EchoLUTION Plant DNA Kit – Protocol

for single-step purification of genomic DNA from plant tissue

This protocol has been developed for fresh, freeze-dried and dried plant tissues like leaves, blossoms, fruits, roots, flour and seed samples.

Materials and equipment needed

- **10-50 mg plant tissue** per sample; 30 mg is generically recommended (for certain plant species, optimization of input amount may be required)
  Dry sample types (e. g., freeze-dried, flour, seeds): 10 mg recommended
- Depending on preferred method of mechanical disruption†:
  Tissue Grinding Pestles (BioEcho product no. 050-004-100)
  Bead-beating Steel Beads (BioEcho product no. 050-006-200)†
- **Microcentrifuge** with rotor for 1.5 and 2 ml reaction tubes
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if not possible, use formula below* to calculate the conversion of round per minute (rpm) into rcf
- **Thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **Vortexer**
- **Pipets** for 10 µl and 200 µl scale, corresponding pipet tips
- One reaction tube (1.5 ml) per sample for the lysis step (preferably safe-lock)
- One reaction tube (1.5 ml) per sample for elution and collection of the purified DNA
- One reaction tube (2 ml) per sample for column preparation
- Ceramic Blade Scalpels. BioEcho product no. 050-002-001
- For fastest procedure (PROTOCOL 1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Heat the thermal shaker, thermo block or heat chamber to 60°C
- Set the microcentrifuge to **1,000 x *g****
- Important: ***Switch to relative centrifugal force, rcf* (x *g****, not rpm)

PROTOCOL 1: Purification **using** the Cap Puncher

BioEcho Cap Puncher

Lysis (including mechanical disruption using a pestle†)

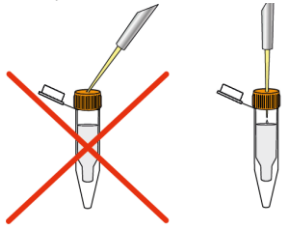
1. Transfer plant sample (30 mg recommended) and **100 µl Plant Lysis Buffer**† (LB) to a 1.5 ml reaction tube (preferably safe-lock).
   Note: If sample type is strongly absorbing liquid (e.g. freeze-dried material, some seeds), the amount of added Lysis Buffer needs to be increased to 200 µl.
2. Resuspend **Grinding Suspension** (GS) by short vortexing and add **10 µl Grinding Suspension**† to each reaction tube containing the plant tissue.
3. Add **2 µl RNase A Plant** (R) to each sample.
4. Homogenize plant tissue with a pestle until completely disrupted.
5. Add **5 µl TurboLyse P Protease** (P) to each sample.
6. Place reaction tube in the thermal shaker and incubate at **60°C** for **30 min** with max. agitation speed (or for **60 min** if agitation is not feasible; in this case, pulse-vortex 3 times during lysis).
   Meanwhile during lysis, proceed with step 8, "Column preparation".
7. After incubation at 60°C, increase temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Column preparation (during steps 6 and 7)

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 clock-wise while pulling out.
   Place the punched spin column back into the 2 ml reaction tube.
10. Centrifuge for **1 min** at **1,000 x *g**.** Discard the flow-through volume ("void volume") collected in the 2 ml reaction tube.
11. Place the prepared **EchoLUTION Spin Column** into a new **1.5 ml** reaction tube for elution of the purified DNA and place back into the rack.
    Continue with "Purification of DNA".

Purification of DNA

12. After having performed step 7, add **25 µl Clearing Solution P** (CS) to each sample. Vortex 3 sec. The sample will become cloudy.
13. Centrifuge for **2 min** at **full speed.**
14. Transfer **lysis supernatant (max. 100 µl)** onto the prepared **EchoLUTION Spin 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.

Note:

- During loading of lysate, do not touch the resin bed with pipette tip!
- Residual sample particles may be loaded and will not interfere with purification.

15. Centrifuge **1 min** at **1,000 *x g**.** The purified genomic DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

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 (T) supplied with the kit as blank.

† Alternative sample disruption by **bead-beating**: Several protocols are feasible. Plant tissues (frozen, lyophilized, fresh) can be beaten either dry or in Plant Lysis Buffer (LB). **DO NOT** add (GS) for bead-beating! If foam has formed during bead-beating, centrifuge for **3 min** at **full speed**. Bead-beating conditions for complete disruption need to be determined experimentally with **3 min** at **30 Hz** being a good starting point.

PROTOCOL 2: Purification **without** a Cap Puncher

Lysis

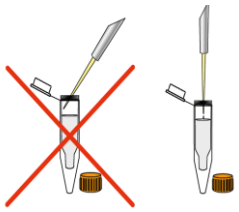
1. Perform steps 1-7 from **PROTOCOL 1**.

Column preparation (during steps 6 and 7)

8. Vortex the **EchoLUTION Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for 10-20 min (recommended to be done during lysis step).
9. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure.**
   Important: Do not re-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 **1,000 x *g**.** Discard the flow-through volume ("void volume") collected in the 2 ml reaction tube.
11. Place the prepared spin column into a new **1.5 ml** reaction tube for elution of the sample DNA and place back into the rack.
    Continue with "Purification of DNA".

Purification of DNA

12. After having performed step 7, add **25 µl Clearing Solution P** (CS) to each sample. Vortex 3 sec. The sample will become cloudy.
13. Centrifuge for **2 min at full speed.**
14. Transfer **lysate supernatant (max. 100 µl)** containing the DNA onto the prepared EchoLUTION Spin 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 re-close the screw cap of the spin column tightly!

Note:

- During loading of lysate, do not touch the resin bed with the pipette tip!
- Residual sample particles may be loaded and will not interfere with purification

15. Centrifuge **1 min** at **1,000 *x g**.** The purified genomic DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

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 matching the g-force using the formula: rpm = 1,000 x $\sqrt{(\frac{g}{1.12 \times r})}$, where r = radius of rotor in mm. and g the required g-force.
E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x g is approx. 2,400 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 Plant, Grinding Suspension, Clearing Solution P, 1x Tris Buffer, Spin Columns		
Related products	Steel Beads 050-006-200 | Ceramic Blade Scalpels 050-002-001 Tissue Grinding Pestles 050-004-100 | Cap Puncher 050-001-001		

Quick PROTOCOL (please, read extended protocol first)

Lysis

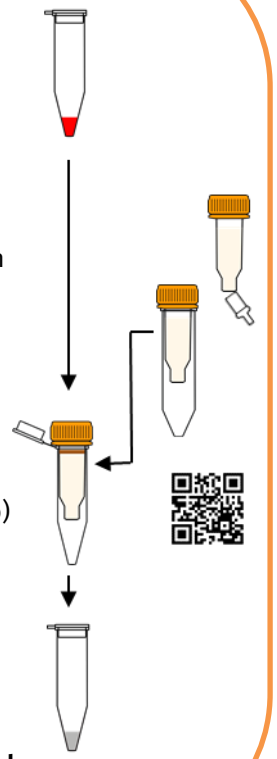
- Transfer **30 mg plant tissue** to reaction tube
- Add **100 µl** (LB)
- Resuspended (GS) by vortexing and add **10 µl**
- Add **2 µl** (R), vortex briefly
- Grind the tissue with a pestle
- Add **5 µl** (P), vortex briefly
- Incubate **30 min/60°C ,** then **10 min/80°C,** max. agitation
- Add **25 µl** (CL) and vortex shortly
- Centrifuge for **2 min** at **max. speed**

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

- Vortex **EchoLUTION column** and place in a **2 ml** tube. Let stand for 10 min
- Punch a hole in the cap using the **Cap Puncher** and break off bottom closure (scan QR code to watch a video)
- Place spin column back into the 2 ml tube
- Centrifuge **1 min** at **1,000 *x g**** to elute column buffer
- Place column in a **1.5 ml** tube

Purification of DNA

- Transfer **lysate supernatant (max. 100 µl)** by pipetting **slowly** through cap hole (scan QR code to watch a video)
- Centrifuge **1 min** at **1,000 *x g**** to elute DNA into **Elution tube**
- Eluted DNA is ready to use



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