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Low-cost tissue collection and genomic DNA extraction for plants

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Low-cost, high-quality ...



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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol describes low-cost methods for the collection and desiccation of plant tissues and subsequent extraction of genomic DNA. It contains modifications of previously described methods found at <https://link.springer.com/book/10.1007/978-3-319-16259-1> and https://link.springer.com/chapter/10.1007/978-3-319-45021-6_14. We have tested this protocol with leaf tissue from flax, *Lupinus luteus*, *Camelina sativa* and *Berberis darwinii*. DNA is suitable for routine molecular biology assays such as PCR and also for next generation sequencing.

Materials

MATERIALS

⊗ Guanidine Thiocyanate **Fisher Scientific Catalog #BP221**

⊗ Sodium Acetate **VWR International (Avantor) Catalog #AA11554-30**

⊗ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

⊗ EDTA **Thermo Fisher Catalog #17892**

⊗ Tris **Thermo Fisher Catalog #17926**

⊗ Silica gel orange **Merck MilliporeSigma (Sigma-Aldrich) Catalog #10087-1KG-R**

⊗ Tissue grinding kit **Bertin Instruments Catalog #CKmix50_7ml**

⊗ Silicagel 60G **Merck Millipore (EMD Millipore) Catalog #107731**

⊗ Celite 545-AW reagent grade **Merck MilliporeSigma (Sigma-Aldrich) Catalog #20199-U**

Prepare the following solutions in advance of DNA extraction:

IMJ 5 Mass Percent NaCl

LYSIS BUFFER IMJ 0.5 Mass Percent SDS IMJ 100 millimolar (mM) TRIS, pH 7.5 IMJ 10 millimolar (mM) EDTA

(0.5% SDS, 10 mM EDTA) add 2.5 ug/ml RNaseA immediately prior to use.

IMJ 3 Mass Percent Sodium Acetate pH 5.2

IMJ 6 Mass Percent Guanidine Thiocyanate

BINDING BUFFER IMJ 6 Mass Percent Guanidine thiocyanate IMJ 67 % volume Ethanol

WASH BUFFER IMJ 50 millimolar (mM) NaCl IMJ 95 % volume Ethanol (dissolve NaCl in water first, then add Ethanol)

SILICA slurry - suspend silica powder (Celite 545 or Silicagel 60G) in an excess (at least an equal volume) of deionized water, centrifuge or let settle, pour off water. Repeat this 3 times, then finally resuspend in equal volume of clean deionized water. This slurry can be used for several months, but should be rewashed 2-3 times prior to use.

Safety warnings

⚠ Standard laboratory safety practices, including wearing a lab coat, disposable gloves and eye protection, should be followed. Please consult the Materials Safety Data Sheets (MSDS) for chemicals used in this protocol. The protocol can be carried out without toxic chemicals. However, we favor the use of guanidine thiocyanate as a chaotropic buffer for DNA binding which is harmful if swallowed, causes serious eye irritation and skin irritation (<https://www.fishersci.com/store/msds?partNumber=BP221250&productDescription=GUANIDINE+THIOCYANATE+250GR&vendorId=VN00033897&countryCode=US&language=en>).

Before start

Read the protocol carefully and prepare solutions in advance of starting the procedure.



Collection and desiccation of plant tissues

2d

- 1 Collect plant tissue and place in a sealed container with silica gel orange, or equivalent. For larger samples, collect tissue in 50 ml falcon tubes. For *Berberis darwinii* leaves, which are tough and spiny, approximately 30 leaves were placed in a 50 ml falcon tube, which was filled with silica. It is best to initially fill tubes partially, then shake to distribute the leaves in the silica. Then fill the remaining space with additional silica. For *Berberis*, tissue was incubated in silica at least 4 days  96:00:00  Room temperature .

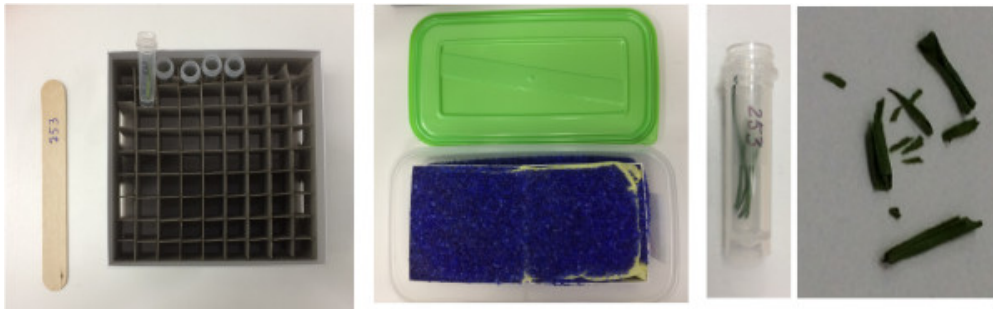
2d





Berberis leaves collected in 50 ml tube.

- 0.2 For smaller amounts of tissue, especially small soft leaves, one can place tissue in eppendorf tubes, and either fill tubes with silica and cap, or place open tubes in a box set inside a sealable container, cover with a breathable material such as cheesecloth or disposable cleaning cloth, then layer the silica gel over the cloth (as pictured below - this avoids the time consuming task of removing silica from tubes). Soft tissue is generally dry after a 2 day incubation 48:00:00 Room temperature .



Tissue dessication in 2 ml tubes. To facilitate field collection, wooden stakes and tubes are labeled with plant numbers prior to collection. Tissue is collected into two boxes (approx 200 samples) before applying silica (approx. 1-2 hours). Tube boxes are placed in a plastic container then covered with a porous material such as a paper towel and covered in silica gel and then sealed (second panel, silica gel with blue indicator is used in this example). Tissue shrinks when dry (third panel) and is brittle when fully desiccated (fourth panel).

- 0.3 Check tissue before grinding. Tissue should be brittle and break into pieces when pinched with fingers. If tissue is soft and bends, incubate with silica gel for a longer period of time. Note that silica gel orange and similar silica gels contain a color indicator. If silica is saturated with water and color changes, replace with fresh silica. Silica gel can be dried and re-used. Tissue can be stored for many months to indefinitely at room temperature as long as silica is not saturated with water.

Grinding and lysis of dry tissue.

20m

- 1 Grind dry tissue to a fine powder.

20m



- 1.1 For Berberis, grinding was performed on a Precellys Evolution tissue homogenizer (Bertin instruments, Montigny-le-Bretonneux, France) using modified Precellys lysing kit "Tissue grinding CKmix50_7ml." Approximately 100 mg of dry tissue (7 leaves) was placed in a 7 ml screw cap tube with 6, 2.8 mm and 2, 5.0 mm ceramic beads. Tissue was ground with 3,  00:00:20 pulses at 4500 rpm, which produced a fine powder. 1m
- 1.2 To grind softer tissue in 2 ml screw cap tubes (for example *L. luteus*, *Camelina sativa*, or flax), use 3, 2.8 mm ceramic beads and grind once for 20s at 4500 rpm. 20s
- 1.3 Alternative grinding methods can be used, including manual grinding with a mortar and pestle, or in-tube grinding with sand, glass, or metal beads on a common lab vortexer (for example, see figure 4.1of https://link.springer.com/chapter/10.1007/978-3-319-16259-1_4). 17m
- 2 Lysis. Add RNase to LYSIS BUFFER (0.5% SDS, 10 mM EDTA, 2.5 µg/ml RNase). 2m
- 2.1 If grinding and lysis is performed in a 7 ml tube, as with Berberis, add  2.4 mL LYSIS buffer and shake or vortex tubes to completely suspend tissue. Shake tubes  00:02:00 on a multi-vortexer, or by hand. 2m
- 2.2 If grinding/lysis is performed in 2 ml tubes, add  800 µL LYSIS buffer . Shake tubes  00:02:00 on a multi-vortexer, or by hand. 2m
- 2.3 Incubate tubes  00:10:00  Room temperature . 10m
- 2.4 Add  600 µL 3M Na Acetate if lysing in 7 ml tubes, or  200 µL 3M Na Acetate if lysing in 2 ml tubes, mix well. Incubate  00:05:00  On ice 5m
- 2.5 If grinding and lysis is performed in a 7 ml tube, distribute lysate slurry into 3 eppendorf tubes. 1h 5m

Binding DNA

- 3 Clear lysate and bind DNA to silica matrix. 1h 5m



3.1 Prepare tubes with binding buffer and silica matrix. Ideally you can do this in advance, or during incubation steps above. Combine  50 μ L SILICA slurry

 700 μ L BINDING buffer (2 M guanidine thiocyanate in 67% EtOH - final concentrations) in a 2 ml eppendorf tube. Prepare one tube for each 2 ml lysis tube, or 3 for each 7 ml lysis tube.

3.2  13000 rpm, Room temperature, 00:03:00 Clear lysate by centrifuging tubes from step 3 for 3 minutes at max speed in a microcentrifuge.

3m

3.3 Transfer lysate (~ 800 ul) to tubes containing BINDING BUFFER and SILICA (prepared in step 4.1). Flick or vortex tubes to resuspend silica. Incubate tubes  00:45:00 to

1h

 01:30:00 at  4 $^{\circ}$ C , then  00:15:00 shaking on a multivortexer at

 Room temperature .

Wash and elute DNA

4 Wash DNA bound silica pellets.

Centrifuge  13000 rpm, Room temperature, 00:03:00 Discard supernatent.

4.1 Add  500 μ L WASH buffer to tubes, flick or vortex to resuspend silica. Centrifuge

 13000 rpm, Room temperature, 00:03:00 Discard supernatent. Repeat two more times for a total of three washes. After final wash, invert tubes briefly on paper towels and tap gently to remove as much liquid as possible. Then leave tubes open on their sides to dry pellets completely, approximately  01:30:00  Room temperature .

5 Elute DNA in TE with 12.5 ug/ml RNase A.

Add  100 μ L TE + RNase to the pellet, flick to resuspend. Incubate tubes

 00:15:00  Room temperature shaking at low speed.

5.1  13000 rpm, Room temperature, 00:03:00 Pipette supernatent into a fresh 1.5 ml eppendorf tube.



5.2  [go to step #5](#) For maximum yield, repeat elution. Combine the second eluant with the first.

5.3 To ensure complete removal of RNA, incubate DNA  Overnight  4 °C . DNA can then be concentrated by EtOH or PEG precipitation. We recommend PEG precipitation as it can help remove any protein impurities. We use a final concentration of 13.3% PEG 6000 and 10 mM MgCl₂, with a  00:10:00 incubation at  Room temperature followed by a  13000 rpm, 00:10:00 centrifugation.

Note

DNA stored in TE buffer can be stable for many years at four degrees Celsius. It is important to test the quality and quantity of your genomic DNA and the stability of the DNA in the chosen storage conditions. For an example of how to do this, please refer to our protocols.io protocol describing quantification of DNA using a home-built low-cost gel documentation system.