Gryllus Techniques and Protocols

gDNA Extraction

To remove the genomic DNA from cricket cells for further use, such as genotyping.

General Notes:
- I have had success using this protocol with cricket eggs, or crickets late instar or older should be used.
- Pestles can be made by briefly heating a p1,000 pipette tip with a Bunsen burner, and then swirling the melty tip in a 1.5mL eppendorf tube. Do not use pestle if there is still an opening in the bottom.

X Instar and Above:
1) Anesthetize crickets using carbon dioxide until they stop moving. Can be done in batches of ~10, depending how quickly you can perform the following steps.
2) Under a dissection scope, lay a KimWipe. Use microscissors to cut off the middle leg from either side of the cricket. Place the leg into a 1.5mL eppendorf tube and return the cricket to its cage to recuperate. Perform serially, cleaning microscissors with 70% EtOH in H_2O in between crickets. Can store continue using fresh, or store frozen at -80°C.

Genomic Prep:
1) Add 100µl Buffer A to each sample.
   → Set dry heat block to 65°C to begin warming.
2) Grind by pestle until sample is visibly disrupted.
3) Add 300µl Buffer A to each sample; rinse pestle over tube as you pipette, to collect sample stuck to pestle.
4) Incubate 30 min at 65°C.
5) Add 800µl LiCl/KAc solution, invert repeatedly to mix.
6) Incubate 10min on ice. Do not incubate over 10 minutes.
7) Spin 15min at 12,000rpm in a microfuge room temperature.
8) Transfer 1mL supernatant to new Eppendorf tube, excluding floating crud.
9) Add 600µl isopropanol and invert several times to mix.
10) Spin 20min at 12,000rpm in a microfuge at room temperature.
11) Aspirate and discard s/n. Quick spin. Aspirate again.
12) Wash pellet with 500µl cold 70% EtOH in H_2O.
13) Spin 10min at 12,000rpm in a microfuge at room temperature.
14) Aspirate and discard s/m. Quick spin. Aspirate again.
15) Air dry pellets ~10min.
16) Resuspend in 50µl TE (or ~10µl per embryo) for several hours at room temperature or overnight at 4°C.
17) Measure concentration with spectrophotometer.
18) Store at -20°C.

Recipes:
Buffer A

	Per prep (µl)
1M Tris-HCl, pH 7.5	40
500 mM EDTA, pH 8.0	80
5M NaCl	8
10% SDS	20
H_2O	To 400

LiCl/KAc Solution

	Per prep (µl)
5M KAc	230
6M LiCl	570