Technical note

EXTRACTING HMW DNA FROM DIVERSE INSECT SAMPLES USING THE NANOBIND PANDNA KIT FOR OPTIMAL HIFI SEQUENCING

Introduction

High-quality, high-molecular-weight (HMW) DNA is key for optimal PacBio® HiFi sequencing yield and performance. In this technical note we describe HiFi sequencing results for insect DNA extracted using the Nanobind® PanDNA kit and the insect DNA extraction workflow.

The Nanobind PanDNA kit contains specific reagents for insect sample processing and lysis. Following lysis, the DNA binds to Nanobind disks which shield bound DNA from damage during extraction, resulting in HMW DNA. A final Short Read Eliminator (SRE) size selection step helps remove DNA fragments below 10 kb. The entire workflow takes approximately 2.5 hours (20 min hands-on time) for Nanobind extraction and 2 hours (10 min hands-on time) for SRE size selection. Insect DNA extracted using the Nanobind PanDNA kit and sequenced on a single Sequel® IIe or Revio™ SMRT® Cell typically generates >30-fold HiFi coverage, which is sufficient for high-quality *de novo* assembly of a diploid genome.

Workflow recommendation and overview

Given the wide diversity of insect species, selection of the starting material should be carefully considered. We recommend using an input amount that will yield 3–30 µg of DNA. A good starting point for pilot extraction is 30 mg, but the optimal input mass will vary from insect to insect and will generally range from 15–100 mg (table 1). An important 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. Another important consideration is the selection of body segment, when possible. For example, when extracting from larger insects, we recommend removing the wings and legs from the thorax and using only the thorax or head + thorax. An exception is for insects such as cricket or grasshopper where the hindlegs contain a substantial amount of muscle and should be used instead. Calibrating the input is important to avoid overloading of the lysis chemistries with too much tissue, which would negatively impact the DNA yield and quality. One should also consider sources of contaminants in different insects such as those with high levels of pigments in the eyes, or non-host DNA found in the digestive tract in the abdomen. These body parts should be avoided when possible.

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Insect or arthropod	Body part(s) recommended	Mass recommended
Drosophila melanogaster fruit fly (adult)	Pooled fruit flies	25–50 mg
Anopheles stephensi mosquito (adult)	Pooled mosquitos	20–35 mg
Acheta domestica cricket (adult)	Single cricket, head, thorax, or 2 hindlegs	30–60 mg
Hippodamia convergens ladybug (adult)	Single beetle	18–35 mg
Reticulitermes flavipes termite (larvae)	Pooled termites	20–45 mg
Dragonfly (nymph)	Pooled head + thorax	40–80 mg
Wax moth (larvae)	Pooled heads	50–60 mg
Millipede	Single head	100–130 mg

Table 1. Insect input recommendations for HMW DNA extraction with the Nanobind PanDNA kit.

Sample storage

We recommend using fresh or flash frozen insects. Storage of insects in ethanol may hinder DNA isolation.

Starting material

Procedure starts with approximately 30 mg of insect tissue (see table above).

Extraction

It is recommended to follow the *Extracting HMW DNA from insects using the Nanobind PanDNA kit* procedure and checklist using the Nanobind PanDNA kit (PN 103-260-000).

Extraction yield

When starting with optimal material and appropriate input mass, yields of 3–30 µg of HMW DNA that are >50 kb in size are typical.

Size selection

It is recommended to use the SRE kit (PN 102-208-300) to remove fragments below 10 kb following the SRE protocol.

DNA QC metrics and sequencing results

HMW DNA from ten different insects was extracted using the Nanobind PanDNA kit following the insect DNA extraction workflow and SRE size selection protocol. The insect sample body parts and input masses used for each of the different insect species are listed in table 2 below.

Following extraction, DNA was quantified using the Qubit dsDNA BR assay kit and sized using the Agilent Femto Pulse system. HiFi libraries were prepared following the SMRTbell® prep kit 3.0 protocol and sequenced on either the Sequel IIe or the Revio systems. Each sample was sequenced on a single SMRT Cell.

Sequencing data for each of the samples is available in table 2. For the given inputs, the samples yielded between 6 to 30 µg of HMW DNA, and the DNA mode size varied from 21 to 164 kb, depending on the species. The HiFi sequencing yield was 13 to 33 Gb on Sequel IIe and 74 to 115 Gb on the Revio system. The average HiFi read length was between 9.7 to 17.2 kb.

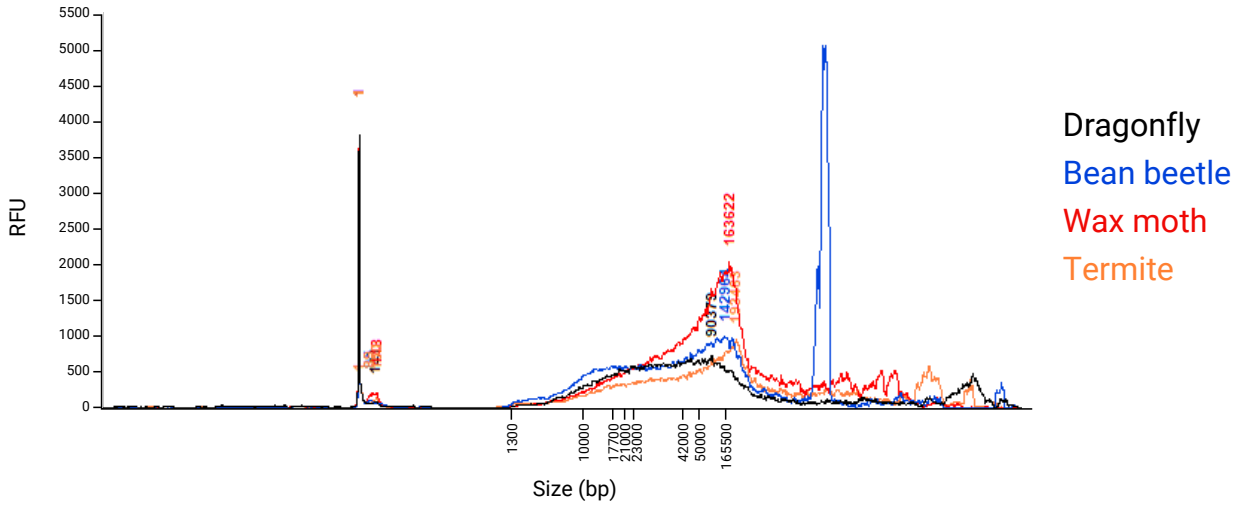

Figure 1. Size distribution of HMW insect DNA extracted with the Nanobind PanDNA kit and quantified on the Femto Pulse system.

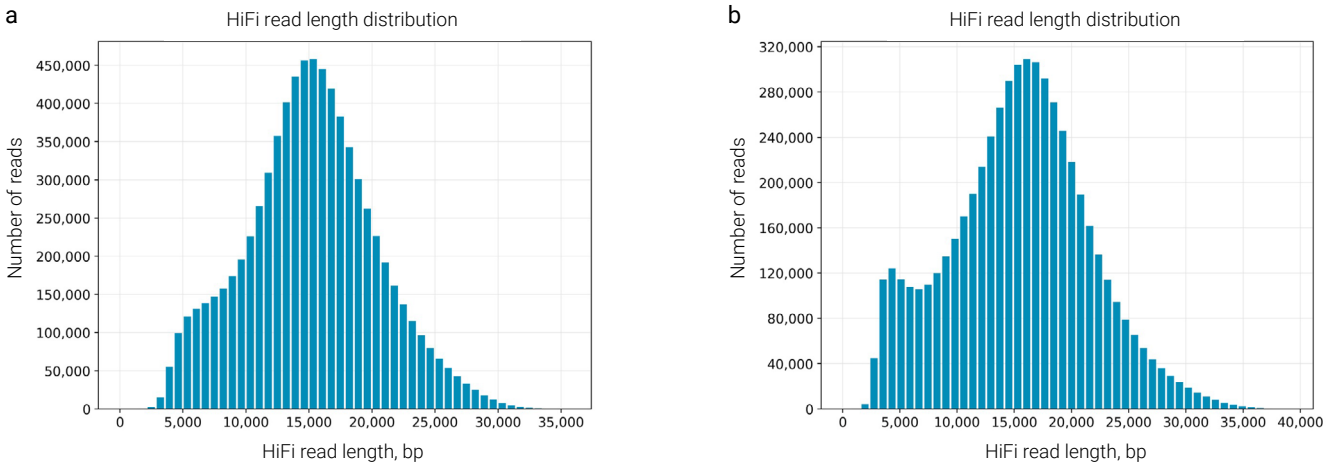

Figure 2. HiFi read length distribution plots for (a) ladybug and (b) bean beetle samples sequenced on the Revio system.

Sample	Insect segments used	Input mass	DNA yield	DNA mode size	Sequencing system	HiFi mean read length	HiFi yield	Median QV	Genome fold-coverage
Cricket	Both hindlegs of 1 adult	44 mg	10.5 µg	118 kb	Revio	15,731 bp	113 Gb	Q35	122×
Dragonfly	Head + thorax of 1 nymph	77 mg	7.3 µg	151 kb	Revio	14,915 bp	106 Gb	Q35	161×
Wax moth	Heads of 4 larvae	50 mg	5.8 µg	164 kb	Revio	17,209 bp	74 Gb	Q35	128×
Ladybug	1 whole adult	29 mg	7.4 µg	67 kb	Revio	15,135 bp	115 Gb	Q36	242×
Bean beetle	20 whole adults	50 mg	30.3 µg	124 kb	Revio	15,389 bp	85 Gb	Q35	57×
Termite	22 whole larvae	37 mg	8.4 µg	27 kb	Sequel IIe	10,117 bp	25.3 Gb	Q36	29×
Butterfly	Thorax of 1 adult	89 mg	7.1 µg	21 kb	Sequel IIe	9,763 bp	21 Gb	Q38	49×
Drosophila	35 whole adults	37 mg	6.9 µg	109 kb	Sequel IIe	14,689 bp	19.8 Gb	Q37	137×
Millipede	Head of 1 adult	95 mg	17.8 µg	80 kb	Sequel IIe	14,722 bp	32.8 Gb	Q32	182×
Mosquito	36 whole adults (starved)	35 mg	11.3 µg	50 kb	Sequel IIe	16,036 bp	13.3 Gb	Q34	54×

Table 2. Sequencing data for insect samples prepared with the Nanobind PanDNA kit.

Conclusion

This technical note demonstrates HiFi sequencing performance from HMW insect DNA extracted using the Nanobind PanDNA kit. Insects are a diverse sample type, and HiFi yield can vary due to species diversity. Some species will be more challenging to extract than others, resulting in variations in both the DNA extraction yield and the HiFi sequencing yield. Given that insect genomes are relatively small (~450 Mb on average), even with some variation in sequencing yield we demonstrate >30× HiFi coverage per SMRT Cell for most of the insect samples, which is sufficient for *de novo* assembly. Insects with small genomes size could also be multiplexed on the Revio system to enable further time and cost savings. Lastly, SRE size selection following DNA extraction helps remove DNA molecules below 10 kb, resulting in improved HiFi read length distribution and HiFi sequencing yield.

KEY REFERENCES

1. Procedure & checklist – Extracting HMW DNA from insects using the Nanobind PanDNA kit
2. Procedure & checklist – Removing short DNA fragments with the Short Read Eliminator (SRE) kit
3. Procedure & checklist – Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0
4. Guide & overview – Nanobind PanDNA kit
5. Brochure – Plant + animal sciences at scale with HiFi sequencing

Order info:
Nanobind PanDNA (103-260-000); SRE kit (102-208-300)
Orders@pacificbiosciences.com

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