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Small-scale DNA Extraction Method for Maize and Other Plants

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[Abstract] Corn, or maize, is a major cereal crop and model monocot. Two of its benefits are ease of pollination and tractable genetics. For a comparison of development between maize and the dicot model, *Arabidopsis*, see Lunde and Hake, 2005. For an example of how this protocol might be used for a double mutant analysis see Lunde and Hake, 2009. Here, we provide a protocol for easy DNA extraction without phenol or chloroform. Therefore, this protocol is suitable for use in schools or laboratories that lack fume hoods.

Keywords: DNA extraction, Corn, Maize, Classroom, Plant Biology

Materials and Reagents

1. 3.2 mm chrome beads (Bio Spec Products, catalog number: 11079132c)
2. Microfuge 1.5 ml tubes (such as USA Scientific, catalog number: 1615-5500)
3. Pipette tip
4. Ice
5. Tris-HCl (CAS 1185-53-1)
6. EDTA (CAS 6381-92-6)
7. Sodium chloride (NaCl) (CAS 7647-14-5)
8. Potassium acetate (KAC) (CAS 127-08-2)
9. SDS (CAS 151-21-3)
10. ddH_2O
11. Isopropanol
12. 70% ethanol
13. RNase (such as Sigma-Aldrich, catalog number: R6513)
14. Extraction buffer (see Recipes)
15. 5 M potassium acetate (KAC) (see Recipes)
16. TE with 1% RNase (see Recipes)

Equipment

1. Ball Mill (such as Retsch, model: Mixer Mill MM 301)
2. QIAGEN adapter (QIAGEN, catalog number: 69982)
3. Microcentrifuge (such as Eppendorf, model: 5417 C)
4. Pipette (such as Eppendorf, model: Research® plus, series in 100-1,000 µl volume)
5. Vortex (such as Scientific Industries, model: Vortex-Genie 2)
6. Water bath (such as Fisher Scientific, model: Isotemp 2025)
7. pH meter (such as Thermo Fisher Scientific, Thermo Scientific™, model: Orion Star™ A111)
8. Standard refrigerator (Such as Fisher Isotemp series)
9. Spectrophotometer (such as Thermo Fisher Scientific, Thermo Scientific™, model: NanoDrop™ 1000, catalog number: ND-1000)
10. Optional equipment
    a. Hand drill (such as DeWalt, model: D21009)
    b. Blue plastic pestle (such as DWK Life Sciences, KIMBLE, catalog number: 749521-1500)

Procedure

1. Load 3.2 mm chrome beads to each tube using the small spoon provided with the beads or your method of choice. Use 4 beads/tube for leaf tissue of grass seedlings less than 4 weeks after germination and 5 beads for tissue of mature leaves of any age as long as they are not senescent. You may collect tissue in advance and store it at -20 °C or -80 °C for at least a year prior to prepping.
2. Collect ~2.0 cm of leaf tip tissue into each tube and add 500 µl of extraction buffer. Less is more! The tissue will grind better if the leaf is no longer than the length of the tube (Figure 1). The maximum number of samples for one run is 48 samples.

Figure 1. Tube containing appropriate amount of maize leaf tip tissue

3. Load tubes into the QIAGEN adapter. Be careful to put the lid on the loose bumpers (Figure 2A) on the bottom and shut each tube completely. The lid with the fixed bumpers goes on top (Figure 2B). Make sure that the microfuge tube lids are not overlapping (Figure 2C). Otherwise, the adapter will not fit into the mixer mill.

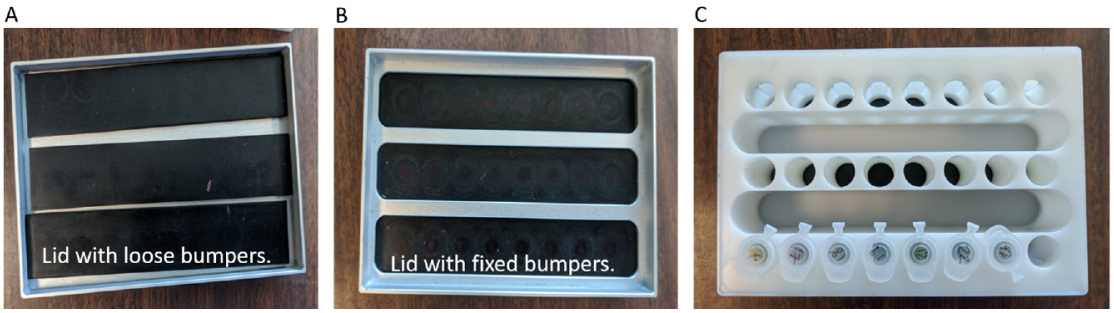

Figure 2. Parts of the QIAGEN adapter. A. The lid with loose bumpers; B. The lid with fixed bumpers; C. Tubes loaded into the center, rack portion of the adapter. Note that lids are not overlapping.

4. Follow the operating instructions for your ball mill. Turn the Retsch Mixer mill on using the toggle power switch in the back. Set the frequency to 30 sec and the time to 2.5 min for tissue of plants older than 4 weeks after germination or previously frozen tissue. Run for 1.5 min for tissue of plants less than 4 weeks after germination.
5. Carefully insert the adapters and tighten but do not overtighten. Make sure each circular section is flush. Do not run only one adapter, it's too much wear on the empty clamp. Run the samples. If samples are not ground, remove adapters, and rotate them 180°, and run for another 2.5 min.
6. Add 130 µl of ice-cold KAC and invert several times.
7. Centrifuge for 15 min at 15,000 *x g*. Do not spin at > 16,100 *x g* or the beads will break the microfuge tubes.
8. Pipet 450 µl of supernatant (doing your best to avoid any loose tissue) (Figure 3) to a new tube and add 350 µl of isopropanol. Mix by inverting.

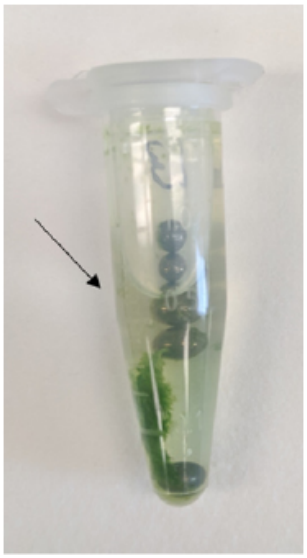

Figure 3. Tube post-grinding and post-centrifuging. After sufficient grinding the tissue is pelleted leaving the clear-to-yellow-green supernatant, containing your DNA, for transfer to a new tube.

9. Centrifuge for 10 min at 16,000 *x g* and discard supernatant without losing the pellet (Figure 4).

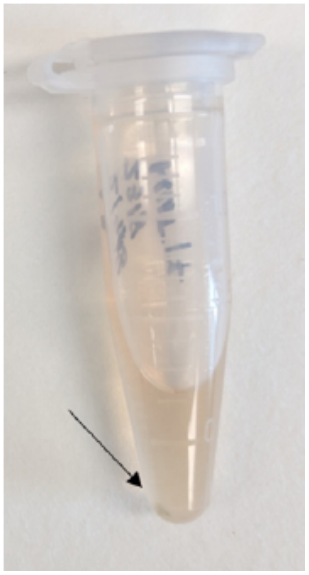

Figure 4. Tube after centrifuging transferred supernatant and isopropanol. The pellet may be white, grey, black green or invisible.

10. Add 100 µl of 70% EtOH, vortex briefly. Spin at 16,000 *x g* for 5 min. Invert to dry until no drops of ethanol are visible. If the pellet slides, push it back to the bottom of the tube with a pipette tip. It is fine to leave the pellets overnight.
11. Add 100 µl of TE with 1% RNase to the tube containing the pellet. To resuspend, leave at 4 °C overnight, flick to resuspend and spin at 16,000 *x g* for 3 min before use. Use 0.5-1 µl in a 20 µl

PCR reaction. Concentration can vary (generally between 100-400 ng/ul) so check a few using a spectrophotometer if you have problems with amplification–you can make a 50-100 ng/µl dilution using ddH_2O for each. For very dilute samples, increase the amount of DNA in your PCR reaction. If you want to perform PCR immediately after DNA extractions, incubate in a water bath at 55-60 °C for 10 min, flick to resuspend and spin at 16,000 *x g* for 3 min before use.

Data analysis

When performing PCR, include appropriate positive (*e.g.*, a DNA sample and primer set that has worked previously) and negative (*e.g.*, a sample with no template) controls.

Notes

1. If a mixer mill is not available, tissue homogenization can be performed using a plastic pestle in place of a bit in a standard, handheld drill (see optional equipment).
2. We have stored tissue in extraction buffer for a year and still extracted DNA suitable for PCR.
3. DNA extracted using this method and stored at -20 °C has amplified reliably 10 years later.
4. This protocol can be used for DNA extraction from *Brachypodium* or *Arabidopsis* as well.
5. This protocol has also been used successfully with 1 cm of maize roots collected 2 weeks after germination as well as maize embryos excised from kernels imbibed overnight in ddH_2O.

Recipes

1. Extraction buffer (1 L)
   100 mM Tris-HCl, adjusted to pH 8.0
   50 mM EDTA
   100 mM NaCl
   0.35% SDS
   in 1 L of ddH_2O
2. 5 M potassium acetate (KAC)
   5 M KAC in ddH_2O, precool on ice for 20 minutes before use or store in -20 °C freezer
3. TE with 1% RNase
   Add 1% RNase (v/v; from a 10 mg/ml stock) to TE buffer

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References

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