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We use this protocol and it's working

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Abstract

The protocol describes a DNA extraction method from cytogenetic suspensions fixed in Carnoy's solution (methanol and acetic acid at a ratio of 3:1) and stored at -20°C for more than 10 years, involving enzymatic digestion, isopropanol precipitation, and ethanol washing. After the final resuspension, the extracted DNA is expected to show good integrity and purity, suitable for downstream molecular analyses such as PCR or sequencing.

Materials

Reagents and Solutions

- Carnoy's fixative solution (3:1 methanol:glacial acetic acid);
- Extraction buffer (prepare 100 mL with):
  NaCl 5 M – 8 mL
  Tris-HCl 1 M pH 8.0 – 1 mL
  EDTA 0.5 M pH 8.0 – 400 µL
  SDS 10% – 20 mL
  Ultrapure (Milli-Q) water – complete to 100 mL (≈70.6 mL);
- Proteinase K (10 mg/mL);
- NaCl 5 M (stock solution);
- 100% isopropanol (ice-cold);
- 70% ethanol (ice-cold);
- Autoclaved Milli-Q water;

Laboratory Materials

- 1.5 mL microcentrifuge tubes (sterile and labeled);
- Micropipette tips (10 µL, 200 µL, 1000 µL; sterile);
- Micropipettes (10 µL, 200 µL, 1000 µL);
- Vortex mixer;
- Bench centrifuge (capable of reaching 10,000 rpm);
- Water bath (set to 55 °C);
- Incubator (set to 37 °C);
- Tube rack;
- Absorbent paper (for drying tubes/pellets);
- Personal protective equipment (PPE): lab coat, gloves, safety goggles
- Spectrophotometer or NanoDrop (to check DNA purity and concentration)
- Agarose gels and electrophoresis equipment (to assess DNA integrity)

1 Homogenize the cytogenetic suspensions fixed in Carnoy's solution (methanol and acetic acid at a ratio of 3:1) .

2 Transfer 150 µL to a 1.5 mL microcentrifuge tube.

3 Centrifuge at 8,000 rpm for 10 minutes.

4 Discard the supernatant and allow the fixative to evaporate in an incubator at 37 °C for 45 minutes (or until no odor remains).

5 Add 440 µL of extraction buffer (NaCl 5 M: 8 mL; Tris-HCl 1 M pH 8.0: 1 mL; EDTA 0.5 M pH 8.0: 400 µL; SDS 10%: 20 mL; ultrapure water: 70.6 mL; final volume: 100 mL) and 16 µL of proteinase K (10 mg/mL).

6 Vortex briefly and incubate in a water bath at 55 °C for 1 hour 30 min.

7 Add 300 µL of NaCl 5 M. Vortex for 30 seconds.

8 Centrifuge at 10,000 rpm for 10 minutes. Transfer 500 µL of the supernatant to a previously labeled 1.5 mL microcentrifuge tube.

9 Add 500 µL of ice-cold 100% isopropanol. Mix by gentle inversion.

10 Centrifuge at 10,000 rpm for 10 minutes. Discard the supernatant.

11 Wash the pellet with 300 µL of ice-cold 70% ethanol.

12 Centrifuge at 10,000 rpm for 5 minutes. Discard the supernatant and allow the pellet to air-dry.

13 Resuspend the pellet in 30 µL of autoclaved Milli-Q water.

Protocol references

Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 1997;25: 4692–4693.