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Low-cost tissue collection and genomic DNA extraction for plants

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1 Works for me dx.doi.org/10.17504/protocols.io.bdg9i3z6

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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.

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KEYWORDS

tissue desiccation, low-cost DNA extraction, silica binding matrix

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MATERIALS

NAME	CATALOG #	VENDOR
Guanidine Thiocyanate	BP221	Fisher Scientific
Sodium Acetate	AA11554-30	Vwr
NaCl	53014	Sigma Aldrich
EDTA	17892	Thermo Fisher
Tris	17926	Thermo Fisher
Silica gel orange	10087-1KG-R	Sigma Aldrich
Tissue grinding kit	CKmix50_7ml	Bertin Instruments

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NAME	CATALOG #	VENDOR
Silicagel 60G	107731	Merck Millipore
Celite 545-AW reagent grade	20199-U	Sigma

MATERIALS TEXT

Prepare the following solutions in advance of DNA extraction:

[M]5 Molarity (M) NaCl

LYSIS BUFFER **[M]0.5 Mass Percent SDS** **[M]100 Milimolar (mM) TRIS, pH 7.5**

[M]10 Milimolar (mM) EDTA (0.5% SDS, 10 mM EDTA) add 2.5 ug/ml RNAseA immediately prior to use.

[M]3 Molarity (M) Sodium Acetate **pH5.2**

[M]6 Molarity (M) Guanidine Thiocyanate

BINDING BUFFER **[M]6 Molarity (M) Guanidine thiocyanate** **[M]67 % volume Ethanol**

WASH BUFFER **[M]50 Milimolar (mM) NaCl** **[M]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 STARTING

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** 2d

Room temperature .

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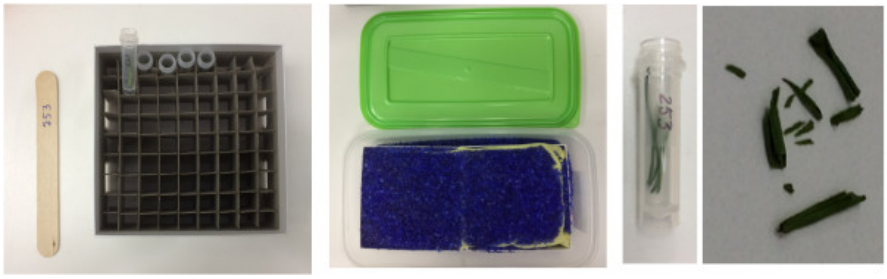
Berberis leaves collected in 50 ml tube.

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

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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).

2 Lysis. Add RNase to LYSIS BUFFER (0.5% SDS, 10 mM EDTA, 2.5 µg/ml RNase). 17m

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.

Binding DNA 1h 5m

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 µl SILICA slurry** **700 µl 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 **01:30:00** at **4 °C** , 1h

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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 µl 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 µl 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.

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.

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