DNA Extraction Protocol

(more details are in DNeasy Blood & Tissue Handbook at www.qiagen.com)

Day 1

1. Prepare 1.5 µl microcentrifuge tubes (n= # samples), label them.
2. Turn on the incubator, check settings (should be 56^0C).
3. Add 180 µl Buffer ATL.
4. Place an insect/tissue in each tube (sterilize forceps between samples, especially if the tissue was cut before).
5. Add 20 µl proteinase K.
6. Vortex the tubes.
7. Place in the incubator at 56^0C overnight. Vortex every 2-3 hours if possible.

Day 2

1. Take the tubes out of the incubator. Turn off the incubator.
2. Vortex the tubes 15 sec.
3. Add 200 µl Buffer AL.
4. Vortex the tubes thoroughly.
5. Add 200 µl ethanol (100%, in the metal cabinet)
6. Vortex the tubes thoroughly.

7. Prepare DNeasy Mini spin columns placed in a 2 ml collection tubes (come with the kit). Label the spin columns.
8. Set up pipet p1000 to 500 µl. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.
9. Centrifuge at 8000 rpm for 1 min
10. Prepare new 2 ml collection tubes (come with the kit).
11. Take the tubes out of the centrifuge. Discard the flow-through and old collection tubes.
12. Place the spin column in a new 2 ml collection tube.
13. Add 500 µl Buffer AW1.
14. Centrifuge at 8000 rpm for 1 min
15. Prepare new 2 ml collection tubes.
16. Take the tubes out of the centrifuge. Discard the flow-through and old collection tubes.
17. Place the spin column in a new 2 ml collection tube.
18. Add 500 µl Buffer AW2.
19. Centrifuge at 14,000 rpm for 3 min

20. Prepare new 1.5 ml mcirocentrifuge tubes. Label them.
21. Take the tubes out of the centrifuge. Discard the flow-through and collection tubes.
22. Transfer the spin column to a new 1.5 ml mcirocentrifuge tube.
23. Add 200 µl Buffer AE directly to the center of the spin column membrane.
24. Incubate for 1 min at room temperature.
25. Centrifuge at 8000 rpm for 1 min.

26. Keep the spin column and microcentrifuge tube.
27. Repeat steps 23-25 (it will increase DNA yield).
28. Discard the spin column. Close the microcentrifuge tube and store it at $+4^0$C short-term (1-2 months) or at - 20^0C long-term.

* After step 28 you can proceed immediately to PCR if needed.

General notes:

- Clean the table with soap water + ethanol before work
- Use designated space for DNA extraction (left bench next to the incubator), PCR preparations (right bench, far end), and gel electrophoresis (right side of the lab)
- Sterilize forceps between samples
- Change pipette tips between samples