Leaf Punch DNA Extraction

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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.

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Before start

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

Protocol

Preparation

Step 1.

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

Solution Preparation

Step 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.

AMOUNT
78.8 g : Tris-HCl
AMOUNT
20 ml : conc. HCl
AMOUNT
500 ml : DI water

NOTES

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.

Solution Preparation

Step 3.

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

AMOUNT
146 g : NaCL
AMOUNT
500 ml : DI water

Solution Preparation

Step 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.

AMOUNT
93.05 g : EDTA
AMOUNT
20 g : NaOH
AMOUNT
500 ml : DI water

REAGENTS
Ethylenediaminetetraacetic acid disodium salt dihydrate E4884 by Sigma Aldrich

NOTES

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

Solution Preparation

Step 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.

AMOUNT
50 g : SDS
AMOUNT
500 ml : DI water

Solution Preparation

Step 6.

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

AMOUNT

200 ml : 1M Tris, pH 8
AMOUNT
50 ml : 5M NaCl
AMOUNT
50 ml : 0.5M EDTA
AMOUNT
50 ml : 10% SDS
AMOUNT
650 ml : DI water

Grind Tissue

Step 7.

Snap freeze the tubes in a dewar of liquid nitrogen (LN2).

Grind Tissue

Step 8.

Freeze a plastic tube wrack by pouring LN2 into it until the LN2 no longer evaporates.

Grind Tissue

Step 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 LN2-containing wells. Make sure that no LN2 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

Step 10.

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

AMOUNT
450 µl : Edward's Buffer
AMOUNT
300 µl : 5M NaCl

Lysis

Step 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.

AMOUNT
750 µl : Edwards/NaCl Solution
DURATION
00:30:00 : at 60ºC with occasional inversion

Lysis

Step 12.

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

DURATION
00:05:00 : spin at 13,000rpm

Precipitation

Step 13.

While the tube are spinning, add 500µL of 100% isopropanol 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.

AMOUNT
500 µl : isopropanol

Precipitation

Step 14.

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

NOTES

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.

Precipitation

Step 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.

DURATION
00:15:00 : optional incubation at -20ºC

Precipitation

Step 16.

Centrifuge for 5 minutes at 13,000 rpm.

DURATION
00:05:00 : spin at 13,000 rpm

Precipitation

Step 17.

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

Wash

Step 18.

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

AMOUNT

300 µl : 70% EtOH

DURATION

00:05:00 : spin at 13,000 rpm

NOTES

There is no need to resuspend the pellet.

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

Wash

Step 19.

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

Wash

Step 20.

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

NOTES

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

Step 21.

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

NOTES

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

Warnings

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