Supplemental Table 1: Sample information for the individuals used in this study. Indiv: voucher identifier number; Technology, sequencing technology used on the individual; reads, number of sequenced reads using each technology, respectively (in millions, M, of thousands, K); Coverage was calculated by dividing total number trimmed, filtered base-pairs by the assembled genome size of 292 Mb.

Species	Sex	Indiv.	Technology	Reads (PE, ia)	Coverage	NCBI SRA
C. glomerata	♂	CgM1	ONT, Illumina	1.6M, 11.6M	16X, 11X	SAMN18340714
C. glomerata	♂	CgM2	Illumina	95.1M	90X	SAMN18340715
C. glomerata	♀	CgF25	ONT	706K	16X	SAMN18340716
C. glomerata	♂	5 males	HiC	139.8M	142X	SAMN18340717

Supplemental Methods:

High Molecular-Weight DNA Extraction by "salting-out—phenol-chloroform—ethanol-precipitation and rapid centrifugation" (SOP-CEPC)

Long-read sequencing is now a staple in genomics labs across the world (e.g. https://vertebrategenomesproject.org/technology). However, this long-read revolution is only really applicable to organisms for which scientists can effectively extract large amounts of high molecular-weight (HMW) DNA. Many current HMW DNA extraction protocols require large amounts of input tissue, spanning from whole vials of nucleated blood to 15mL tubes of ground tissue (with liquid N_2). Indeed, many organisms are simply too small to cost-effectively sequence single individuals or sample a non-destructive subset of an organism. This has led to common practices of pooling multiple individuals for long-read sequencing (e.g. Solares et al. 2018). However, for in-depth genomic analysis of single individuals we need methods to cost-effectively accommodate individual small animals.

Animals across the tree of life are the results of adaptations to being both big and small. In amniotic vertebrates, for instance, animals range from >100,000kg (blue whales; http://marinemammalcenter.org) to ~140mg (dwarf geckos; Hedges and Thomas, 2001). Indeed, invertebrates have also taken this to extreme. In fact, the lab-staple *Drosophila melanogaster* has an average dry mass of ~0.3mg (https://bionumbers.hms.harvard.edu), which is representative of the weight of their distant their relatives braconid wasps. Small vertebrates and invertebrates face similar issues in the face of long-read genome sequencing. In vertebrates, preserving a voucher specimen or preserving other segments of the organism for other experiments, while with invertebrates, researchers may want to analyze the 'genomic architecture' of a single individual from a wild population instead of a homogenous lab-variety strain. Indeed, the ability to reduce raw input material inherently expands the ability of researchers to ask questions or limit wasteful practices when addressing multi-faceted questions, e.g. generating RNAseq and whole genomes from a single individual.

Here, we provide a DNA extraction protocol, basically a composite of other extraction protocols (Aitken, 2012; Mayjonade et al. 2016; Miller et al. 1988), for which we've compiled, modified, and optimized for the cost-effective extraction of large amounts of high molecular-weight DNA from small amount of tissue, such as a single insect.

Table 1: List of necessary and recommended reagents.

Reagent Name	Manufacturer	Usage temperature
Proteinase K	Qiagen®	20˚C
RNase A	Thermo Fisher®	-20˚C
5 M NaCl	N/A.	4˚C
6X gel loading dye	Thermo Fisher®	4-20˚C
3 M Sodium Acetate	N/A.	4˚C
100% Ethanol	N/A.	-20˚C
Phenol	EMD®	4˚C
Chloroform	Fisher Chemical®	20˚C
Phase lock or vacuum grease (optional)	N/A.	20˚C
EB Buffer	Qiagen®	37-60˚C
10mM Tris-Cl (pH 7.0)	N/A.	20˚C
200µl wide-bore pipette tips	N/A.	N/A.
1000µl wide-bore pipette tips	N/A.	N/A.

Step 1) *Mechanical Lysis*:

Tissue must be washed of any storage medium (e.g. RNA Later, EtOH, etc.) using nuclease-free water, if applicable. Using a disposable, sterilized plastic pestle in a 1.5mL microcentrifuge tube, crush tissues into a smooth paste. Proceed to Step 2.

Step 1 Recommendations:

- For nucleated blood in anticoagulant (e.g. 1:1 with 0.5M EDTA; more EDTA is generally better, e.g. 2:1), skip to step 2.
- For keratinized tissues (e.g. skin, muscle, etc.), slice tissue into small, thin pieces, and place directly into labeled 1.5mL tube. Carefully and using PPE, lower tube into a small aliquot of LN_2 and use a disposable pestle to crush tissue, iteratively grind tissue into a fine powder. Proceed to Step 2.

Table 2: Lysis buffer recipe (adapted from Mayjonade et al. 2016). Make fresh lysis buffer and incubate at 60.0˚C for ≥30 minutes prior to use (only 'good' for ~48 hours).

Chemical	Amount	Final %
Polyvinylpyrrolidine 40	0.1 g	1%
Sodium metabisulfate	0.1 g	1%
Sodium Chloride	0.29 g	0.5 M
1 M Tris – pH ≈7.5-8.0	1 mL	100 mM
0.5 M EDTA	1 mL	50 mM
20% SDS	1 mL	2.0%
Nuclease-free H_2O	Adjust to 10 mL	--

Step 2) *Cell Lysis*:

To disrupt cellular membranes and release DNA into solution, add 500-600µL of warm lysis buffer (Table 2) to each aliquot of tissue and aggressively flicked tube to prevent the tissue 'paste' from congealing to the tube. Then, add 15µl of Proteinase K and 6-8µL of RNase A, before incubating at 60˚C for >30 minutes (but no more than an hour), inverting the tubes 10-20 times every ~5 minutes, until the solution becomes homogenous and bubbly when flicked excessively (i.e. bubbles reflect protein content of fluid). The exact time-frame is strictly dependent upon the specific sample, but largely scales from nucleated blood tissue (~35-40 min.) to structured/keratinized tissues (50-60 min.). Here, we incubated liver and whole-insect samples for 45 minutes. Proceed to Step 3.

Step 3) *Cellular DNA Removal (salting out)*:

To extract the free DNA from the cellular solution, add 200µL of **5 M NaCl** (Table 1) to cellular solution and incubate at 4˚C for 10 minutes. After incubation, immediately centrifuge tubes at 5,000Xg at 4˚C (~8,000 rpm) for 12 minutes. Subsequent to centrifugation, cellular debris should form a pellet at the bottom of the tube. Proceed to Step 4.

Step 4) *Phase Lock Purification* (*Phenol/Chloroform)*:

To clean DNA extract from protein and lipid contaminants, remove the supernatant and add it to the 2ml phase lock tubes, while avoiding cellular debris pellet (at this point in step 4, you will generally need to split the extraction into two phase lock tubes to accommodate the 2X increase in volume later in this step). Add 200µl of 10mM Tris-Cl buffer and 10µl of 6X loading dye to ensure proper salt concentration to separate phases. Then, add an equal volume (1:1) of phenol/chloroform† (make sure to mix phenol adequately) to the DNA solution at the same ratio (1:1), i.e. adding 200µl of phenol and chloroform to 400µl of DNA solution for a final volume of 800µl. Mix tubes by ***vigorously*** shaking and flicking (see Aitken, 2012) until extremely homogenized *but do not vortex any samples.* Immediately centrifuge at maximum speed (~13,000 rpm) in a 4˚C environment for 5 minutes; return to fume hood and add an additional 200µl of chloroform; mix by inverting 2-5 times; and centrifuge again at maximum speed for 2 minutes. Next, remove the aqueous phase (top) and place into new 2ml tube and add 500µl of chloroform†; mix by inverting 5-10 times, then centrifuge at maximum speed in 4˚C for an additional 2 minutes. Proceed to Step 5.

Step 4 Tips:

- During phenol/chloroform steps†, the organic phase is dyed blue, separated by the phase lock gel layer. If two phase lock layers form (i.e. above and below the

aqueous phase) after first centrifugation (5 min), remove the top phase lock layer using a pipette tip.

† – any step where a tube or bottle containing phenol or chloroform should use appropriate PPE and work in a fume hood.

Step 5) *Ethanol precipitation*:

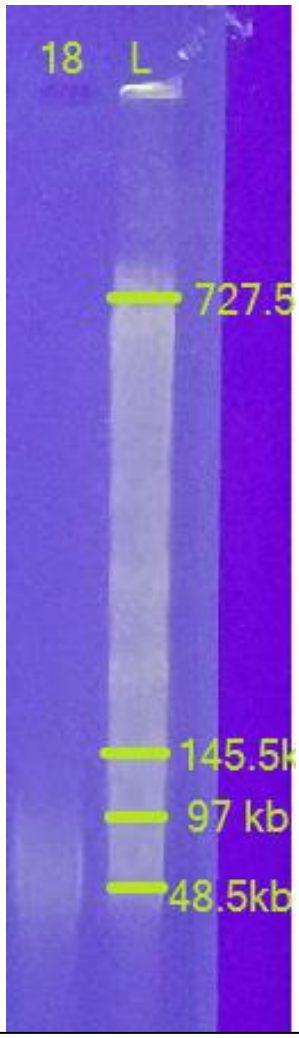

Figure 1: Pulsed-field gel electrophoresis of HMW DNA extracted from a gecko, *Sphaerodactylus* sp. Ladder size is annotated along Lane "L" and lane "18" contains HMW DNA.

To purify and concentrate DNA extract for downstream applications, remove the aqueous phase (top) from organic phase (bottom), *without disturbing the organic phase* (chloroform), dispense into new 2ml tube and discard tube with organic phase. Then, add 3M Sodium Acetate to DNA solution (your newly dispensed aqueous phase) to achieve a final concentration of 0.3M Sodium Acetate (usually between 40-60µl) and add 2 volumes (2:1) of ice-cold 100% EtOH. Incubate tubes at -20˚C for 60-90 minutes‡ and then centrifuge at maximum speed in 4˚C for 2-4 minutes [xx]. Proceed to Step 6.

Step 5 Tips:

- After adding ice-cold ethanol, hold the tubes to a light source and rock tubes to look for the emergence of long DNA strands catching the light.

‡ – this time can be extended, or reduced, up to overnight at -20˚C or 30 minutes at -80˚C, respectively. However, I do not recommend overnight at -80˚C as this seems to pull too much salt out of solution and into the precipitate, reducing the final purity and elution efficiency in the next step.

[xx] – this time can be extended to exceptionally increase yield at the expense of maximum DNA fragment size where ~30 minutes is a completely sheared DNA extraction.

Step 6) *DNA elution*:

To elute DNA, remove EtOH supernatant (this can be spun down more for low molecular-weight DNA) while preserving the DNA pellet the bottom of the tube and let the pellet dry in fume hood for ~10 minutes, without over-drying. Elute pellet with [XXX]µl of warm EB buffer (volume of EB

buffer will vary), flick and snap tubes ~2-3 times to mix pellet into solution. If pellets don't resuspend instantly, incubate at 37˚C for 10-15 minutes and then let incubate at 4˚C overnight (recommended). Next, pipette eluted DNA solution into a new labeled, low-bind 1.5ml tube using wide-bore pipette tips. Proceed to Step 7.

Step 7) *Quantification and quality-control*:

It should be noted that the HMW DNA will not be fully suspended into solution until a few days post-extraction, the values taken the following day are expected to be far below the true values. For more accurate estimates of the DNA quantity wait 4-7 days post-extraction before quantification.

To quantify and assess the size distribution of the extracted DNA, use Qubit™ dsDNA BR Assay Kit (Table 3) and visualize via gel electrophoresis against GeneRuler® High Range DNA Ladder (Figure 1 and 3), or equivalent methods. For greater resolution, use pulsed-field gel electrophoresis to examine size distribution (Figure 2) and, if desired, use a nanodrop to examine purity (not shown).

Single Insect Adjustments

As outlined previously, we optimized the extraction protocol for single, small insect extractions by slightly modifying the previously-outlined extraction protocol. These modifications increased yield by ≥5X. Modifications between "Round 1" and "Round 2" (Table 3):

1. **Step 2)** Adjust to 100µl lysis buffer, 10µl Proteinase K, and 5µl RNase.
2. **Step 3)** Adjust to 50µl 5M NaCl solution.
3. **Step 4)** Adjust to 100µl Tris-HCl solution.
4. **Step 6)** Adjust to elution volume to 30µl EB Buffer.

Additional SOP-CEPC References:

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