Appendix 1. Protocol for extracting DNA from feathers

Day 1

1. Cut the cannon of the feathers
2. Cut the cannon into small pieces and place it in a tube with beads

Note: The use of the mortar and liquid nitrogen is omitted, since their use did not show better results

3. Add 180 µl of Buffer ATL
4. Add 20 µl Proteinase K and DTT 30 µl
5.

Note: If Proteinase K and DTT are not used, a greater DNA quantification can be obtained at the end, however, it presents a large number of salts (i.e., contaminants)

5. Place in the homogenizer for 5 minutes at a frequency of 30.0
6. Incubate the samples whole night (Time: HLD, RPM: 20 and Temperature: 56°C)

Day 2

Note: If the incubation lasts a short time, almost no DNA will be obtained

7. Vortex for 15 seconds, and centrifuge at 14,000 rpm for 5 minutes
8. Add 200 µl Buffer AL and vortex for 30 seconds
9. Add 200 µl ethanol (96 - 100%) and vortex for 30 seconds

Note: Assays are performed being carried out with 100% ethanol

10. Pipette the resulting liquid and place it on the column that comes with the Kit
11. Centrifuge at 8,000 rpm for 1 minute
12. Discard the collection tube
13. Place the column in a new collection tube (2 ml)
14. Add 500 µl of Buffer AW1
15. Centrifuge at 8,000 rpm for 1 minute
16. Discard collection tube
17. Place column in a new collection tube (2 mL)
18. Add 500 µl Buffer AW2
19. Centrifuge at 14,000 rpm for 3 minutes
20. Change collection tube and centrifuge again at 14,000 rpm for 3 minutes
21. Discard the collection tube
22. Place the column in an eppendorf tube (1.5 ml or 2 ml)
23. Add 25 µl Buffer AE
24. Incubate at room temperature for 1 minute
25. Centrifuge at 8,000 rpm for 1 minute
26. Add 25 µl Buffer AE

27. Centrifug ar at 8,000 rpm for 1 minute
28. Discard the column and store the properly labeled eppendorf tube

Note: The total volume of the DNA sample is 50 µl

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