Mar 07, 2019

Working

Leaf Punch DNA Extraction

Version 2

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dx.doi.org/10.17504/protocols.io.yxufxnw

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ABSTRACT

This is a quick (and very cheap) genomic DNA extraction protocol for fresh leaf or seed tissue in many plant species. It should be use preferably for extractions that do not require exceptional lengths of DNA, and is suitable for routine PCR, sanger sequencing, and restriction digests. The protocol is modified from Edwards *et al. Nucleic Acids Research* 19(6): 1349, and has been adapted since then by Zach King, Jonathan Serrano, and Cecilia McGregor. Typical yeilds of DNA are ~200ng/µL.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Edwards *et al. Nucleic Acids Research* 19(6): 1349

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Ethylenediaminetetraacetic acid disodium salt dihydrate	E4884	Sigma Aldrich

SAFETY WARNINGS

All reagents listed here can be disposed of in the sink.

BEFORE STARTING

Collect and freeze tissue before starting, and make up the stock solutions in advance.

Preparation

1 Collect young leaf (the size of a penny) into a 2 mL Eppendorf tube. Store tubes at -80°C until extraction.

Solution Preparation

2 Make 500mL of a 1M Tris solution by dissolving 78.8g of Tris-HCl into 500mL of DI water, adjusting the pH to 8 with HCl, and autoclaving.

78.8 g Tris-HCl

20 ml conc. HCl

500 ml DI water

This recipe has approximate volumes for HCl and water. Also, confirm that you are using Tris-HCl and not Tris-Base. Either is fine, but substitutions will change the mass of Tris needed and also alter the starting pH of the solution.

3 Make a 5M NaCl solution by dissolving 146g of NaCl in ~400mL of DI water, bringing to 500mL, and autoclaving.

146 g NaCL

500 ml DI water

4 Make a 0.5M EDTA solution by dissolving 93.05g of EDTA in ~400mL of DI water. Adjust the pH to 8 by adding ~20g of NaOH. Bring to 500mL and autoclave.

Ethylenediaminetetraacetic acid disodium salt dihydrate
by Sigma Aldrich
Catalog #: E4884

93.05 g EDTA

20 g NaOH

500 ml DI water

The disodium salt of EDTA will not dissolve until the pH is near 8.

5 Make a 10% SDS solution by dissolving 50g of sodium dodecyl sulphate in ~400mL of DI water. Heat it to 65ºC to dissolve, and then bring to 500mL. There is no need to autoclave this solution.

50 g SDS

500 ml DI water

6 Prepare the Edward's Buffer by adding the following compenents, bringing to 1L with DI water, and then autoclaving.

200 ml 1M Tris, pH 8

50 ml 5M NaCl

50 ml 0.5M EDTA

50 ml 10% SDS

650 ml DI water

Grind Tissue

7 Snap freeze the tubes in a dewar of liquid nitrogen (LN_2).

8 Freeze a plastic tube wrack by pouring LN_2 into it until the LN_2 no longer evaporates.

9 Quickly remove an Eppendorf tube with the leaf tissue from the dewar, warm the tube hinge with your fingers to prevent it from breaking, open the tube, and place it in one of the LN_2-containing wells. Make sure that no LN_2 gets into the tube. Using a plastic pestle, grind the tissue to a fine powder, transferring the tube to a new well every ~10 seconds to prevent it from thawing. Close the tube, return it to the dewar, and process the next sample.

Lysis

10 Prepare 750µL of 60% Edwards buffer and 40% 5M NaCl for each tube.

450 µl Edward's Buffer

300 µl 5M NaCl

11 Add 750µL of this Edwards/NaCl mixture to each tube, vortex, and place at 60ºC for 30 minutes, inverting 2-3 times during the incubation to mix.

750 µl Edwards/NaCl Solution

00:30:00 at 60ºC with occasional inversion

12 Centrifuge the tubes for 5 min at 13,000 rpm. During this spin, proceed to the next step.

00:05:00 spin at 13,000rpm

Precipitation

13 While the tube are spinning, add 1mL of 100% ethanol into a new tube. This is the tube you will store the samples in. Clearly label it with your initials, the sample ID and date on side, and the sample ID on the lid.

1 ml 100% ethanol

14 Once the tubes are done spinning, transfer 500µL of the supernatant into the ethanol tube, and gently invert several times to mix.

During the transfer to not disturb the pellet at the bottom of the tube.

During the inversion, you may see your DNA precipitate as a white-ish, wispy cloud that that looks like snot. This is good.

15 Optional, allow the tubes to sit at -20ºC for at least 15 minutes. This step can go overnight but is normally a good place to break for lunch.

00:15:00 optional incubation at -20ºC

16 Centrifuge for 5 minutes at 13,000 rpm.

00:05:00 spin at 13,000 rpm

17 Carefully pour-off the ethanol. Turn the tube upside down for no more than 2 seconds, as pellets can be slippery, and blot with paper towels.

Wash

18 Add 300µL of 70% ethanol and centrifuge for 5' @ 13,000 rpm.

300 µl 70% EtOH

00:05:00 spin at 13,000 rpm

There is no need to resuspend the pellet.

The extraction can pause here, just put the tubes at 4ºC.

19 Carefully pour-off ethanol. Turn the tubes upside down and blot as above (speeds drying of EtOH greatly).

20 Allow the plate to sit ~10 minutes at 60ºC in to evaporate ethanol.

Check the tubes to make sure all ethanol has evaporated by visual inspection or wafting before you proceed to the next step. You should not see or smell any ethanol. Pellets are clearly visible.

Resuspend

21 Resuspend the pellet in 200 µl of 1x TE buffer (or water). Rotate plate upside down gently several times to mix.

Optional, put tubes on a shaker at 200rpm for ~30 minutes to resuspend.

We have noticed that this method tends to co-extract RNA, so spec/nanodrop concentrations of DNA might be artificially inflated by RNA. Addition of RNase A helps with this, but this extraction does not seem to effectively remove RNase.

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