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Quick & Dirty DNA Extraction

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Sam Mantel[1], Andrea Sweigart[1]

[1]University of Georgia

Mimulus

Sam Mantel

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

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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 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

Troubleshooting

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 [M] 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.