DNA extraction (Salting out)

Version 4

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ABSTRACT

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

BEFORE START

1. In microtubes containing tissue fragments, add 440µL of lysis buffer (10mM Tris-HCl, 2mM EDTA, 400mM NaCl, 2% SDS) and 10 µL of proteinase K (10mg/mL);
2. Incubate in a water bath at 55°C for approximately 1:30h or overnight;
3. Add 300µL of NaCl (5M) and centrifuge for 10min at 10.000rpm.
4. Transfer supernatant containing the DNA to microcentrifuge tube (1,5mL);
5. Add 500µl of 100% isopropanol.
6. Centrifuge for 10min at 10.000rpm;
7. Discard the supernatant and reuse microcentrifuge tube;
8. Add 700µL of 70% ethanol;
9. Centrifuge for 3min at 13.000rpm;
10. Discard the supernatant and dry microcentrifuge tube;
11. Add 30 µL of sterile H_2O and 5µL of RNAse (10mg/mL);
12. Incubate at 37°C for 30 min o overnigth and stored at -20ºC.

MATERIALS TEXT

NaCl; Tris-HCl; EDTA; Proteinase K; Ethanol; Sterile H_2O; RNAse; Isopropanol; Microcentrifuge capable of at least 13.000rpm; Incubator

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