EchoLUTION Plant DNA 384 Kit - Protocol

for 384-well plate purification of genomic DNA from **fresh** plant tissue samples

This protocol has been developed for fresh plant tissues like leaves, blossoms, fruits, roots, flour and seed samples. For freeze-dried and dried plant tissues, please use the corresponding protocol also supplied with this kit.

Note:

The **EchoLUTION Plant DNA 384 Kit** lyses the plant tissue in a **96 well** Lysis plate with the same buffer and reagent volumes as for a conventional 96 well purification. The difference is the loading volume of **25 µl** lysate to the **384 well** purification plate (in contrast to 100 µl lysate loading volume to a 96 well purification plate).

Materials and equipment needed

Plant sample: 10 - 30 mg are recommended for fresh plant tissue samples etc. (depending on plant species).

Supplied with the kit:

- **384 Purification Plate:** 384-well plate to be filled with 130 µL of the provided **bulk resin** per well
- **384 Elution Plate:** 384-well plate for the collection of the purified DNA. Make sure the Elution plate is suitable for centrifugation at 1,000 x g
- **Adhesive Foil** for plate sealing during lysis
- **All needed reagents and solutions**

Not supplied with the kit:

- **Lysis Plate:** 96-well plate for the lysis of freeze-dried or dried plant tissue in a 96-well bead-beating device followed by incubation in a 96-well thermo shaker
  Note: The use of Bioecho's Tube & Cap Strips is recommended to prevent sample leakage during beat-Beating. BioEcho product no. 060-002-024
- **Sealing option:** Plastic or silicone mat suitable for sealing the lysis plate during homogenization in the 96-well bead-beating device
- **384 Conditioning Plate**: 384-deep well plate with minimum of 100 µl well volume for the collection of void volume during preparation of **384 Purification Plate**. Reusable!
- **96 (or 384)-well swing-out centrifuge** (preferentially with switch option to rcf (x g*))
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- **96-well plate thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **8-channel pipets** for 200 µl scale
- **Wide Bore Pipette Tips** for 200 µL scale
- **Troughs** for Master Mix preparation holding > 20ml
- **Plate(s) to be used as *tara*** in the centrifuge in case an odd number of plates are processed
- **Bead-beater device** for 96-deep well plates
- **Steel beads**. BioEcho product no. 050-006-200
- **Ceramic blade scalpel** for cutting plant samples. BioEcho product no. 050-002-001

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:

Lysis

1. Transfer plant samples and steel beads in each cavity of the 96-well **Lysis Plate** (if using BioEcho`s steel beads, add one bead for each well)
2. Prepare the **Bead-beating Master Mix** for 96 bead-beating reactions with 20% excess volume in a reagent trough:

Bead-beating Master Mix

No. of samples	1	96 (+20%)	Yours
BB Buffer BB	99	11,400	
R RNAse A Plant (µl)	1	115	
Final volume (µl)	**100**	**11,515**	

3. Add **100 µl of the Bead-beating Master Mix** from step 2 to each well of the **Lysis Plate** containing the plant samples and the steel beads with an 8-channel pipette.
4. Seal **Lysis Plate** with the Sealing option of choice **(e.g. Plastic or Silicone mat)**
5. Place the sealed **Lysis Plate** in the bead-beater and beat **3 min at 30 Hz or** until the plant tissues are completely disrupted.
6. Centrifuge **Lysis Plate** for **1 min at 1,000 x *g*** with the **Sealing mat** attached to collect the lysate at the bottom of the well.
7. Prepare the **Lysis Master Mix** for 96 lysis reactions with 20% excess volume in reagent trough:

Lysis Master Mix

No. of samples	1	96 (+20%)	Yours
LB 96 Plant Lysis Buffer	100	11,520	
P TurboLyse P Protease (µl)	5	580	
Final volume (µl)	**105**	**12,100**	

8. Remove **Sealing mat** and add **105 µl** of the **Lysis Master Mix** from step 7 to each well.
   Note: If sample type is strongly absorbing liquid (e.g. seeds etc.), the amount of added Lysis Buffer needs to be increased to 200µl.
9. Seal **Lysis Plate** tightly with the **Adhesive Foil** (supplied with the kit).
10. Place the sealed **Lysis Plate** 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).
    Meanwhile during lysis, proceed with step 12, "Preparation of Purification Plate"
11. After incubation at 60°C, increase temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Preparation of Purification Plate (during steps 10 and 11)

12. Please follow the instructions in the attached manual "**Instructions for the filling of 384-well purification plates with BioEcho`s purification matrix**" to fill the **384 Purification Plate** with bulk resin.
13. Plate preparation: Place the prepared **384 Purification Plate** on top of a 384-deep well plate ("384 Conditioning Plate", not supplied, minimum well volume of 100 µl) and centrifuge for **1 min** at **1,000 x *g**** to collect the void buffer from the purification resin in the **384 Purification Plate**. Discard the flow-through volume ("void volume") collected in the lower **384 Conditioning Plate** (Conditioning Plate can be re-used).
14. Place the conditioned **384 Purification Plate** on top of the **384 Elution Plate** for elution of the purified DNA. Continue with "Purification of DNA".

Purification of DNA

15. Detach **Adhesive Foil** from the incubated **Lysis Plate** and **add 25 µl Clearing Solution P** (CS) to each well of the **Lysis Plate** and mix by pipetting up & down. The sample will become cloudy.
16. Centrifuge **Lysis Plate** for **3 min** at **full speed**.
17. Transfer the **lysis supernatant (max. 25 µl)** onto the **384 Purification Plate**.

    ### Important loading instructions:

    - Do not touch the cellular debris at the bottom of the well while removing the supernatant to avoid clogging of the pipet tip (preferentially, wide bore tips).
      Residual tissue particles may be loaded and will not interfere with purification.
    - During the loading step, make sure that the 8-channel pipette releases the lysate solution **slowly and vertically**, non-angular onto the middle of the resin surface!
    - Do not punch pipette tip into the resin bed during loading of lysate!
18. After completion of the loading step, centrifuge **384 Purification Plate** on top of an **384 Elution Plate** as "plate sandwich".
19. Centrifuge for **1 min** at **1,000 x *g**.
20. The purified DNA elutes into the **384 Elution Plate** 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.

Product use limitation

The EchoLUTION Plant DNA 384 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 $\sqrt{(\frac{g}{1.12\,x\,r})}$, 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 384 Kit

for 384-well purification of genomic DNA from **fresh** plant tissue samples

Product no. (plates)	010-303-002 (2x 384)	010-303-008 (8x 384)
Kit contents	384 Purification Plate, 384 Elution Plate, Adhesive Foil, Buffer BB, 96 Plant Lysis Buffer, TurboLyse P Protease, RNAse A Plant, Clearing Solution P, 1x Tris Buffer, Tube & Cap Strips (not included in the 8 x 384 Kit), bulk resin	
Related products	384 Conditioning Plate 060-006-008 | Steel beads 050-006-200 | Ceramic Blade Scalpels 050-002-001 |Tube & Cap Strips 060-002-024	

Quick PROTOCOL (please read protocol first)

Bead-beating, Sample Lysis and Clearing

- Load **Lysis Plate** with plant samples and steel beads
- Prepare **Bead-beating Master Mix** for 96 reactions + 20% excess vol.
- Add **100 µl Bead-beating Master Mix** per well to the **Lysis Plate** and seal plate with the Sealing option of choice (e.g. **Plastic or Silicone mat**)
- Bead-beat for **3 min** at **30 Hz**
- Centrifuge **Lysis Plate** for **1 min** at **1,000 x *g**
- Prepare **Lysis Master Mix** for 96 rxns. + 20% excess vol.
- Remove **Sealing mat** from **Lysis Plate**, add **105 µl** Lysis Master Mix per well
- Seal **Lysis Plate** tightly with the **Adhesive Foil**
- Incubate **Lysis Plate** for **30 min at 60°C** with max. agitation
- Incubate **Lysis Plate** for **10 min at 80°C** with max. agitation
- Detach **Adhesive Foil** and add **25 µl** of (CS) per well to the **Lysis Plate** and mix by pipetting up & down
- Centrifuge **Lysis Plate** for **3 min** at **full speed**

Preparation of Purification Plate (during 60°C / 80°C incubation)

- Fill **384 Purification Plate** according to the attached manual
- Place **384 Purification Plate** on top of a **384 Conditioning Plate**
- Centrifuge **1 min** at **1,000 *x g**** to elute void buffer from **384 Purification Plate**
- Place prepared **384 Purification Plate** on top of **384 Elution Plate**

Purification of DNA

- Transfer **lysis supernatant (max 25 µl per well)** to **384 Purification Plate**
- Centrifuge **1 min** at **1,000 *x g**** to elute DNA into **384 Elution Plate**

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EchoLUTION Plant DNA 384 Kit - Protocol

for 384-well plate purification of genomic DNA from **dried** plant tissue samples

This protocol has been developed for dried and freeze-dried plant tissues like leaves, blossoms, fruits, roots, flour and seed samples. For fresh plant tissues, please use the corresponding protocol also supplied with this kit.

Note:

The **EchoLUTION Plant DNA 384 Kit** lyses the plant tissue in a **96 well** Lysis plate with the same buffer and reagent volumes as for a conventional 96 well purification. The difference is the loading volume of **25 µl** lysate to the **384 well** purification plate (in contrast to 100 µl lysate loading volume to a 96 well purification plate).

Materials and equipment needed

Sample: 2 - 10 mg freeze-dried or dried plant tissue samples recommended

Supplied with the kit:

- **384 Purification Plate:** 384-well plate to be filled with 130 µL of the provided **bulk resin** per well
- **384 Elution Plate:** 384-well plate for the collection of the purified DNA. Make sure the Elution plate is suitable for centrifugation at 1,000 x g
- **Adhesive Foil** for plate sealing during lysis
- **All needed reagents and solutions**

Not supplied with the kit:

- **Lysis Plate:** 96-well plate for the lysis of freeze-dried or dried plant tissue in a 96-well bead-beating device followed by incubation in a 96-well thermo shaker
  Note: The use of Bioecho's Tube & Cap Strips is recommended to prevent sample leakage during beat-Beating. BioEcho product no. 060-002-024
- **Sealing option:** Plastic or silicone mat suitable for sealing the lysis plate during homogenization in the 96-well bead-beating device
- **384 Conditioning Plate**: 384-deep well plate with minimum of 100 µl well volume for the collection of void volume during preparation of **384 Purification Plate**. Reusable!
- **96 (or 384)-well swing-out centrifuge** (preferentially with switch option to rcf (x g*))
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- **96-well plate thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **8-channel pipets** for 200 µl scale
- **Wide Bore Pipette Tips** for 200 µL scale
- **Troughs** for Master Mix preparation holding > 20ml
- **Plate(s) to be used as *tara*** in the centrifuge in case an odd number of plates are processed
- **Bead-beater device** for 96-deep well plates
- **Steel beads**. BioEcho product no. 050-006-200
- **Ceramic blade scalpel** for cutting plant samples. BioEcho product no. 050-002-001

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:

Lysis

1. Transfer plant samples and beads in each cavity of the 96-well **Lysis Plate** (if using BioEcho`s steel beads, add one bead for each well)
2. Seal **Lysis Plate** with the Sealing option of choice **(e.g. Plastic or Silicone mat)**
3. Place the **Lysis Plate** in the bead-beater and beat **3 min at 30 Hz** or until the plant tissue is completely disrupted.
4. Centrifuge Lysis Plate for **1 min at 1,000 x *g*** with the **Sealing mat** attached to collect the disrupted sample at the bottom of the well.
5. Prepare the **Lysis Master Mix** for 96 lysis reactions with 20% excess volume in a reagent trough:

Lysis Master Mix

No. of samples		1	96 (+20%)	Yours
BB	Buffer BB (µL)	94	11,400	
R	RNAse A Plant (µl)	1	115	
LB	96 Plant Lysis Buffer (µL)	100	11,520	
P	TurboLyse P Protease (µl)	5	580	
Final volume (µl)		**200**	**23,615**	

6. Add **200 µl of the Lysis Master Mix** to each well of the **Lysis Plate** containing the plant samples and resuspend the homogenized plant tissue in the Lysis Master Mix.
7. Seal **Lysis Plate** tightly with the **Adhesive Foil** (supplied with the kit).
8. Place the **Lysis Plate** 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).
   Meanwhile during lysis, proceed with step 10, "Preparation of Purification Plate"
9. After incubation at 60°C, increase temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Preparation of Purification Plate (during steps 8 and 9)

10. Please follow the instructions in the attached manual "**Instructions for the filling of 384-well purification plates with BioEcho`s purification matrix**" to fill the 384 Purification Plate with provided bulk resin.
11. Plate preparation: Place the prepared **384 Purification Plate** on top of a 384-deep well plate ("Conditioning Plate", not supplied, minimum well volume of 100 µl) and centrifuge for **1 min** at **1,000 x *g**** to collect the void buffer from the purification resin in the **384 Purification Plate**. Discard the flow-through volume ("void volume") collected in the lower **384 Conditioning Plate** (not supplied).
12. Place the conditioned **384 Purification Plate** on top of the 384 well **384 Elution Plate** for elution of the purified DNA. Continue with "Purification of DNA".

Purification of DNA

13. Detach **Adhesive Foil** from the incubated **Lysis Plate** and **add 25 µl Clearing Solution P** (CS) to each well of the **Lysis Plate** and mix by pipetting up & down. The sample will become cloudy.
14. Centrifuge **Lysis Plate** for **3 min** at **full speed** to sediment debris.
15. Transfer the **lysis supernatant (max. 25 µl)** onto the **384 Purification Plate**.
    Important loading instructions:
    - Do not touch the cellular debris at the bottom of the well while removing the supernatant to avoid clogging of the pipet tip (preferentially, wide bore tips). Residual tissue particles may be loaded and will not interfere with purification.
    - During the loading step, make sure that the 8-channel pipette releases the lysate solution **slowly and vertically**, non-angular onto the middle of the resin surface!
    - Do not punch pipette tip into the resin bed during loading of lysate!
16. After completion of the loading step, centrifuge **384 Purification Plate** on top of an **384 Elution Plate** as "plate sandwich".
17. Centrifuge for **1 min** at **1,000 x *g**.
18. The purified DNA elutes into the **384 Elution Plate** 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.

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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 384 Kit

for 384-well purification of genomic DNA from **dried** plant tissue samples

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Related products	Conditioning Plate 384 060-006-008 | Steel beads 050-006-200 | Ceramic Blade Scalpels 050-002-001 |Tube & Cap Strips 060-002-024	

Quick PROTOCOL (please read protocol first)

Bead-beating, Sample Lysis and Clearing

- Load **Lysis Plate** with plant samples and steel beads
- Seal plate with the Sealing option of choice (e.g. **Plastic or Silicone mat**)
- Bead-beat for **3 min** at **30 Hz**
- Centrifuge **Lysis Plate** for **1 min** at **1,000 x *g***
- Prepare **Lysis Master Mix** for 96 reactions + 20% excess vol.
- Add **200 µl Lysis Master Mix** per well to the **Lysis Plate** and resuspend homogenized plant tissue
- Seal **Lysis Plate** tightly with the **Adhesive Foil**
- Incubate **Lysis Plate** for **30 min at 60°C** with max. agitation
- Incubate **Lysis Plate** for **10 min at 80°C** with max. agitation
- Detach **Adhesive Foil** and add **25 µl** of (CS) per well to the **Lysis Plate** and mix by pipetting up & down
- Centrifuge **Lysis Plate** for **3 min** at **full speed**

Preparation of Purification Plate (during 60°C / 80°C incubation)

- Fill **384 Purification Plate** according to the attached manual
- Place **384 Purification Plate** on top of a **384 Conditioning Plate**
- Centrifuge **1 min** at **1,000 *x g**** to elute void buffer from **384 Purification Plate**
- Place prepared **384 Purification Plate** on top of **384 Elution Plate**

Purification of DNA

- Transfer **lysis supernatant (max 25 µl per well)** to **384 Purification Plate**
- Centrifuge **1 min** at **1,000 *x g**** to elute DNA into **384 Elution Plate**

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