GEroNIMO

Rapid high-salt genomic DNA extraction from bird red blood cells

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1. Summary

The aim of this protocol is to obtain DNA from bird red blood cells (e.g. chicken or quail) using a high-salt extraction method.
Adapted from Roussot et al. (2003), Genet. Sel. Evol. 35: 559–572. DOI: 10.1051/gse:2003039

2. Protocol description

1.1 Required reagents and instruments

Reagents:

- EDTA 0.5M (Invitrogen - AM9261)
- NaCl 3M (Merck-Sigma Aldrich - S3014)
- Tris-HCl 1M (Invitrogen – 15568025)
- Sodium dodecyl sulfate solution (Merck-Sigma Aldrich – 71736)
- Proteinase K 20 mg (Eurobio - GEXPRK01-B5)
- TE Buffer (Invitrogen – 12090015)
- Saturated NaCl solution 6M (Merck-Sigma Aldrich - S301)
- Absolute ethanol (Carlo Erba 4146012)

Consumables:

- 2mL tubes
- 5mL tubes
- 1.5mL tubes

Equipment:

- Centrifuge 14500 rpm
- Multichannel pipettes
- Nanodrop
- Agarose gel electrophoresis system

1.2 Preparatory step

Label the tubes. Dispense 50µL of red blood cells per tube.

Prepare the lysis buffer:

- 10mM EDTA
- 10mM NaCl
- 10mM Tris HCl
- 0.5% SDS

1.3 DNA extraction procedure

1. Add 800µL lysis buffer to each 50µL red blood cells, add 4µl Proteinase K.

2. Gently mix by inverting the tube

3. Incubate overnight at 56°C.

4. Add 650µL saturated (6 M) NaCl solution; mix vigorously to precipitate proteins during at least 5min. Centrifuge for 30 minutes at 14500 rpm at 4°C

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5. Transfer the supernatant to a 5mL tube containing 3mL of absolute ethanol to precipitate DNA.

6. Collect DNA using an inoculation loop.

7. Put it on a 1.5mL tube containing 250µL TE buffer

9. Incubate overnight at 37°C to dissolve completely the DNA.

1.4 Quality control

Assess DNA quantity (Nanodrop) and quality by agarose gel electrophoresis.

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