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Quick & Dirty DNA Extraction

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ABSTRACT

Modified from Cheung et al., 1993

MATERIALS

Liquid nitrogen

Quick & Dirty extraction buffer with detergent (350 µL per sample)

Nanopure H₂O

1M Tris base (pH 8.0)

0.5M EDTA (pH 8.0)

NaCl

Sodium Metabisulfite (NA₂S₂O₅)

5% sarkosyl solution (50 g/L N-lauroyl-sarcosine, sodium salt)

10 M ammonium acetate (150 µL per sample)

100% isopropanol (200 µL per sample)

70% ethanol (500 µL per sample)

95% ethanol (500 µL per sample)

Nanopure H₂O/TE

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.baf7ibrn

External link:
<https://genome.cshlp.org/content/3/1/69.long>

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MANUSCRIPT CITATION: Cheung WY, Hubert N, Landry BS (1993) A simple and rapid DNA microextraction method for plant, animal, and insect suitable for RAPD and other PCR analyses. PCR Methods Appl 3:69-70

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Protocol status: Working
We use this protocol and it's working

Created: Dec 12, 2019

Make Quick & Dirty extraction buffer with detergent (500mL)

- 1 Add roughly 200 mL nanopure H₂O to a 1 L glass bottle with a stir bar and stir.
 - 1.1 Transfer 80 mL 1 M Tris base to bottle.
 - 1.2 Transfer 56 mL 0.5 M EDTA to bottle.
 - 1.3 Transfer 46.7 g NaCl to bottle.
 - 1.4 Transfer 3 g sodium metabisulfite to bottle.
 - 1.5 Stir until dissolved.
 - 1.6 Transfer solution to graduated cylinder and add nanopure H₂O to bring volume to 400 mL.

2 Return to 1 L bottle and autoclave for 00:25:00 .

2.1 Allow solution to cool to room temperature.

3 Gently add 100 mL 5% sarkosyl solution to Q&D buffer.

Note: Sarkosyl solution is a detergent and will foam if poured too quickly.

3.1 Swirl solution gently to mix.

Note: Once detergent has been added, do not autoclave, detergent will degrade at high heat.

Prep

4 Pre-heat Quick & Dirty extraction buffer with detergent in a 60 °C incubator.

5 Place isopropanol, 70% ethanol, and 95% ethanol in the freezer to chill.

6 Place one steel ball bearing into each sample tube.

7 Remove plate from -80 and quickly remove bottom from plate and float in liquid nitrogen to keep tissue frozen.

Extract and Wash DNA

- 8 Snap bottom back on to plate and shake in tissue homogenizer for 00:00:30 on highest seed.

Note: Shake plate without lid so that casing of the homogenizer holds caps in place.
Distribute samples evenly across plate so that samples are ground evenly.

- 9 When tissue is ground to a fine powder centrifuge briefly to remove powder from the inside of caps.

- 9.1 Open tubes and discard caps.

- 10 Add 350 μ L pre-heated Q&D extraction buffer with detergent to each sample.

- 10.1 Add 150 μ L 1 M ammonium acetate to each sample.

- 10.2 Cover with clean caps and invert gently to mix.

- 11 Incubate samples at 60 °C for 00:15:00 to 01:00:00 .

Note: To prevent caps from popping off while incubating, place inverted plate bottom over caps and hold down with a weight.

- 11.1 Invert samples gently every 00:05:00 .

Note: Caps will likely be loose, close tubes completely before inverting.

- 12 Centrifuge 3700 rpm, 22°C, 00:20:00 to pellet tissue.
- 13 Near the end of the spin, transfer 200 µL per sample ice cold isopropanol to a new plate.
- 14 Transfer 300 µL , or as much as you can, of supernatant from each sample to isopropanol plate.
- 15 Recap tubes and invert several times to mix.
- 15.1 Incubate at room temperature for 00:05:00 to 00:15:00
- 16 Centrifuge 3700 rpm, 4°C, 00:20:00 to pellet DNA.
- 17 Slowly pour off supernatant without disturbing pelleted DNA.
- 18 Add 500 µL ice cold 70% ethanol to each sample.

18.1 Cover with clean caps and invert gently to mix.

19 Centrifuge 3700 rpm, 4°C, 00:10:00 to wash pellet.

20 Slowly pour off supernatant without disturbing pelleted DNA.

21 Add 500 µL ice cold 95% ethanol to each sample.

21.1 Cover with clean caps and invert gently to mix.

22 Centrifuge 3700 rpm, 4°C, 00:05:00 to wash pellet.

23 Slowly pour off supernatant without disturbing pelleted DNA.

Dry and Re-suspend DNA

24 Place the flat on a paper towel and cover the open tubes with a KimWipe.

24.1 Allow pellets to dry overnight.

25 Once pellet is dry, and no residual liquid remains in the tubes, re-suspend pellets with 20 µL - 100 µL of TE buffer or nanopure H₂O depending on pellet size, desired concentration, and future uses.