



Stool Extraction Protocol



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Extraction of genomic DNA from carnivore fecal samples using QIAamp DNA Stool Mini Kit.

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(Modified from QIAamp[®] DNA Stool Handbook)

DAY 1:

Before starting

- ⇒ If a precipitate has formed in Buffer ASL, dissolve by incubating at 55°C or 70°C.
- ⇒ Clean the bench and the pipettes with DNA Away or bleach.
- ⇒ UV 2.0 ml tubes (2 tubes per sample)
- ⇒ UV filtered tips.

You should never use the same tip or anything else with two different samples. Cross contamination is a big issue with scat samples, because of low amount and bad DNA quality.

Procedure

1. Shave (thin slices) ~ 200mg (0.5ml) of fecal material from the outside of the scat using a new razor blade, sterilized forceps, and a Petri dish.

The amounts of fecal material you will use depend on the amount you have. If you have just ~ 200mg (0.5ml) of fecal material or less, start you extraction with half of what you have. It is always important to preserve at least a little of the sample to re-extract if needed.

2. If your sample is solid, put the fecal material into the tube and add 1.5ml of Buffer ASL in each tube.
3. If your sample is made mostly from hairs, pour about 4.0ml of Buffer ASL in the Petri dish with the fecal material. Let it soak a little bit to dissolve the solid material attached to it then chop the hairs. Collect the liquid and aliquot 1.5 ml in each 2ml tube.
4. Repeat the steps 1 to 3 to each sample. Clean the work area with bleach and change gloves between samples.
5. Vortex the tubes continuously for 15 sec.
6. Incubate at 55-65°C on a rotator (~22rpm) overnight.
7. Wash the forceps with bleach and sterilize them before put them back into the forceps box.

Note: when using frozen stool samples, wait until they are totally defrosted, but do not thaw until Buffer ASL is added. Otherwise the DNA in the sample may degrade.

DAY 2:

Before starting

- ⇒ Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels.
- ⇒ If a precipitate has formed in Buffer AL, dissolve by incubating at 55°C or 70°C.
- ⇒ Heat Buffer AE at 70°C and continue heating during the extraction.
- ⇒ Place 100% ethanol in freezer for use in step 11.
- ⇒ Clean the bench and the pipettes with DNA Away or bleach.
- ⇒ UV filtered tips and tubes.
- ⇒ Make the labels with sample number and put them in your final tubes.

PS: You should never use the same tip or anything with two different samples.

Procedure

1. Centrifuge the samples during 3 min at 13,300 rpm to pellet particles. In this step you will have 2 tubes per sample.

2. Transfer 1.5ml of the supernatant to a fresh 2.0ml reaction tube, if more, use a second tube.

At this point the supernatants from the sample duplicate tubes are joined together.

3. Add one InhibitEX tablet to the tube filled with 1.5ml of the supernatant. Add a proportional piece of tablet to the second tube. Cut the tablet using a new razor blade and forceps on a Petri dish, avoiding touching it.

1ml = use 2/3 tablet
750ml = use ½ tablet
500ml = use 1/3 tablet

4. Vortex continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.

5. Centrifuge 12 min at 13,300 rpm in order to pelletize the tablet.

More spins may be necessary to recover the max supernatant. Always remove just 4-6 tubes at a time from the centrifuge, and while processing those spin the others an additional 4 min.

6. While spinning, prepare 2 sets of tubes (2ml) with 25 ul of Proteinase K.

7. Transfer 600 ul supernatant to 2ml reaction tube with ProtK and, if more, use the second tube. Gently mix manually to form a homogeneous solution.

At this point the supernatants from the sample duplicate tubes are joined together.

8. Add 600 ul of AL Buffer to the tube with 600 ul of supernatant+ProtK. The amount of AL Buffers should be the same as the amount of supernatant. Therefore, AL Buffer needs to be scaled if supernatant in the tube is less than 600 ul.

9. Vortex for 15 sec.

10. Incubate at 70°C for 15 min.
11. Add 600 ul of 100% Ethanol (or as much Ethanol as AL Buffer added in step 8).
12. Vortex for 15 sec.
13. Label the lid of a new QIAamp spin column provided in a 2ml collection tube.
14. Transfer 600ul lysate from step 12 to the QIAamp spin column placed over a collection tube.
15. Centrifuge at 13,300 rpm for 1 min and discard the filtrate after each transfer.
Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.
16. Repeat the steps 14 and 15 until all lysate from the tube is filtered.
17. Transfer the QIAamp spin column to a fresh collection tube and add 500 ul of AW1 Buffer.
18. Centrifuge at 13,300 rpm for 1 min.
19. Transfer the QIAamp spin column to a fresh collection tube and add 500 ul of AW2 Buffer.
20. Centrifuge at 13,300 rpm for 2 min.
21. Transfer the QIAamp spin column to a fresh collection tube, centrifuge again at 13rpm for 2 min to dry the column.
This step helps to eliminate the chance of possible Buffer AW2 carryover.
22. Transfer the QIAamp spin column into a new, labeled 1.5 ml Eppendorf tube.
23. Carefully open the QIAamp spin column and pipet 60ul Buffer AE (heated) directly onto the QIAMembrane. Close the cap and incubate for 40 min at room temperature.
24. Centrifuge at 8,000 rpm for 3 min.
25. In the same tube, pipet more 60ul Buffer AE directly onto the QIAMembrane. Close the cap and incubate for 15 min at room temperature.
26. Centrifuge at 8,000 rpm for 3 min.
27. Still in the same tube, pipet more 60ul Buffer AE directly onto the QIAMembrane. Close the cap and incubate for 15 min at room temperature.
28. Centrifuge at 8,000 rpm for 3 min.
29. At this step you will have 180 ul of DNA in one tube with the same concentration.

30. Transfer 90 ul of DNA to a new 1.5ml Eppendorf tube.

31. Now that you have 2 tubes with DNA ready to use, label one as your STOCK and another as your BACK UP.

If you forget to do any step or do it wrong, consult the Global Felid Project specialist. You may have to start the extraction again. DNA from scat samples is very sensitive to mistake, because of low amount and bad DNA quality.