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Quick & Dirty DNA Extraction ↗

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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 H₂O

1M Tris base pH8.0

0.5M EDTA pH8.0

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 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 speed.
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.

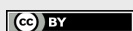
23 Slowly pour off supernatant without disturbing pelleted DNA.

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 H₂O depending on pellet size, desired concentration, and future uses.



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