**Purification of total DNA from single ants using the DNeasy Blood & Tissue Kit**

With modifications by Max John

*Note* - ants can be fresh or frozen at -80C (but must be fresh when frozen).

*Note* - part 1 calls for immersing open Eppendorf tubes into liquid nitrogen. Take great care to not allow the liquid into the tube (look to see how far the tube is immersed). Liquid nitrogen in the tube will carry off animal tissue (which is then a contamination risk for other tubes).

*Note* - centrifuge speeds are calculated for 5415C Eppendorf centrifuge.

*Required*

* Sterile pestles
* Sterile long forceps (enough length to manipulate Eppendorf in liquid nitrogen)
* Liquid nitrogen
* Vortex
* Water bath and (optionally) shaker in 56 C room
* 100% EtOH
* 1.5 ml Eppendorf tubes
* DNeasy Blood & Tissue kit with EtOH added to reagents where required

**Part 1 - Lysis**

1. Insert a tightly closed 1.5 ml Eppendorf tube containing a single ant into liquid nitrogen until temperature is equalised (i.e. nitrogen stops boiling). Remove the tube, open it, and re-insert the lower end of the tube into the liquid nitrogen, gripping the tube by the arm of the open lid. When temperature has equalised again, remove and insert a sterile pestle into the tube. Manipulate the animal inside the tube such that it is caught between the pestle and the wall of the tube (avoid getting the animal between the pestle and the bottom of the tube). Push down hard to crush the animal (there should be an audible pop/snap noise and the animal should be completely pulverised into white powder).
2. Leaving the pestle in the tube, and gripping the tube by the arm of the open lid, again insert the lower part of the tube into the liquid nitrogen and allow the temperature to equalise. Remove the tube and grind hard with the pestle. The main grinding motion is around the axis of the pestle but use some lateral motion too. When grinding well there is a distinctive squeaking noise. After no more than 15-20 seconds, insert the tube back into the liquid nitrogen and, when the temperature is equalised, remove the tube and continue grinding. Repeat this until the animal is as finely powdered as possible (typically 3-5 immersions in liquid nitrogen). Always err on the side of caution, it is essential that the animal does not thaw during this process.
3. Still leaving the pestle in the tube, insert the bottom of the open tube back into the liquid nitrogen and, when temperature has equalised, remove the tube and immediately pipette in **180 ul Buffer ATL**. ATL contains PBS, and will quickly go opaque, white, and gelatinous. Manipulate the solution with the pestle to disrupt the gelatinous texture and use the walls and edge of the tube to scrape anything stuck to the pestle back into the main body of the solution.
4. Pipette **20 ul Proteinase K solution** into the tube (try to pipette deep into the bottom of the tube). Continue manipulating the solution to disrupt the gelatinous texture, and scraping the pestle against the inside of the tube to avoid losing any material stuck to the pestle.
5. Vortex at **½ power for 5 seconds** and spin down.
6. Place tube(s) in water bath at **56 C overnight**. Alternatively, place the samples on a gentle shaker at 56 C overnight.

**Part 2 - Purification (the next day)**

1. Remove samples from water bath and vortex at **½ power for 5 seconds**. Return samples to the **56 C water bath for >= 10 minutes**.
2. Remove the samples from the water bath and add **4 ul RNase A solution (25 mg/ml)**. Vortex at **½ power for 5 seconds** and incubate at **room temperature for 5 minutes**.
3. While incubating, transfer sufficient buffer AE for elution of n+1 samples to a 1.5 ml Eppendorf tube. **160 ul per sample** is required. If necessary prepare more than 1 tube. Place tubes containing buffer AE in water bath at **60 C**.
4. Pre-mix **1:1 100% EtOH with Buffer AL** (**400 ul mixed required per sample**, prepare enough for n+1 samples). Ensure the new solution is well-mixed (vortex violently).
5. Add **400 ul of the mixed EtOH + Buffer AL solution** to each sample tube and immediately vortex vigorously for 15 seconds.
6. Spin down.
7. Transfer *all* contents of the sample tubes (including any precipitate or solid matter) to DNeasy spin columns placed inside 2 ml collection tubes.
8. Centrifuge at **8,500 rpm for 1 minute**.
9. Discard flow-through and collection tube.
10. Place the spin column in a new collection tube. Pipette **500 ul Buffer AW1** onto the membrane and centrifuge at **8,500 rpm for 1 minute**.
11. Discard flow-through and collection tube.
12. Place the spin column in a new collection tube. Pipette **500 ul Buffer AW2** onto the membrane and centrifuge at **14,000 rpm for 3 minutes**.
13. Discard flow-through and reassemble the collection tube and spin column. Centrifuge again at **14,000 rpm for 1 minute**. *At all times avoid allowing the column to come into contact with the flow-through. If contact occurs, discard flow-through and spin again.*
14. Discard flow-through and collection tube.
15. Place spin column in a 1.5 ml Eppendorf tube and pipette **60 ul of the pre-warmed (60 C) Buffer AE** directly onto the membrane. Return the unused Buffer AE to the 60 C water bath. *The volume is small compared to the surface area of the membrane. Try to get the Buffer AE in the middle and not at the side, where surface tension will keep it from seeping into the membrane. Be very careful not to pierce the membrane with the pipette tip.*
16. Incubate at **room temperature for 5 minutes**.
17. Centrifuge at **1,000 rpm for 1 minute** and then immediately at **8,500 rpm for 1 minute**.
18. Pipette **100 ul of the pre-warmed (60 C) Buffer AE** directly onto the membrane.
19. Incubate at **room temperature for 5 minutes**.
20. Centrifuge at **1,000 rpm for 1 minu**te and then immediately at **8,500 rpm for 1 minute**, and then immediately at **14,000 rpm for 1 minute**.

*Note* - elution volumes can vary. Greater volumes typically lead to greater overall yield at reduced concentration.

*Note* - 1st and 2nd elutions can be done into the same or different Eppendorf tubes. Do not elute more than 200 ul into a single Eppendorf tube or the eluate will come into contact with the tube (undesirable).