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SCSR - Isolation of Genomic DNA from Exfoliated Colonic Cells

We currently recommend isolating genomic DNA from colonocytes using the standard phenol/chloroform extraction protocol for eukaryotic cell lysates[1].

Note that fecal isolates contain inhibitors that attenuate qPCR signals. We are currently evaluating inhibitor removal systems. Please contact us for the latest information.

Materials

1. Digestion Buffer:
    a. 100 mM NaCl
    b. 10mM Tris-Cl, pH 8.0
    c. 25mM EDTA, pH 8.0
    d. 0.5% (w/v) SDS
    e. 0.1 mg/ml Proteinase K
2. PBS, ice-cold
3. 25:24:1 phenol/chloroform/isoamyl alcohol
4. 7.5 M ammonium acetate
5. 100% and 70% ethanol
6. TE buffer, pH 8

Procedure

1. Begin with 50K cells, based on the 5-8 µm count. *(The expected yield is ~1-2 µg DNA)*
2. Centrifuge cells 5 min at 900 x *g* and discard supernatant.
3. Resuspend cells in 1 ml PBS. Centrifuge 5 min at 900 x *g*, discard supernatant, and repeat. Resuspend cells in 1 vol (0.5-1.0 ml) digestion buffer.
4. Incubate samples, shaking, in tightly capped tubes, 12 to 18 hr at 55ºC.
5. Extract samples with an equal volume of phenol/chloroform/isoamyl alcohol. Centrifuge 10 min at max speed. If phases do not resolve well, add another volume digestion buffer, omitting Proteinase K, and repeat centrifugation. If thick white material appears at interface, repeat organic extraction. Transfer top layer (aqueous) to a new tube.
6. Add 1 vol of chloroform. Centrifuge 10 min at max speed
7. Add ½ vol of 7.5 M ammonium acetate and 2 vol of 100% ethanol. Centrifuge 10 min at max speed. Carefully remove supernatant because the pellet might not be visible.
8. Wash with 70% ethanol being careful not to disturb the pellet, centrifuge 10 min at max speed, air dry, and resuspend in TE buffer at ~1 mg/ml.
9. (Optional) Remove residual RNA by adding 0.1% SDS and 1µg/ml DNase-free RNase, incubating 1 hr at 37 ºC, repeating steps 5-7.

[1] Adapted from "Preparation of Genomic DNA from Mammalian Tissue," *Short Protocols in Molecular Biology 3rd Ed.* Frederick M. Ausubel et. al. John Wiley & Sons, 1997.