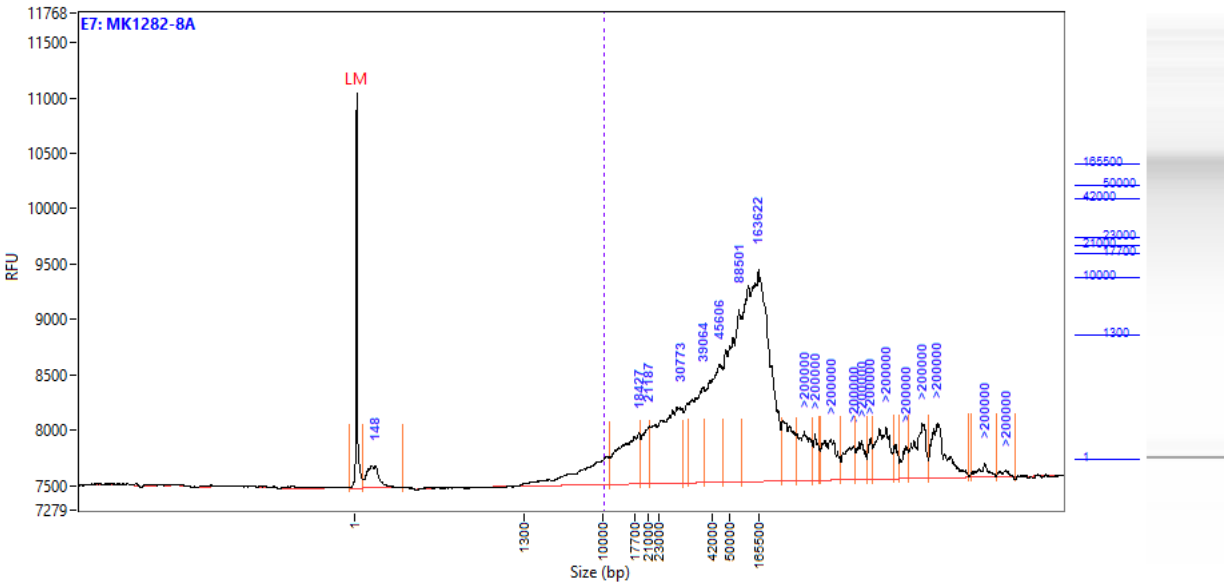

Figure 1. DNA size distribution of gDNA isolated from wax moth larvae heads on the Femto Pulse system (Agilent Technologies).

Revision history (description)	Version	Date
Initial release	01	February 2024
Updated references to PanDNA kit documentation	02	January 2025