

IHMS DNA extraction protocol Q

Fecal DNA extraction with the use of Qiagen QIAamp DNA stool kit

1. Homogenize the 150 to 200mg frozen feces with 1.0mL ASL lysis buffer of the kit by vortexing for 2min in a 2mL tube containing 0.3g of sterile zirconia beads \varnothing 0,1mm zirconia (BioSpec, Cat. No. 11079101z). [if buffer shows precipitate, heat at 70°C before use]
2. Incubate for 15min at 95°C.
3. Cells are mechanically lysed by running the Fastprep™ Instrument for 8min15sec (series of beating 1 min and resting 5 min are preferable).
4. Samples are allowed to cool down on ice for 2min.
5. Samples are centrifuged at 16000 x g, 4°C, for 5min.
6. Supernatant is transferred to a new 2mL tube.
7. The pellet is mixed with 300µL ASL lysis buffer of the kit, and steps 2-5 are repeated.
8. Supernatants are pooled in the new 2mL tube.

9. Add 260µl of 10M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min.
10. Centrifuge at 16000 g, 4°C, for 10min.
11. Transfer the supernatant to two 1.5mL Eppendorf tubes, add one volume of isopropanol, mix well, and incubate on ice for 30 min.
12. Centrifuge at 16000 g, 4°C, 15min, remove the supernatant using aspiration, wash nucleic acids pellet with 70 % EtOH (0,5mL) and dry the pellet under vacuum for 3min.
13. Dissolve the nucleic acid pellet in 100µL of TE (Tris-EDTA) buffer and pool the two aliquots.
14. Add 2µL of DNase-free RNase (10mg/mL) and incubate at 37°C, 15 min.
15. Add 15µL proteinase K and 200µL AL buffer to the supernatant, vortex for 15sec and incubate at 70°C for 10 min.
16. Add 200µL of ethanol (96-100%) to the lysate, and mix by vortexing.
17. Transfer to a QIAamp spin column and centrifuge at 16000 g for 1min, at Room Temperature (RT).
18. Discard flow through, add 500µL buffer AW1 (Qiagen) and centrifuge at 16000 g for 1min, at RT.
19. Discard flow through, add 500µL buffer AW2 (Qiagen) and centrifuge at 16000 g for 1min, at RT
20. Dry the column by centrifugation at RT for 1min.
21. Add 200µL Buffer AE (Qiagen), incubate for 1min at RT
22. Centrifuge for 1min at 16000 g to elute DNA.

Quality control: use 1% agarose gel

Sample concentration: use Nanodrop or Qubit