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Quick & Dirty DNA Extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.baf7ibrn

Mimulus

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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

MATERIALS TEXT

Liquid nitrogen

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

- Nanopure H_2O
- 1M Tris base pH **8.0**
- 0.5M EDTA pH **8.0**
- NaCl
- Sodium Metabisulfite ($NA_2S_2O_5$)
- 5% sarkosyl solution ([M] **50 g/L** *N*-lauroyl-sarcosine, sodium salt)

[M] **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 H_2O/TE

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

1 Add roughly **200 ml** nanopure H_2O to a **1 L** glass bottle with a stir bar and stir.

1.1 Transfer **80 ml** [M] **1 M** Tris base to bottle.

1.2 Transfer **56 ml** [M] **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 H_2O 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 a detergent and will foam if poured to 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.

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 H_2O depending on pellet size, desired concentration, and future uses.