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Rapid Single-Tube Plant DNA Isolation Suitable for PCR

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We use this protocol and it's working

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Abstract

Described is an inexpensive single-tube mini-prep method of plant DNA isolation from small amounts of starting plant tissue. Isolated DNA is suitable for PCR applications such as Marker Assisted Selection or transgene detection. Fresh tissue is first disrupted in STE buffer, centrifuged and then lysed in standard Edwards buffer to release DNA, followed by a single 2-propanol precipitation and then re-suspension in TE buffer. The protocol is relatively quick, requires no hazardous organic solvents, and also minimizes plastic waste and transfer steps. The protocol reduces co-precipitation of PCR inhibitors and is an improvement on several previously published rapid protocols. The protocol has been used successfully on tomato leaf but the protocol should be applicable to other species as well.

Attachments

Background.pdf
130KB

Figures.pdf
444KB

Materials

Edwards Buffer: 200mM Tris-HCl pH 7.5, 250mM NaCl, 25mM EDTA, 0.5% SDS.

STE Buffer: 0.25M sucrose, 0.03M Tris pH 7.5-8.0, 0.05M EDTA.

TE buffer pH 8.0: 10mM Tris-HCl pH 8.0, 1mM EDTA

Procedure

1 Clip ½ tomato cotyledon into a 1.5 mL microcentrifuge tube (or 1-2 leaf punches, or leaf disk clipped with the 1.5 mL microcentrifuge tube top).

2 Add 600 uL STE buffer and grind fully with microcentrifuge tube pestle. No big pieces should be left. Invert gently to mix well after grinding. Inversion can be done for all samples at one time in the tube rack.

3 Spin 1 minute at top speed in microcentrifuge. Gently decant STE buffer to waste without dislodging the pellet. Invert tube on paper towel for 1 minute and tap gently to remove as much STE as possible, again not disturbing the pellet. Some STE will remain in the tube.

4 Resuspend pellet in 400 uL Edwards buffer. Either vortex or drag the bottom of the tube along the microcentrifuge tube rack several times to dislodge the pellet of organelles and cell debris. Make sure there are no big clumps of organelles and debris; vortexing a few times over ~5 minutes might be needed to fully resuspend and lyse the organelles.

5 Add 400 uL 2-propanol. Vortex gently and/or invert multiple times. Incubate room temp 2-5 minutes. Spin in microcentrifuge at top speed for 3 minutes.

6 Gently decant the supernatant to waste. Dry the large pellet, containing cell debris and DNA, by inverting the tube onto a paper towel. Tap gently to help drain. Dry the pellet for 10-15 minutes. A fan, Speed-vac, or 37°C oven could also be used, if available.

7 Resuspend the pellet in 100 uL TE buffer pH 8.0 by vortex or dragging across the tube rack.

8 Spin the tubes in the microcentrifuge for several minutes to help pellet the debris. It may not form a compact pellet.

9 Use 1 uL in a 10 uL PCR. Avoid the cell debris when pipetting.

Notes

10 Notes

1. Volume of STE in step 2 is not crucial, but use a minimum of 600 uL. The idea is to dilute and then remove the soluble inhibitors that will precipitate with DNA and inhibit downstream PCR.
2. 400 uL of Edwards buffer works well, I would not go lower but more should be fine. Scale 2-propanol appropriately in the next step.

3. Heating (50-60^{O}C) for a few minutes might help the Edwards buffer breakup the pellet in step 4. Let cool to room temp before precipitation with 2-propanol.
4. Cell pellet in step 4 can also be resuspended using a sterile wood toothpick by twirling with fingers.
5. If using 91% rubbing alcohol, increase the volume in step 5 to 500 uL. (500uL*91%/900uL= 50.5% final 2-propanol concentration)

Protocol references

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