DNA extraction (Salting out) V.4

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BEFORE START

1. In microtubes containing tissue fragments, add 440 μL 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 H2O and 5μL of RNase (10mg/mL);
12. Incubate at 37°C for 30 min o overnigh and stored at -20°C.

MATERIALS

NaCl; Tris-HCl; EDTA; Proteinase K; Ethanol; Sterile H2O; RNase; Isopropanol;
Microcentrifuge capable of at least 13.000rpm; Incubator
BEFORE START INSTRUCTIONS

1. In microtubes containing tissue fragments, add 440 μL of lysis buffer (10 mM Tris-HCl, 2 mM EDTA, 400 mM NaCl, 2% SDS) and 10 μL of proteinase K (10 mg/mL);
2. Incubate in a water bath at 55°C for approximately 1:30h or overnight;
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4. Transfer supernatant containing the DNA to microcentrifuge tube (1.5 mL);
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7. Discard the supernatant and reuse microcentrifuge tube;
8. Add 700 μL of 70% ethanol;
9. Centrifuge for 3 min at 13,000 rpm;
10. Discard the supernatant and dry microcentrifuge tube;
11. Add 30 μL of sterile H₂O and 5 μL of RNAse (10 mg/mL);
12. Incubate at 37°C for 30 min overnight and stored at -20°C.