Quick & Dirty DNA Extraction

Sam Mantel¹, Andrea Sweigart¹
¹University of Georgia

ABSTRACT
Modified from Cheung et al., 1993

EXTERNAL LINK
https://genome.cshlp.org/content/3/1/69.long

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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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MATERIALS TEXT

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

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 1M Tris base to bottle.

1.2 Transfer 56 mL 0.5M 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.

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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 µl pre-heated Q&D extraction buffer with detergent to each sample.

10.1 Add 150 µl 1 M ammonium acetate to each sample.

Cover with clean caps and invert gently to mix.
10.2

11 Incubate samples at \(60 \, ^\circ C\) 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 \(3700 \, \text{rpm, } 22^\circ C, 00:20:00\) to pellet tissue.

13 Near the end of the spin, transfer \(200 \, \mu l\) per sample ice cold isopropanol to a new plate.

14 Transfer \(300 \, \mu l\), 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 \(3700 \, \text{rpm, } 4^\circ C, 00:20:00\) to pellet DNA.

17 Slowly pour off supernatant without disturbing pelleted DNA.

18 Add \(500 \, \mu l\) ice cold 70% ethanol to each sample.

18.1 Cover with clean caps and invert gently to mix.

19 Centrifuge \(3700 \, \text{rpm, } 4^\circ C, 00:10:00\) 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.