Jusino & Banik et al. Supporting Information

Appendix S2 - DNA extraction details for the modified bat guano extraction

Modified from QIAamp DNA Stool Handbook 06/2012

1. Weigh 100 mg of bat guano and add to a labeled 2 ml microcentrifuge tube (not in kit). If samples are frozen, this step should be performed quickly, or samples should be kept on ice.
2. Add 1.4 ml Buffer ASL to each guano sample.

   Do not pipette directly from large buffer ASL supply, make sure to aliquot buffer out to avoid contamination. Change pipette tips between each sample as well.
3. Vortex sample in buffer continuously for 1 min or until the guano sample is thoroughly homogenized.
4. Heat the sample for 10 min at 70° C
5. Vortex the sample for 30s.
6. Centrifuge the sample at full speed for 7 min to pellet the guano particles.
7. Pipet 1.2 ml of the supernatant into a new labeled 2 ml microcentrifuge tube (not in kit) and discard the pellet. (Note, you may not get 1.2 ml, 1.0 ml is okay). A transfer of small quantities of pelleted guano will not affect the procedure.
8. Add 1 inhibitEX tablet to each sample and vortex immediately and continuously for 2 min or until the tablet is completely suspended. When adding the tablet to each sample, do not touch the pellet, simply break the seal and guide the pellet into the tube. (Note, it may be helpful to break the pellet in half prior to adding to tube, but make sure to do this sterilely.)
9. Incubate suspension for 2 min at room temperature to allow inhibitors to absorb to the inhibetEX matrix.
10. Centrifuge the sample at full speed for 5 min to pellet inhibitors bound to the inhibitEX matrix.
11. Pipet all of the supernatant into a new labeled 1.5 ml microcentrifuge tub (not in kit) and discard the pellet.
12. Centrifuge the sample at full speed for 3 min.
13. Pipet 15 µl of proteinase K (in fridge) into a new labeled 1.5 ml tube (not in kit).
14. Pipet 200 µl of supernatant from step 12 into the corresponding 1.5 ml tube containing proteinase K.
15. Add 200 µl Buffer AL to the tube containing the supernatant and proteinase K and vortex for 15s.

    Note, do not add Buffer AL directly to proteinase K, follow the order of these steps.
16. Incubate sample at 70° C for 10 min

17. Centrifuge briefly to remove drops from the inside of the tube lid
18. Add 200 µl of 100% ethanol to the lysate and mix by vortexing.
19. Centrifuge briefly to remove drops from the inside of the tube lid
20. Label the lid of a new QIAamp spin column placed in a 2ml collection tube. Carefully pipet the liquid from step 18 to the QIAamp spin column without moistening the rim.
21. Close the cap and centrifuge at full speed for 1 min. If the lysate has not completely passed through the column after centrifugations, centrifuge again until the QIAamp spin column is empty.
22. Place the spin column in a new 2ml collection tube, and discard the tube containing the filtrate.
23. Carefully open the spin column and add 500 µl Buffer AW1.
24. Close the cap and centrifuge at full speed for 1 min.
25. Place the spin column in a new 2ml collection tube, and discard the tube containing the filtrate.
26. Carefully open the spin column and add 500 µl Buffer AW2.
27. Close the cap and centrifuge at full speed for 3 min.
28. Place the spin column in a new 2ml collection tube (from the bag labeled “extra”), and discard the tube containing the filtrate.
29. Centrifuge at full speed for 1 min. (This step helps eliminate the chance of possible buffer carryover).
30. Transfer the spin column into a new 1.5 ml microcentrifuge tube with the cap removed (not in kit).
31. Carefully open the spin column and pipet 200 µl Buffer AE directly onto the QIAamp membrane. (we may change this to water, and perhaps 100 instead of 200)
32. Close the cap and incubate for 1 min at room temperature.
33. Centrifuge at full speed for 1 min to elute DNA.
34. Pipet DNA in collection tube into a new labeled 1.5 microcentrifuge tube.
35. Store at -20° C