**Protocol for DNA extraction from pooled bees for genome sequencing**

**(*modified from Brock Harpur*)**

**DAY 1:**

**A. Homogenization**

1. Record ID of bees onto the daily extraction log chart ([see here](https://docs.google.com/spreadsheets/d/1pylX467fjaMxyDpCpR3iQMVbX_I-egJlEM_tRAn6sFQ/edit#gid=1782088543)).
2. Take at least 30 worker bees from each colony and flash freeze them in liquid nitrogen.
3. Remove the thorax used for extraction by decapitating bee with a razor and then cutting off the abdomen. Cut the thorax in half and place one half into the labeled ziplock bag.
4. Add 5ml of CTAB buffer to the ziplock bag
5. Using a rolling pin, roll samples ~20 times to ensure they are thoroughly homogenized.
6. Transfer 700 ul of the homogenate to a labeled tube.
7. Add 4 uL RNase A (100 mg/ml), incubate at room temperature for 5 minutes.
8. Add 10 ul Proteinase K (10 mg/ml) and let the tissue digest for at least 2 h at 50oC in a water bath; gently stir occasionally.

**B. Extraction and purification**

1. UNDER THE HOOD: Briefly centrifuge the tubes; add 650 ul phenol-chloroform-isoamylalcohol (25:24:1), close the tubes, place them into a tube rack. Cover them with a paper towel, put another rack on top, and shake the samples for 5 min thoroughly by hand (better mixing than by vortexing).
2. Centrifuge for 10 min at 13’200 rpm at room temperature. Carefully remove tubes from the centrifuge.
3. With a 10-200 ul pipette set to 200 ul, transfer as much as possible of the upper, aqueous layer (**avoid the middle, white layer!**) to a new tube. **Avoid contamination by making sure that the liquid does not go into the pipette! (You can use filtered tips for this step)**
4. Add 650 ul chloroform-isoamylalcohol (24:1) and shake the samples for 2-3 min by hand.
5. Centrifuge for 5 min at 13’200 rpm.
6. Transfer as much as possible of the upper aqueous layer to a new tube
7. Add 650 ul chloroform-isoamylalcohol (24:1) and mix thoroughly.
8. Centrifuge for 5 min at 13’200 rpm.

**C. DNA precipitation**

1. Transfer as much as possible of the upper, aqueous layer to a new tube (you should have approximately 400 ul of volume)
2. Add 1000 ul (2.5 x the volume) ice cold (-20oC) ethanol (96%).
3. Add 40 ul (1/10 of the volume) 3 M sodium acetate (pH 5.2)
4. Mix gently by inverting the tubes ~20 times.
5. Place the tubes into the -20 freezer for the next day **after ensuring the labels of the tubes have not been washed off by EtOH**

**END OF DAY 1**

**DAY 2:**

**D. DNA clean up**

1. Centrifuge for 30 min at 13’200 rpm at 4oC (use centrifuge in the cold room).
2. Discard the liquid phase (carefully turn the tube on side opposite to the pellet, dry top of tube with a clean paper towel).
3. Wash the pellet with 1000 ul ice cold (-20oC) ethanol (70%).
4. Gently shake, do not vortex.
5. Centrifuge for 10 min at 13’200 rpm at 4oC.
6. Discard ethanol (carefully turn the tube on side opposite to the pellet and remove as much as you can by drying the tube on a clean paper towel). **Ensure tube labels have not been washed off with EtOH.**
7. Dry the DNA pellet by leaving the tubes open 5-10 minutes at 50°C, ideally under the fume hood (a vacuum centrifuge can also be used). The pellet should not become totally dry, but ideally gelatinous (If necessary, leave tubes overnight at room temperature in fume hood).
8. Add 50 ul dist. TE buffer, PH 8 (autoclaved).
9. Do not vortex. Close tubes, let DNA dissolve for 5 minutes.
10. Use the SpectraMax iD3 plate reader (spectrophotometer) to measure quantity (ng/uL) and quality (260/280) of the sample and record results in the chart provided.

**END OF DAY 2**

**Materials**

* liquid N2
* ice
* Eppendorf tubes (1.5 ml) and pestles (sterile, autoclaved)
* rolling pin
* razors
* vortex
* Ziplock bags
* 10 ul tips + pipette
* 200 ul tips + pipette
* 1000 ul tips + pipette
* Molecular-grade distilled H2O (Milli-Q)
* **RNase A (100 mg/ml dist. H2O); for example Ribonuclease A from bovine pancreas, Sigma-Aldrich, R4875**
* **Proteinase K (10 mg/ml dist. H2O): for example Proteinase K from Tritirachium album, Sigma-Aldrich, P2308**
* **Phenol-chloroform-isoamylalcohol (25:24:1): for example Sigma-Aldrich, 77617**
* **Chloroform-isoamylalcohol (24:1): add 1 ml isoamyalcohol (also called 3-methylbutanol, for example Sigma, H9151) to 24 ml chloroform**
* **Sodium acetate (3 M, pH 5.2): see bellow**
* Ethanol (96%, -20° C) (use molecular grade ethanol)
* Ethanol (70%, -20° C) (use molecular grade ethanol)
* **2x CTAB buffer: see below.**

**RECIPE 2x CTAB extraction buffer (0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 0.02 M EDTA, 2% CTAB)**

Recipe for 250 ml (from Bryan Danforth):

25 ml 1M Tris-HCl (pH 8.0)

20.45 g NaCl

10 ml 0.5 M EDTA

5 g CTAB (Sigma-Aldrich, H9151)

0.5 ml 2-mercaptoethanol (Sigma-Aldrich, M6250)

to 250 ml. w/ dH2O.

Note: the CTAB is hard to get into solution; slightly heat up mixture (approx. 50-60 °C); add mercaptoethanol once the solution has cooled down.

**RECIPE 3M Sodium Acetate**

Mix 12.24 g Na-acetate in 30 ml dH2O in a Falcon tube.

COMMENTS BROCK:

so I'd say leave a little bit more of the top layer during phenol steps and maybe use the pink phenol if you're not already to increase contrast between layers.

Check the Qiagen protocol for clean-up protocol but definitely check out my tips on how to do the spins: use hot EB, spin it really dry, etc.

but it looks good. We only need like 1ug of DNA