Appendix 2. Protocol for DNA extraction from the footpad of a museum oilbird

Day 1

1. Place the sample in a microcentrifuge tube
2. Add 100 µl of absolute ethanol (i.e., 100%) to each tube
3. Incubate at room temperature for 5 minutes
4. Remove by pipetting the ethanol and discard it
5. Add 200 µl of buffer AE to each tube
6. Incubate at room temperature for 5 minutes
7. Remove by pipetting the buffer AE and discard
8. Remove the samples and leave them on a paper towel until dry
9. Cut the sample into small pieces with a razor blade and place in a tube with beads

Note: The razor blade must be passed through ethanol and distilled water each time a new sample is cut

10. Add 40 µl proteinase K and 20 µl DTT
11. Place in homogenizer for 15 min at a frequency of 30.0
12. Incubate samples overnight (Time: HLD, RPM: 20 and Temperature: 56°C)

Day 2

1. Vortex for 15 seconds, and centrifuge at 14,000 rpm for 5 minutes
2. Add 200 µl of Buffer AL and mix for 30 seconds on vortex
3. Incubate for 10 minutes at 70°C
4. Add 200 µl of ethanol (96 - 100%) and mix for 30 seconds on vortex

Note: ethanol must be cold, preferable to - 20°C

5. Incubate at 4°C for one hour
6. Pipette the resulting liquid and place in the column that comes with the Kit
7. Incubate for 10 minutes at room temperature
8. Centrifuge at 8,000 rpm for 1 minute
9. Discard the collection tube
10. Place the column in a new tube collector tube (2 ml)
11. Add 500 µl Buffer AW1
12. Centrifuge at 8,000 rpm for 1 minute
13. Discard collector tube
14. Place column in a new collector tube (2 ml)
15. Add 500 µl Buffer AW2
16. Centrifuge at 1 4,000 rpm for 3 minutes
17. Change collecting tube and centrifuge again at 14,000 rpm for 3 minutes
18. Discard collecting tube
19. Place column in eppendorf tube (1.5 ml or 2 ml)

20. Add 25 µl Buffer AE

Note: Buffer AE should be at 56°C

21. Incubate at room temperature for 24 hours

Day 3

1. Centrifuge at 8,000 rpm for 1 minute
2. Add 25 µl Buffer AE
3. Centrifuge at 8,000 rpm for 1 minute
4. Discard the column and store the eppendorf tube properly marked

DNA quantification

1. Turn on the computer where the NanoDrop 2000 is located
2. Open the NanoDrop 2000 program
3. Enter “Nucleic Acid”
4. in “Type” put “DNA”
5. Open and clean the NanoDrop 2000 with alcohol
6. Put 2 µl of Buffer AE in the NanoDrop 2000
7. Click on “Blank”
8. It is placed in the name of the sample “Control”
9. Click on “Measure”, with the same Buffer AE that was placed in step 6
10. Values are accepted in a range from 0 to 1 or from 0 to -1, if it gives a different value, place "Control" again and click on "Measure"
11. Open the NanoDrop 2000 and clean with water
12. Place the samples, clean with water between samples
13. Clean with alcohol at the end