Supplementary File 2.1. Laboratory protocol for extracting and quantifying DNA from primate feces

Genomic DNA extraction from primate feces stored in RNA Later

Adapted from a protocol by Simone Loss Chaves, Isabela Dias, Cristina Pomilla and QIAamp®

You will need:
Your samples stored in RNAlater. See the main text for collection technique tips.
QIAamp Stool kit
Petri dishes
Razor blades
Forceps
2 mL tubes
Gloves and a respirator mask
DNA Away or bleach
100% ethanol
1 mL, 200 µL, and 20 µL pipetters
200 Centrifuge
Vortexer
An incubator set to 55°C with a rotator that can fit 2 mL tubes
A heat block set to 70°C
Bunsen burner (for forcep sterilization)

Day 1 Steps:

1. Clean bench and pipetters using 50% DNA Away or bleach. Be sure to wear appropriate personal protective equipment while handling samples.
2. UV 2.0 ml tubes (2 tubes per sample), filtered tips, petri dishes, and razor blades or use sterile ones.
3. Heat ASL buffer to 70°C to ensure suspension.
4. Thaw samples.
5. Once no ice remains, homogenize sample by briefly vortexing its falcon tube.
6. Pour roughly 0.5ml of fecal sample into a sterilized Petri dish. Use forceps to remove large solids if needed.
7. Add an equivalent amount of ASL buffer or enough to cover the bottom of the Petri dish, whichever is more. Use a razor blade to chop the fecal material as small as possible. Slowly, carefully rotate the Petri dish to mix the sample and the buffer.
8. Tilt the Petri dish, collect the liquid, and aliquot 1.5 ml per 2ml tube, as many times as necessary to aliquot all the liquid. Discard the solids and Petri dish in a biohazard container and razors in a sharps container.
9. Clean bench using 50% DNA Away or bleach and change gloves. Repeat until 12 samples have been processed.
10. Vortex all tubes.
11. Incubate at 55°C on a rotator at ~22rpm for 12 or more hours.
12. Wash the forceps with Liquinox or bleach. Next, sterilize them by dipping them in alcohol lighting them on fire with a Bunsen burner.

Day 2 Steps:

1. Clean bench and pipetters using 50% DNA Away or bleach. All tubes from this day may be discarded in the normal trash as the lysis buffer from yesterday has rendered them non-hazardous.
2. UV 2.0 ml tubes (2 tubes per sample), 1.5 mL tubes, and filtered tips or use sterile ones.
3. Heat AL and AE buffers to 70°C to ensure suspension.
4. Place 100% ethanol in the freezer.
5. Centrifuge the samples from yesterday for 3 min at max speed (13,300 rpm) to pellet particles.
6. Transfer 1.5ml of the supernatant to a fresh 2.0ml reaction tube, using as many tubes as necessary to remove all the supernatant.
7. Add one InhibitEX tablet to each tube of supernatant. Add a proportional piece of tablet to any tube containing less than 1.5 mL of supernatant. Cut each tablet using a new razor blade in a sterilized Petri dish. Avoid touching the tablet even with gloved hands.
8. Vortex each tube until the tablet is completely suspended.
9. Incubate the suspension for 1 min at room temperature.
10. Centrifuge tubes for 12 min at max (13,300 rpm).
11. Prepare 2 sets of 2 mL tubes with 25 µL of Proteinase K (ProK) for each 1.5 mL tube of sample. Be sure to keep the ProK covered as you work with it because it is light sensitive.
12. Transfer 600 µL of sample to each tube with ProK. Pipette up and down to mix.
13. Add 300 µL of AL Buffer to the tube with 600 µL of supernatant and ProtK.
14. Vortex sample thoroughly.
15. Incubate at 70°C for 15 minutes.
16. Add 300 µL of 100% Ethanol.
17. Vortex.
18. Transfer 600µL of sample to a QIAamp spin column placed over a collection tube. Do not overfill!
19. Centrifuge at max speed (13,300 rpm) for 1 min and discard the filtrate.
20. Repeat steps 18 and 19 until all the sample has been filtered.
21. Transfer the QIAamp spin column to a fresh collection tube and add 500 µL of AW1 Buffer.
22. Centrifuge at max speed (13,300 rpm) for 1 min.
23. Transfer the QIAamp spin column to a fresh collection tube and add 500 µL of AW2 Buffer.
24. Centrifuge at max speed (13,300 rpm) for 2 min.
25. Transfer the QIAamp spin column to a fresh collection tube, centrifuge again at max speed (13,300 rpm) for 2 min to dry the column. It is important that no liquid is left on the spin column membrane at this point.
26. Transfer the QIAamp spin column into a new 1.5 ml Eppendorf tube.
27. Carefully pipet 60µL of heated AE buffer directly onto the spin column's membrane. Avoid pipetting the buffer onto the walls of the column. Close the cap and incubate for at least 40 min at room temperature.
28. Centrifuge at 8,000 rpm for 3 min.
29. In the same tube, again pipet 60 µL of heated AE Buffer directly onto the spin column membrane. Close the cap and incubate for at least 15 min at room temperature.
30. Centrifuge again at 8,000 rpm for 3 min.

31. Still in the same tube, pipet another 60 µL heated AE Buffer directly onto the spin column membrane. Close the cap and incubate for at least 15 min at room temperature.
32. Centrifuge at 8,000 rpm for 3 min one final time.
33. Discard the spin column and label the tube. Your DNA is ready to freeze or for an AMPure clean up.

Purification of extracted gDNA with AMPure Beads

Adapted from a protocol by the Tung Lab and AMPure® (AgenCourt®)

<u>You will need</u>:
A magnetic rack for 1.5-2 mL tubes
100% ethanol
Nuclease free water
AMPure beads
1.5 mL tubes

<u>Steps</u>:
1. Make a fresh dilution of 80% ethanol. The volume depends on the number of samples you intend to process.
2. Ensure AMPure beads are at room temperature and pipette up and down to resuspend them prior to each use.
3. Add 90 µL beads per 50 µL sample; mix thoroughly by pipetting up and down. If your sample is a different volume, add enough beads to have 1.8x beads per sample.
4. Incubate at room temperature for at least 5 minutes.
5. Place sample on magnetic stand at room temperature for at least 5 minutes, until the liquid is completely clear.
6. Remove and discard most of liquid without disturbing the beads. Err on the side of having a little liquid left over at this time if necessary.
7. Keeping the sample on the magnetic stand and without disturbing the beads, add enough 80% ethanol to completely cover beads.
8. Remove and discard the ethanol.
9. Repeat steps 7 and 8 for a total of two washes with 80% ethanol.
10. Without disturbing the sample on the magnetic stand, allow beads to dry at room temperature or in a biosafety hood until completely dry and shiny but not cracked (approximately 15 minutes).
11. Remove samples from the magnetic rack. Add 40 µL of water and mix by pipetting up and down. Incubate at room temperature for at least 5 minutes.
12. Place sample on magnetic stand at room temperature for at least 5 minutes, until the liquid is completely clear.
13. Recover your DNA, which is suspended in the supernatant, without disturbing the beads and transfer it to new 1.5 mL tubes.
14. Set aside 5 µL DNA in a separate tube for Qubit Fluorometric Quantification and qPCR analyses to avoid unnecessarily thawing your stock DNA. It is okay to freeze your DNA and stop here.

Qubit Fluorometric Quantification of fDNA

Adapted from the Thermo Fisher Scientific Qubit® protocol

You will need:
0.5 mL clear Qubit assay tubes, one for each sample and the two standards
Qubit dsDNA High Sensitivity assay kit
Qubit Fluorometer

Steps:
1. Set up your 0.5 mL tubes, one for each sample and the two standards, and label the tube lids. Do not mark the sides of the tubes as this will interfere with the fluorometer.
2. Make your master mix: (1 µL dye + 199 µL buffer) * (n samples + 2 standards + 1-3 extra to ensure you have enough master mix). Keep the dye covered while not in use because it is light sensitive.
3. Aliquot 199 µL of master mix into each sample tube, and 190 µL into the standard tubes.
4. Add 10 µL of each standard to the appropriate tubes and 1 µL. Given this small amount, make sure your DNA is well mixed by pipetting up and down several times before pipetting directly into the master mix. Then mix by vortexing for 2-3 seconds. Be careful not to scuff the sides of the tubes as this will interfere with the fluorometer. Remove any bubbles for the same reason by gently tapping the tube on the benchtop or by popping them with a pipette tip. The final volume in each tube should be 200 µL.
5. Incubate at room temperature for 2 minutes.
6. On your Qubit machine, select DNA, then dsDNA High Sensitivity.
7. Insert your first standard and read it as prompted, doing the same with your second standard. Discard tubes.
8. Read your samples, recording the amount of DNA in ng/µL.

Optional: quantitative PCR for assessing amounts of target DNA

Adapted from the QIAgen® QuantiNova protocol and
auxilliary protocol B of FecalSeq (Chiou and Bergey, 2018)

You will need:
The SYBR Green-based qPCR kit of your choice. I used QuantiNova from QIAgen.
Primers for your target species. See text for design tips.
qPCR machine
Reaction tubes that fit your qPCR machine
High-quality, uncontaminated DNA (e.g. extracted from a blood sample) for your target species
Nuclease free water

Steps:
1. Quantify the amount of DNA is your positive control sample (e.g. extracted from blood or tissue) using the Qubit methods described above.
2. Create a dilution series of your high-quality, known quantity DNA – including 10 ng/µl, 1 ng/µl, 0.1 ng/µl, and 0.01 ng/µl.
3. Calculate the volume of each primer at 0.5 mM concentration.

4. Make your master mix for a total volume of 19 µl including both primers in the amount you calculated above, with the remainder being 1X of SYBR Green master mix. Make enough to run two reactions for each sample, two for each dilution of your high-quality DNA, and two negative controls.
5. Fill all your tubes with 19 µL of master mix, and 1 µL of sample, dilution, or nuclease free water.
6. Run on your qPCR machine at 95 °C for 15 minutes, followed by 50 cycles of 94 °C for 15 seconds, 60 °C for 25 seconds, and 72 °C for 20 seconds. Discard tubes after the run.
7. Look at the curves generated by the qPCR instrument. Label the curves associated with your high-quality DNA dilutions. Categorize your samples based on where they fall relative to the dilution curves (e.g. a sample curve that takes off in between the 1 ng/µl and 0.1 ng/µl dilutions contains between 1 ng/µl and 0.1 ng/µl target species DNA). If the replicates for any sample or dilution are wildly different (more than 5 cycles, for example), you should consider this unreliable and rerun the sample instead of averaging the values.

Proceed to FecalSeq (Chiou and Bergey, 2018)

Chiou KL, Bergey CM. 2018. Methylation-based enrichment facilitates low-cost, noninvasive genomic scale sequencing of populations from feces. Scientific Reports 8: 1975.