EchoLUTION Plant DNA Kit – Protocols

for single-step purification of genomic DNA from plant tissue

This protocol has been developed for plant leaves and root tissues.

Materials and equipment needed

- 10-100 mg plant tissue per sample depending on species. 50 mg generically recommended, species-dependent adjustment of input amounts may be needed. Ultra-sharp ceramic scalpels (various blade shapes) for cutting plant samples can be ordered from BioEcho.
- Depending on preferred method of mechanical disruption: Pestle (disposable pestles can be ordered from BioEcho) or beads for bead-beating.
- Microcentrifuge with rotor for 1.5 and 2 ml reaction tubes. If available, switch to *relative centrifugal force* (rcf)*.
- For fastest performance: Thermomixer, capable of heating to 60°C and 80°C with agitation; pre-heated to 60 °C. Alternatively, use a heating block pre-heated to 60°C.
- Vortexer
- One reaction tube (1.5 ml) per sample for the lysis step (preferably safe-lock).
- One reaction tube (2 ml) per sample for column preparation.
- One reaction tube (1.5 ml) per sample for elution and collection of the purified DNA.
- Pipets for 10 µl and 200 µl scale, corresponding pipet tips.
- For fastest procedure (PROTOCOL1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Heat the thermomixer or thermo block to 60°C.
- Set the microcentrifuge to 750 x g.*

BioEcho cap puncher

PROTOCOL 1: Purification using the Cap Puncher

Lysis (including mechanical disruption using a pestle)

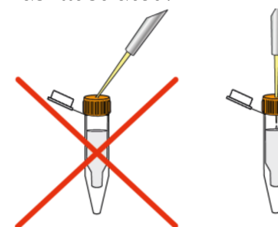
1. For each sample, transfer 10-100 mg (50 mg recommended) of plant tissue and 100 µl Plant Lysis Buffer (PL) to a 1.5 ml reaction tube (preferably safe-lock).
2. Resuspend the Grinding Suspension (GS) by short vortexing and add 10 µl to the plant tissue.
   Note: Don't add (GS) if you perform mechanical disruption by bead-beating!
3. Grind plant tissue with a pestle (disposable pestles can be ordered from BioEcho).
4. Add 2 µl RNAse A (R) to each sample and vortex briefly.
5. Add 5 µl TurboLyse P Protease (TLP) to each sample.
6. Place the tube in the thermomixer and incubate at 60°C for 30 min with agitation at full speed. Alternatively, incubate on a heating block for 60 min and pulse-vortex 3 times during lysis.
   Meanwhile, proceed with "Column preparation" **(step 8)**.
7. Increase the temperature to 80°C and incubate for additional 10 min with agitation at full speed.

Column preparation (to be performed during Lysis)

8. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for 10-20 min.
9. Use of the cap puncher (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2 ml collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting clockwise.
   Place the punched spin column back into the 2 ml reaction tube.
10. Centrifuge for 1 minute at 750 x *g**. Discard the 2 ml reaction tube containing the column buffer.
11. Place the prepared spin column into a new 1.5 ml reaction tube for elution of the sample DNA and place back into in the rack.
    Continue with "Purification" (below).

Purification

12. After having performed step 7, add 25 µl Clearing Solution (CS) to each sample. Vortex 3 sec. The sample becomes cloudy.
13. Centrifuge for 2 minutes at maximum speed.
14. Transfer 90 – 110 µl supernatant containing the DNA onto the prepared column from step 11 as illustrated:



Insert pipet tip vertically through the hole in the column cap and pipet the sample slowly (~5 sec) into the column.

15. Centrifuge 1 minute at 750 *x g**. The purified genomic DNA (90–100 µl; 10 mM Tris·Cl, pH 7.8) flows through the column into the 1.5 ml elution tube. Discard the spin column.

The eluted DNA can be used immediately or stored at 4°C or for long-term storage at −20°C. For spectrophotometric analysis, use 1x Tris Buffer as blank after 1:10 dilution of the 10x Tris Buffer (T) supplied with the kit.

PROTOCOL 2: Purification without a Cap Puncher

Lysis

1. Perform steps 1-7 from PROTOCOL 1.

Column preparation

8. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. Let stand for 10-20 min (recommended during lysis step).
9. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure**. Important: Do not close the screw cap of the spin column. The screw cap must stay loosened **half a turn** to avoid generation of a vacuum. Place the column back into the 2 ml collection tube and both into the centrifuge.
10. Centrifuge for 1 min at 750 x *g**. Discard the 2 ml reaction tube containing the column buffer.
11. Place the prepared spin column into a new 1.5 ml reaction tube for elution of the sample DNA and place back into in the rack.
    Continue with "Purification" (below).

Purification

12. Upon completion of step 7, add 25 µl Clearing Solution (CS) to each sample.
    Vortex 3 sec.
13. Centrifuge for 2 minutes at maximum speed.
14. Transfer supernatant containing the DNA (90-110 µl) onto the prepared column from step 11 as illustrated:

Open cap and pipet the sample slowly (~5 sec) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.
Important: Do not close the screw cap of the spin column tightly!

15. Centrifuge 1 minute at 750 *x g**. The purified DNA (90–100 µl) flows through the column into the 1.5 ml elution tube. Discard the spin column.

Product use limitation

The EchoLUTION Plant DNA Kit is for research use only. It is not registered or authorized to be used for diagnosis, prevention or treatment of a disease.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm corresponding to 750 x g using the formula: rpm = 1000 x $\sqrt{(\frac{750}{1.12\,x\,r})}$, where r = radius of rotor in mm. E.g., with a radius of 150 mm, the corresponding rpm to 750 x g is approx. 2,200 rpm.

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EchoLUTION Plant DNA Kit

for single-step purification of genomic DNA from plant tissues

Product no. (rxn's)	010-003-010 (10)	010-003-050 (50)	010-003-250 (250)
Kit contents	Plant Lysis Buffer, TurboLyse P Protease, RNAse A, Grinding Suspension, Clearing Solution, 10x Tris Buffer, Spin Columns		
Related products	Cap Puncher 050-001-001	Pestles 050-004-100	Scalpels 050-004-00x

Quick PROTOCOL 1

Lysis

- Transfer 50 mg plant tissue to a reaction tube and add 100 µl Plant Lysis Buffer (PL)
- Add 10 µl resuspended Grinding Suspension (GS)
- Grind the tissue with a pestle
- Add 2 µl RNAse A (R), vortex briefly
- Add 5 µl TurboLyse P Protease (TLP), vortex briefly
- Incubate 20 min/60°C → 20 min/80°C, max. agitation

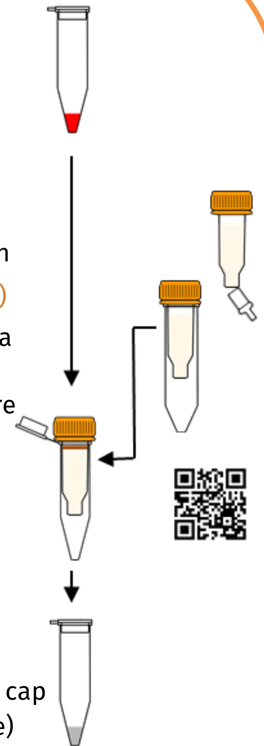
Column preparation (during 60°C and 80°C incubation)

- Homogenize column resin by vortexing and place in a 2 ml tube, let stand for 10-20 min
- Punch a hole in the cap, and break off bottom closure (scan QR code to watch a video)
- Place spin column back into 2 ml tube
- Centrifuge 1 min at 800 *x g** to elute column buffer
- Place column in a 1.5 ml tube for sample loading

Purification of DNA

- Add 25 µl (CS) and vortex shortly
- Centrifuge for 2 minutes at max. speed
- Transfer 90-110 µl lysate by pipetting slowly through cap hole – see PROTOCOL 1 or watch video (scan QR code)
- Centrifuge 1 min at 750 *x g**

Purified DNA is ready to use



BioEcho Life Sciences GmbH
Nattermannallee 1
50829 Köln (Cologne)/Germany

Phone: +49 (0) 221-99 88 97-0
E-Mail: contact@bioecho.de
www.bioecho.de