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# HMW DNA extraction for insects

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High molecular weight DNA extraction from all kingdoms
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**Protocol status:** Working
**This has been tested with Argentine Stem Weevil, but grinding time should be optimised for other insects.**

**Created:** April 23, 2018

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**Protocol Integer ID:** 11702

## Abstract

Adapted from Qiagen's **genomic DNA handbook** and the **user protocol for mosquito DNA extraction.**

## Materials

STEP MATERIALS

- 3.2 mm stainless steel beads, RNase free **Catalog #**NEXSSB32-RNA
- RNase A **Qiagen Catalog #**19101
- Buffer G2 **Qiagen Catalog #**1014636
- Proteinase K **Qiagen Catalog #**19131
- Buffer QBT
- Buffer QC
- Buffer QF
- 2-Propanol (IsoPropanol) **Bio Basic Inc. Catalog #**PC8601.SIZE.4L
- Ethanol
- Buffer EB **Qiagen Catalog #**19086
- 3.2 mm stainless steel beads, RNase free **Catalog #**NEXSSB32-RNA
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## Tissue homogenization

1 Place insects in a 1.7 mL tube with two 3.2 mm steel balls (RNase-free).

Snap freeze in liquid nitrogen.

3.2 mm stainless steel beads, RNase free **Catalog #**NEXSSB32-RNA

2 The goal of this step is to pulverise the insects without allowing them to thaw. Grinding time requires optimisation to balance DNA yield against physical damage to the DNA.

Grind insects using a Retsch mixer mill MM 400 / Qiagen TissueLyser or similar with **blocks pre-cooled in liquid $N_2$** for 90 seconds at 20 Hz.

After grinding, immediately return the tubes to liquid nitrogen. Ground samples can be stored at -80°C until you're ready to continue.

## Lysis

3 Prepare the lysis buffer by adding 1.5 µL of RNase A (100 mg/mL) to 1438.5 µL of Buffer G2 per sample.

RNase A **Qiagen Catalog #**19101

Buffer G2 **Qiagen Catalog #**1014636

4 Remove the powdered sample from liquid nitrogen or the -80°C freezer and immediately add 1440 µL of lysis buffer from step 3.

5 Incubate for 30 minutes at 37°C with inversion.

37 °C

00:30:00

6 Add 60 µL of Proteinase K (20 mg/mL) and incubate with inversion at 50°C for 2 hours.

Proteinase K **Qiagen Catalog #**19131

02:00:00

50 °C

## Pellet debris and recover supernatant

7 Centrifuge at max speed for 20 minutes to pellet the debris. Using a P1000, **slowly** pipette 1200 µL of the supernatant into a 15 mL Falcon tube.

8 Dilute the lysate to around 3 mL. The volume doesn't matter, but if the sample is more dilute it will run through the column faster.

## DNA binding and washing

9 Equilibrate a QIAGEN Genomic-tip 20/G with 1 mL Buffer QBT. Wait for the Genomic-tip to drain by gravity. You can do this during step 7.

Buffer QBT

10 Carefully apply the lysate to the Genomic-tip. Don't vortex the sample. Gently invert it a few times to mix it, then pour as much as you can from the 15 mL tube into the Genomic-tip. Pulse spin the 15 mL tube to collect any remaining lysate, and **slowly** pipette it into the Genomic-tip with a P1000. Wait for the Genomic-tip to drain by gravity. All of the draining steps can take a long time, and this step is particularly slow if the sample is viscous (30–60 minutes), but resist the urge to apply positive pressure.

11 Wash the QIAGEN Genomic-tip with 1 mL Buffer QC.

Repeat this step 3 times for a total of 4 mL of Buffer QC.

Buffer QC

## DNA recovery

12 Elute the DNA into a new 15 mL tube with 2 mL of Buffer QF. Wait patiently for the tip to drain.

Buffer QF

13 After elution I split the sample into three because our lab doesn't have a fast centrifuge for 15 mL tubes.

**Slowly** pipette 667 µL of the eluate into three clean 1.7 mL tubes with a P1000. Add 467 µL of room-temperature isopropanol to each tube, mix by inversion about 10 times, and centrifuge for 20 min at 15,000g at 4°C to pellet DNA

2-Propanol (IsoPropanol) **Bio Basic Inc. Catalog #**PC8601.SIZE.4L

14 The pellet can be hard to see at this point. **Slowly** pour off the supernatant. I usually keep the supernatant from this step and the ethanol wash until I've tested the final yield of my preps. Pulse spin the tubes, and remove any remaining traces of supernatant with a P10.

15 Add about 1 mL of ice-cold, 70% ethanol. Invert a few times, and centrifuge at 15,000g for 10 min at 4°C. Pour off the ethanol, pulse spin the tubes, and remove any remaining traces of ethanol.

Repeat this step once for a second wash with 70% ethanol.

Ethanol

16 Air dry the pellet **briefly**. It can be difficult to resuspend, so don't leave it too long. I try to get all the drops of ethanol with a P10, and then watch the tubes until the traces evaporate.

17 Resuspend each pellet in 55 µL of Qiagen EB (Tris-Cl pH 8). Don't pipette the sample up and down to resuspend it. Leave the tubes overnight at room temperature with inversion to resuspend.

Resuspention volume is up to you, but if you're doing a MinION run, this leaves you around 5 µL for QC.

Buffer EB **Qiagen Catalog #**19086

## Sample QC

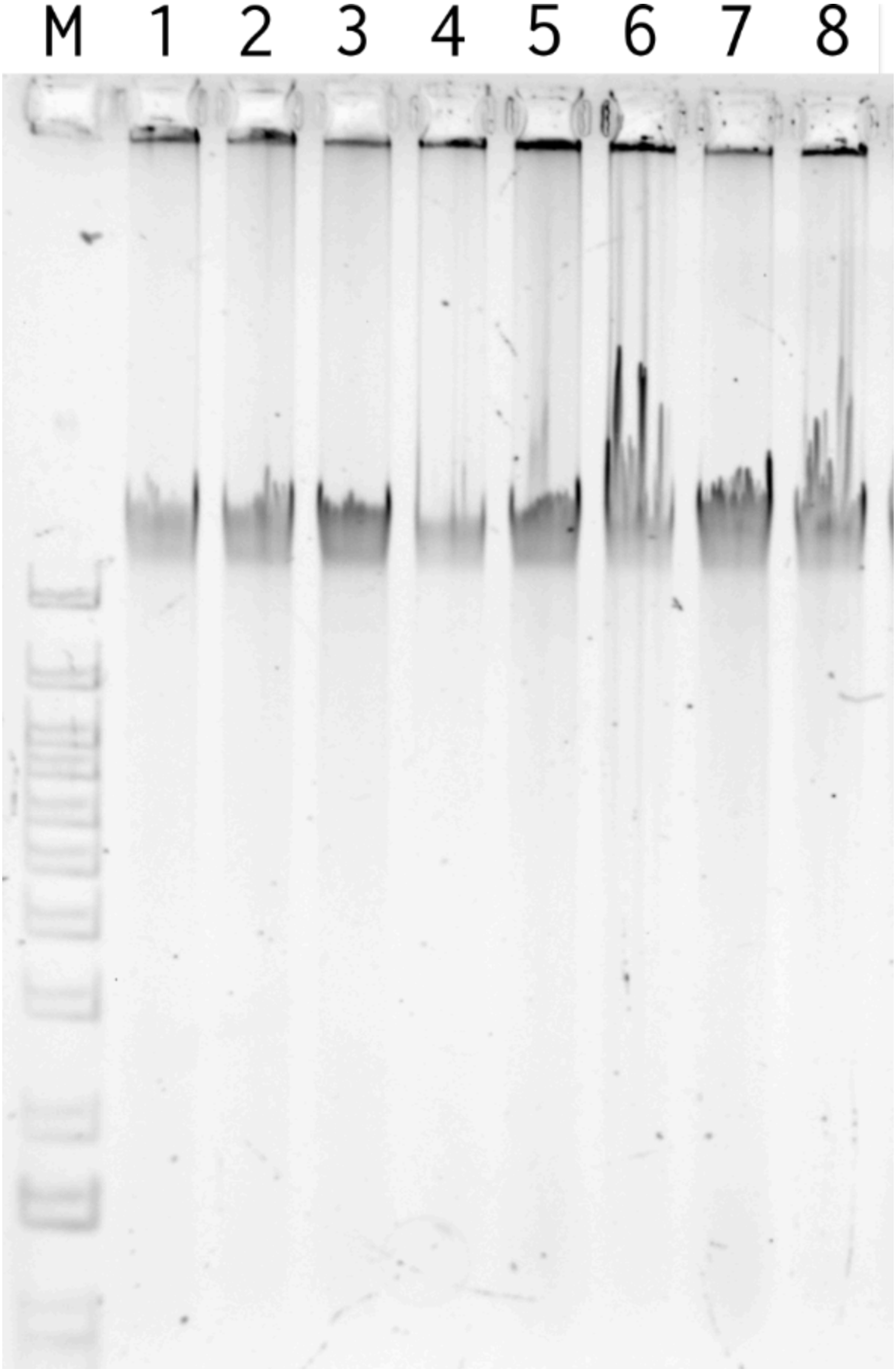
18 Visualise 1 µL of sample to estimate the molecular weight. We don't have PFGE or a TapeStation / Fragment Analyzer, so I use 0.7% Agarose.

Quantify your sample with a Qubit. If you're using the HS kit, you may have to dilute the sample 1:10.

Analyse 1 µL in a UV spectrophotometer (e.g. Nanodrop).

## Results

19 This gel shows 10 samples extracted with this method. Each sample is one third of a single sample that was run through a Genomic-tip and split into three at the precipitation stage. Each original sample was a pool of 20 adult **Argentine stem weevils** (Coleoptera: Curculionidae). DNA extractions from these insects with other methods have resulted in low 260/230 ratios.


M
1
2
3
4
5
6
7
8


0.7 % Agarose in TBE, run at 80 V for 60 minutes and post-stained for 30 minutes in 0.1 mg / mL EtBr. We ran 1 µL of each sample.

20 These are the qubit and nanodrop results for the same 10 samples.

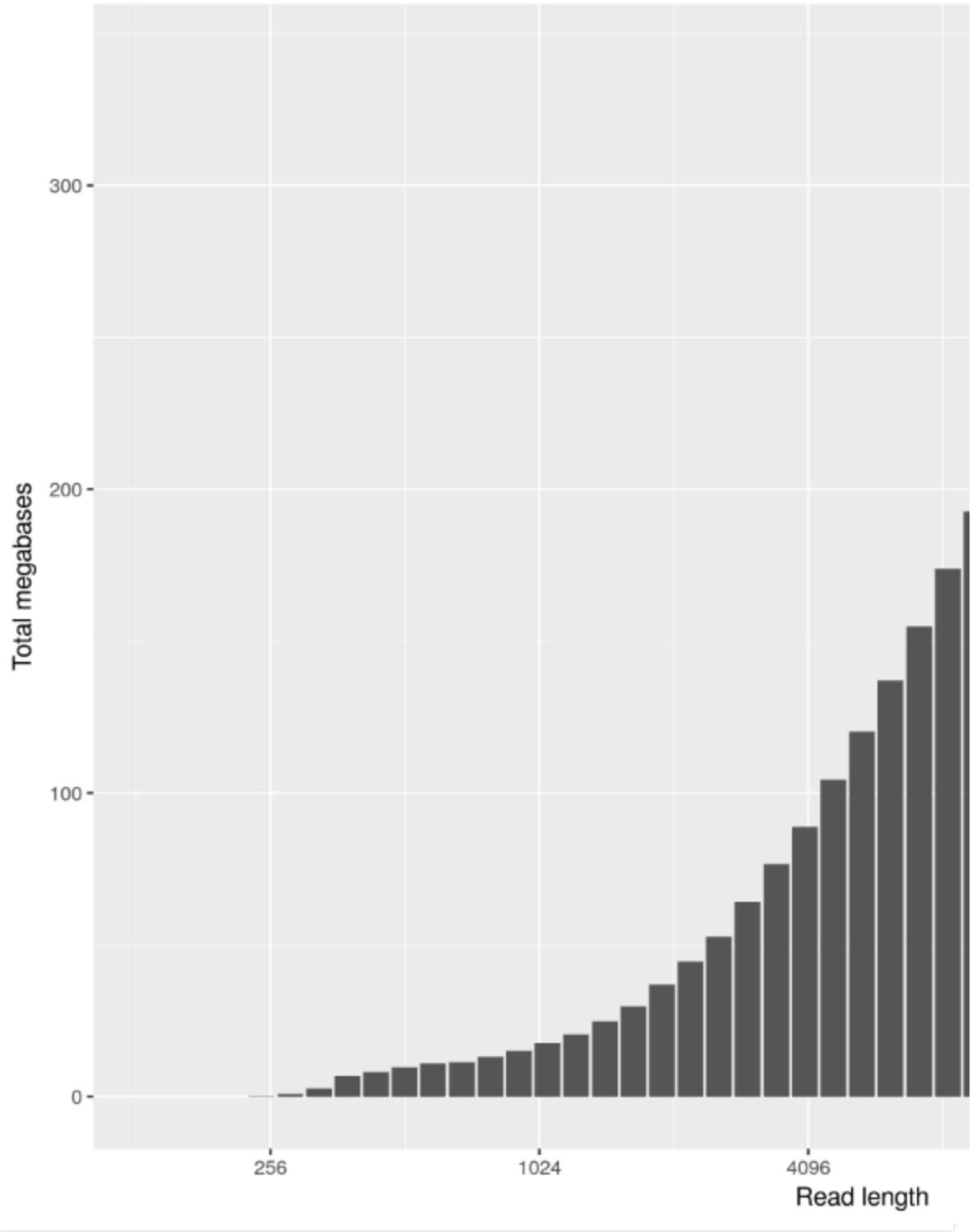
| | ID | Qubit (ng/µL) | Nanodrop (ng/µL) | 260/280 | 260/230 |
|---|---|---|---|---|---|
| | 1 | 52 | 35.4 | 1.87 | 1.96 |
| | 2 | 56 | 113.1 | 1.85 | 2.24 |
| | 3 | 59 | 122.7 | 1.88 | 2.28 |
| | 4 | 30.4 | 61.4 | 1.8 | 2.1 |
| | 5 | 53 | 54 | 1.78 | 2.19 |
| | 6 | 53.3 | 104.2 | 1.81 | 2.28 |
| | 7 | 79.6 | 214.6 | 1.78 | 2.18 |
| | 8 | 132 | 133.7 | 1.88 | 2.41 |
| | 9 | 210 | 127.7 | 1.85 | 2.36 |
| | 10 | 114 | 233.8 | 1.8 | 2.18 |

21 We used all of sample 8 for a library prep with the Oxford Nanopore SQK-LSK108 kit. At the end of the prep we recovered 1536 ng of DNA. We loaded the whole library onto a R9.4.1 flowcell and ran it for 40 hours.

Here are some metrics from the basecalling:

| | GB data called | GB >Q7 | N50 KB >Q7 | Mean KB >Q7 | Median KB >Q7 | Max KB >Q7 | GB >20KB >Q7 | GB >50KB >Q7 | GB >100KB >Q7 |
|---|---|---|---|---|---|---|---|---|---|
| | 5.5 | 5.2 | 21.4 | 8.6 | 3.7 | 216 | 2.8 | 0.8 | 0.1 |

Here is a weighted read length histogram:


300
200
100
0
Total megabases
256
1024
4096
Read length