Cat. # 9194

For Research Use

TaKaRa

Plant DNA Isolation Reagent

Product Manual

v201908Da

Table of Contents

Takara plant DNA Isolation Reagent is a ready-to-use kit that uses benzyl chloride for extracting plant genomic DNA. Benzyl chloride causes disintegration of plant cell walls by benzylation of the hydroxyl groups in cellulose and other components of the cell wall. This kit takes advantage of this property in a procedure which involves freeze thawing, and then physical grinding the plant tissue with a pipette tip several times in a microcentrifuge tube to achieve degradation of the cell wall. Traditional methods of plant cell wall disruption, which rely on pulverization in liquid nitrogen using a mortar and pestle, are not needed. This makes preparing multiple samples at the same time more convenient. Furthermore, improvements in reagent composition have reduced the heat treatment to only 15 minutes, and the entire process through aqueous phase collection can be completed within 30 minutes. The genomic DNA obtained can be used directly in PCR reactions, or for restriction endonuclease digestion.

I. Components（100 Preps）

Extraction Solution 1	40 ml
Extraction Solution 2	8 ml
Extraction Solution 3（100% Benzyl Chloride）	15 ml

II. Storage

Room Temperature

Storage of Extraction Solution 2 at a low temperature may cause a precipitate to form. This precipitate may be dissolved by heating to 50℃. Then store the solution at room temperature.

＊ 2 years from date of receipt when unused and stored properly at -20℃. Once opened or thawed, store at 4℃ and use sooner to avoid contamination.

III. Materials Required but not Provided

- Isopropanol
- 70% Ethanol
- TE Buffer
- RNase A（Optional）
- Micropipettes
- Micropipette tips
- 1.5 ml microcentrifuge tube
- Microcentrifuge (Capable of 12,000 rpm）
- 50℃ heat block or water bath
- Vortex mixer

IV. Precautions

Extraction Solution 3 contains Benzyl chloride and may cause severe eye irritation and possible eye injury. May be fatal if inhaled. Harmful if swallowed. Causes skin and respiratory tract irritation. Follow the storage and handling conditions listed on the attached Material Safety Data Sheet (MSDS).

V. Protocol

＜ Workflow ＞

Cut the plant tissue into 3mm-squares, weigh, and freeze at -20℃.
（10 - 100 mg plant tissue /1.5 ml microcentrifuge tube）

↓

Place at room temperature for 5 min to thaw.

↓

Physically grind the plant tissue by using a pipette tip to crush the sample into bottom of the tube 10 times.

↓ ← Extraction Solution 1　400 μl

Vortex (maximum speed) 5 sec
Spin down

↓ ← Extraction Solution 2　80 μl

Vortex (maximum speed) 5 sec
Spin down

↓ ← Extraction Solution 3　150 μl

Vortex (maximum speed) 5 sec
Spin down

↓

50℃ incubation：15 min

↻ 12,000 rpm, 15 min, 4℃

Transfer aqueous (upper) phase to a fresh tube.

↓ ← Add an equal volume of isopropanol, mix gently.

↻ 12,000 rpm, 10 min, 4℃

Remove the supernatant, add 1 ml of 70% EtOH to wash.

↻ 12,000 rpm, 3 min, 4℃

Remove the supernatant, dry the pellet, and dissolve in an appropriate volume of TE buffer.

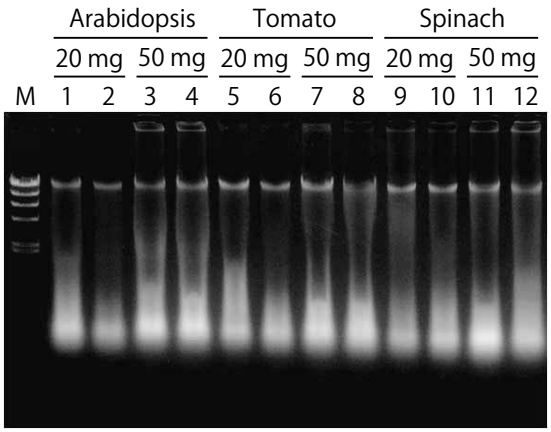
1. Using a scissors, or other cutting utensil, cut the plant tissue into 3 mm-squares, weigh 10 - 100 mg of clippings in a 1.5 ml microcentrifuge tube, and freeze at -20℃.
   Note: Use 70% Ethanol to wipe the edge of the cutting utensil when processing the plant samples.
2. Place the frozen plant tissue at room temperature for 5 min until thawed.
3. Spin down briefly to collect the plant tissue at the bottom of the tube.
4. Physically grind the plant tissue by using a pipette tip to crush the sample into bottom of the tube 10 times.
5. Add 400 μl of Extraction Solution 1, and vortex at maximum speed for 5 sec. If the plant tissue sticks together in the bottom of the tube, flick the bottom of the tube with your finger to resuspend.
6. Spin down briefly, add 80 μl of Extraction Solution 2, and vortex at maximum speed for 5 sec. With the addition of Extraction Solution 2, a white precipitate should form that will cloud the solution when vortexed. If the plant tissue sticks together in the bottom of the tube, flick the bottom of the tube with your finger to resuspend.
7. Spin down briefly, add 150 μl of Extraction Solution 3, and vortex at maximum speed for 5 sec. If the plant tissue sticks together in the bottom of the tube, flick the bottom of the tube with your finger to resuspend.
8. Spin lightly for less than two seconds, and incubate at 50℃ for 15 min.
   Note: Long centrifugation will cause Extraction Solution 3 to separate. Centrifuge for no more than two seconds in a table-top centrifuge.
9. Centrifuge at 12,000 rpm for 15 min at 4℃ to separate the aqueous (upper) phase from the organic (lower) phase.
10. Collect as much of the upper clear aqueous phase as possible, while taking care not to collect the lower phase and the tissue debris. About 400 μl of liquid can be recovered.
11. Add an equal volume of isopropanol to the collected aqueous phase, and mix gently.
    Note: Storage of the sample, or suspending the protocol for long periods at this stage will cause the accumulation of impurities. Please proceed to the next step as quickly as possible.
12. Centrifuge at 12,000 rpm for 10 min at 4℃.
13. Remove the supernatant, taking care not to disturb the pellet.
    Note: In some cases the pellet may not be visible.
14. Add 1 ml of 70% ethanol to wash the pellet.
15. Centrifuge at 12,000 rpm for 3 min at 4℃.
16. Remove the supernatant, taking care not to disturb the pellet.
17. Dry the pellet, and resuspend in a suitable amount of TE buffer (about 20 μl)
    Note: Take care not to over dry the pellet, as it can make resuspension difficult.

＊ The recovered DNA solution may be used 'as is' for restriction endonuclease digestion, or as PCR template.
Approximately 0.5 - 2 μl of the recovered 20 μl DNA can be used as template for PCR (25 μl total reaction), or for digestion (20 μl total reaction).
Store DNA at 4℃ if not used immediately.

VI. Experimental Examples

【 Example 1: Extraction of DNA from plant tissue 】

Arabidopsis sprouts, tomato sprouts, or spinach leaves were cut into 3 mm-squares. 20 or 50 mg of each were weighed in microcentrifuge tubes. After freezing at -20℃, genomic DNA was extracted according to protocol. The precipitate obtained was dissolved in 20 μl of TE buffer, and analyzed by UV spec and gel electrophoresis (N=2).



5 μl of the genomic DNA solution was loaded.
M： λ-*Hin*d III digest（150 ng loaded)

1% Agarose L03「TAKARA」

Figure 1. Electrophoresis of DNA extracted from plant tissue.

Table 1. Purity of DNA extracted from plant tissue

	Tissue	Sample No.	A_{260}/A_{280}	A_{260}/A_{230}
Arabidopsis sprouts	20 mg	1	2.2	1.4
	20 mg	2	2.2	1.4
	50 mg	3	2.1	1.7
	50 mg	4	2.1	1.7
Tomato sprouts	20 mg	5	2.0	1.4
	20 mg	6	2.0	1.6
	50 mg	7	1.8	1.4
	50 mg	8	1.7	1.4
Spinach Leaves	20 mg	9	2.2	1.6
	20 mg	10	2.2	1.8
	50 mg	11	2.1	1.8
	50 mg	12	2.0	1.7

【 Example 2: Extraction of DNA from tobacco BY-2 cell culture 】

50 mg of tobacco cell culture was weighed in a microcentrifuge tube, washed with PBS, and then frozen at -20. Crushing the cells with a pipette tip was omitted. Genomic DNA was extracted according to protocol. The precipitate obtained was dissolved in 20 μl of TE buffer, and analyzed by UV spec and gel electrophoresis (N=2).

5 μl of genomic DNA solution was loaded.
M： λ-*Hin*d III digest（150 ng loaded)
1% Agarose L03「TAKARA」

Figure 2. Electrophoresis of DNA extracted from tobacco BY-2 cell culture.

Table 2. Purity of DNA extracted from tobacco BY-2 cell culture.

	Culture	Sample No.	A_{260}/A_{280}	A_{260}/A_{230}
Tobacco BY-2 cell culture.	50 mg	1	2.2	2.1
		2	2.2	2.2

【 Example 3: PCR amplification using genomic DNA extracted in Example 1 and 2 】

PCR was conducted using genomic DNA extracted in Example 1 and 2 as the template.

Template : 0.5 μl of genomic DNA solution extracted in Example 1 and 2.
Polymerase : *TaKaRa Ex Taq*® Hot Start Version
Total volume : 25 μl

Target gene and amplicon size.

Arabidopsis	MERI5B gene	(about 1.0 kb)
Tomato	XET gene	(about 0.6 kb)
Spinach	coxl gene	(about 0.5 kb)
Tobacco	EXT gene	(about 2.2 kb)

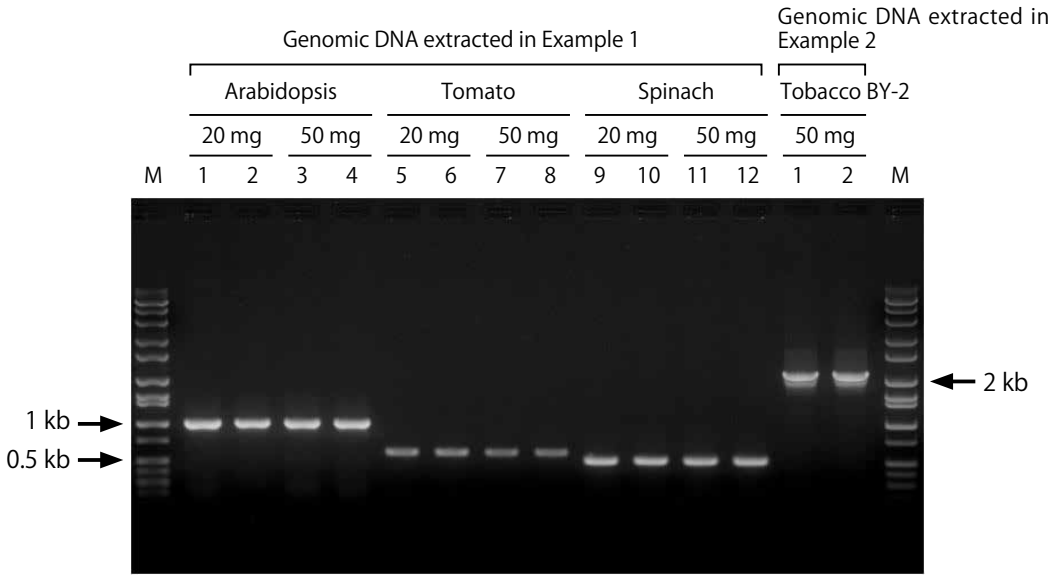
PCR Conditions

Arabidopsis, Spinach

98℃	10 sec	30 cycles
60℃	30 sec	
72℃	1 min/kb	

Tomato, Tobacco

98℃	10 sec	35 cycles
55℃	30 sec	
72℃	1 min/kb	



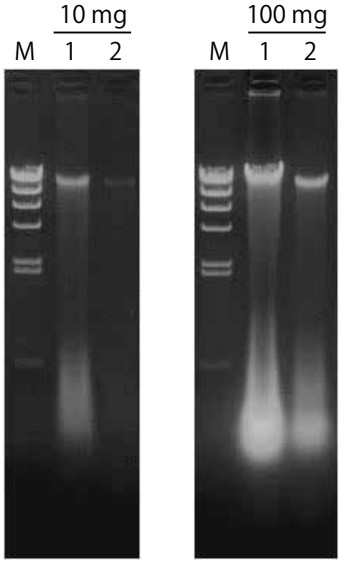
4 μl of each PCR reaction was loaded.
M : Wide-Range DNA Ladder (50 - 10,000 bp)

1% Agarose L03「TAKARA」

Figure 3. Electrophoresis of PCR products amplified from genomic DNA extracted in Example 1 and 2

【Example 4：Extraction of DNA from Spinach（10 mg,100 mg）】

Spinach leaves were cut into 3 mm-squares. 10 or 100 mg were weighed in microcentrifuge tubes. After freezing at -20℃, genomic DNA was extracted according to the protocol. The precipitate obtained was dissolved in 20 μl of TE buffer, and analyzed by UV spec and gel electrophoresis (N=2).



1 ： 5 μl of genomic DNA solution was loaded
2 ： 0.5 μl of genomic DNA solution was loaded
M ： λ-*Hin*d III digest (150 ng loaded)

1% Agarose L03「TAKARA」

Figure 4. Electrophoresis of DNA extracted from spinach leaves

Table 3. Purity of DNA extracted from spinach leaves

	Tissue	A_{260}/A_{280}	A_{260}/A_{230}
Spinach leaves	10 mg	2.2	1.9
	100 mg	2.1	2.1

【 Example 5: PCR amplification using genomic DNA extracted 】

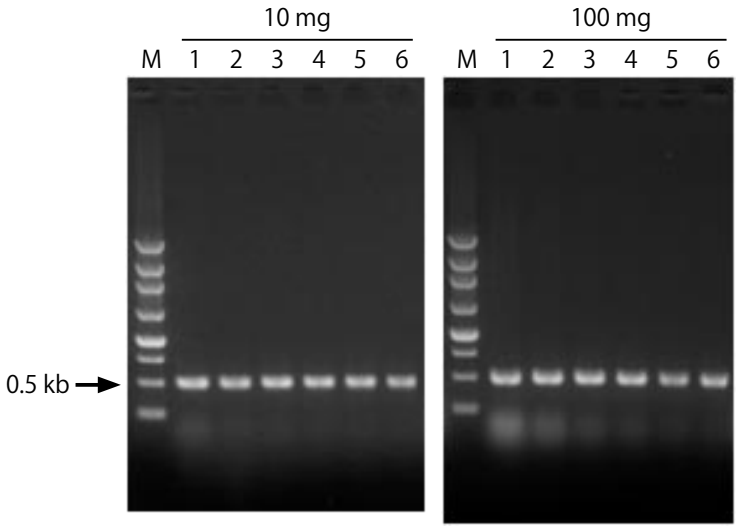
PCR amplification was conducted using genomic DNA extracted in Example 4 as the template.

Template : 2 μl of diluted, or undiluted DNA solution extracted in Example 4.
Polymerase : *TaKaRa Ex Taq* Hot Start Version
Total volume : 25 μl

Target gene and amplicon size
coxI gene (about 0.5 kb)

PCR Conditions

98℃	10 sec	30 cycles
60℃	30 sec	
72℃	30 sec	



1 : Genomic DNA undiluted
2 : Genomic DNA 2 fold dilution
3 : Genomic DNA 5 fold dilution
4 : Genomic DNA 10 fold dilution
5 : Genomic DNA 20 fold dilution
6 : Genomic DNA 40 fold dilution
M : 250 bp DNA Ladder (Dye Plus)

1% Agarose L03「TAKARA」

Figure 5. Electrophoresis of PCR products amplified from genomic DNA extracted in Example 4

VII. Troubleshooting

1. There is little or no DNA.

- The amount of DNA depends on the type and amount of the plant tissue used. Please increase the initial amount of tissue.
- Grinding in liquid nitrogen may be used for hard leaves such as pine and camellia.
- Yield can be increased by using microcentrifuge tube pestles, instead of using pipette tips for grinding the plant .
- After adding isopropanol or 70% ethanol, yield can also be increased by lengthening the centrifugation steps. After adding isopropanol, centrifuge for an hour, instead of 10 minutes, or after adding 70% ethanol, centrifuge for a 30 minutes, instead of 3 minutes.
- The yield may be reduced depending on how long the plant tissues have been stored, or their condition, so please use fresh samples.

2. PCR amplification does not work.

- The extracted DNA solution may contain PCR inhibitors originating from the plant tissue. Dilute the DNA solution, and then use it as template.
- There may be too much initial tissue. Try decreasing the amount of tissue and re-extracting.
- If the DNA solution has an orange/brown color, or is gelatinous, use the High-Salt Solution for Precipitation (Plant) (Cat #9193) to remove PCR inhibitors. Although the High-Salt Solution for Precipitation (Plant) is an RNA preparation reagent, use it to purify the extracted DNA solution according to the standard protocol. For samples which do not contain PCR inhibitors, using this purification method may not be helpful.
- We recommend using *TaKaRa Ex Taq* Hot Start Version (Cat # RR006A) for amplification.

3. The extracted DNA solution contains too much RNA.

- RNA cannot always be excluded when processing with this product. In this case, please treat with RNase.

  Protocol for RNase treatment:

  1. Add RNaseA* to a 10 - 20 μg/ml final concentration in the DNA solution.
  2. Incubate for 30 minutes at 37℃.
  3. Extract with phenol/chloroform if necessary

  * Powdered RNaseA should be prepared to a 1mg/ml solution in 10mM Tris-HCl pH 7.5, and 15 mM NaCl. If it is not DNase Free, heat treat for 15 minutes at 100℃ to inactivate DNases (A DNase-free solution is available for purchase).

VIII. References

Zhu H, Qu F, and Zhu LH. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. *Nucleic Acids Research*. (1993)**21** (22): 5279-5280.

IX. Related Products

High-Salt Solution for Precipitation (Plant) (Cat #9193)
TaKaRa Ex Taq® Hot Start Version (Cat #RR006A)