Cat. # 9768

For Research Use

TaKaRa

TaKaRa MiniBEST Plant Genomic DNA Extraction Kit

Product Manual

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I. Description

TaKaRa MiniBEST Plant Genomic DNA Extraction Kit is designed to purify genomic DNA from plant tissue. This kit contains two unique lysis system, which are useful for genomic DNA extraction effectively from both general plant tissue and polysaccharides, polyphenols-rich plant tissue. After the plant tissue grind in liquid nitrogen, this system employs a unique lysis Buffer in combination with DNA preparation membrane to efficiently purify genomic DNA from the biological sample. The protocol provides a simple method to achieve the rapid isolation of highly purified genomic DNA and to be accomplished within 40 minutes after tissue ground in liquid nitrogen. Using the kit, 1 - 10 μg of highly purified genomic DNA can be extracted from 50 - 500 mg plant tissues. Genomic DNA prepared is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular biology experiments.

II. Components (50 reactions)

The kit contains Part I and Part II.

Part I (stored at -20℃)	
50X DTT Buffer	700 μl
RNase A	500 μl

Part II (stored at room temperature)	
Buffer HS I*1, 3	28 ml
Buffer HS II*1	28 ml
Buffer KAC	1.8 ml x 2
Buffer GB*1	28 ml
Buffer WA*1	28 ml
Buffer WB*2	24 ml
Elution Buffer	14 ml
Spin Column	50
Collection tube	50

*1: Contains strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.
*2: Before using the kit, add 56 ml of 100% ethanol. Mix well.
*3: When Buffer HS I form precipitate by storage bellow room temperature (15 - 25℃), redissolve the precipitate by incubating at 37℃ before used.

Reagents not supplied in this kit

1. 100% ethanol
2. Sterile distilled water

III. Storage and shipping

1. Part I can be stored at -20℃, and Part II can be stored at room temperature (15 - 25℃).
2. Part I can be shipped at -20℃, and Part II can be shipped at room temperature (15 - 25℃).

IV. Preparation before experiment

1. Adjust a water bath to 56℃.
2. Add 56 ml of 100% ethanol to Buffer WB and mix well before using it.
3. Pre-heat the Elution Buffer or sterile distilled water to 65℃ will improve elution efficiency.

V. Protocol

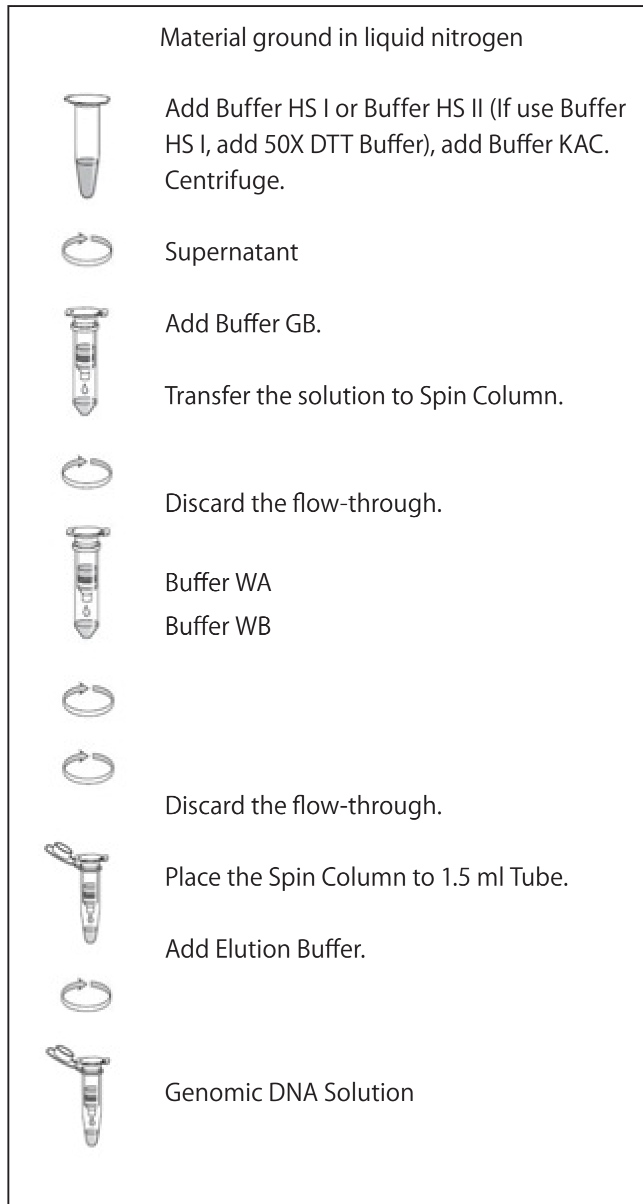

Figure 1. Flow chart

Protocol overview is in Figure 1. The procedure can be accomplished in 40 minutes after grinding plant tissue in liquid nitrogen. The whole procedure includes tissue lysis, DNA binding with column and DNA purification. Protocol in detail is as below.

1. Lysis of plant tissue
   This kit employs two unique lysis system for plant tissue. DNA extraction from the general plant tissue refers to Protocol I; DNA extraction from plant tissue rich in polysaccharides and polyphenols refers to Protocol II.

   - For general plant tissue (Protocol I)
     General plant tissue are such as plant roots, stems and leaves, which are less or no polysaccharide, polyphenols, and fat content.
     (1) Add 500 μl of Buffer HS I and 10 μl of 50X DTT Buffer for 100 mg plant tissue into 1.5 ml tube. In low DNA content plant tissue, the starting amount may increase.
     (2) Weigh accurately and grind plant tissue in liquid nitrogen.
     (3) Quickly take the tissue powder into the tube of step (1), add 10 μl RNase A (10 mg/ml), mix well and incubate it into 56℃ water bath for 10 minutes.

   - Plant tissue rich in polysaccharides, polyphenols, or fat (Protocol II):
     Plant tissues rich in polysaccharides, polyphenols, and fat are from plant flowers, fruits, seeds, tubers and leaves of some ornamental plants.
     (1) Add 500 μl of Buffer HS II for 100 mg plant tissue into 1.5 ml tube. In low DNA content, plant material may increase the starting amount.
     (2) Weigh accurately and grind plant tissue in liquid nitrogen.
     (3) Quickly take the tissue powder into the tube of step (1), add 10 μl RNase A (10 mg/ml) , mix well and incubate it into 56℃ water bath for 10 minutes.

Processed samples to the following:

2. Add 62.5 μl Buffer KAC (1/8 volume of Buffer HS I or Buffer HS II) and mix well. Place on the ice for 5 minutes. Centrifuge at 12,000 rpm for 5 minutes. Transfer the supernatant in a new tube and add an equal volume of Buffer GB, mix well.
3. Transfer the solution to Spin Column (by twice). Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
4. Add 500 μl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
5. Add 700 μl of Buffer WB into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Take care to add Buffer WB along the tube wall of Spin Column to wash off any residual salt.
   Note: Make sure the amount of 100% ethanol specified on the bottle label has been added to the Buffer WB.
6. Repeat Step 5.
7. Place Spin Column into Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
8. Place Spin Column into a new 1.5 ml tube. Add 30 - 50 μl of Elution Buffer or sterile distilled water to the center of the membrane. Let it stand for 5 minutes at room temperature.
   Note: Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.

9. Centrifuge at 12,000 rpm for 2 minutes to elute DNA.
   If more yield is needed, the flow-through can be re-added into the center of the membrane or add 30 - 50 μl of Elution Buffer or sterile distilled water and let it stand for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute DNA.
10. Quantification of the genomic DNA.
   The genomic DNA can be quantitatively determined by electrophoresis or absorbance measuring. If the genomic DNA is stored for a long time, it is recommended to save in the Elution Buffer.

VI. Experimental examples

1. Purification of genomic DNA from general plant tissue
   Approximate 1.5 μg, 1.2 μg, 9 μg, 1.8 μg or 3 μg of highly purified genomic DNA was extracted from 100 mg of Celery leaves, maize seedling leaves, wheat seedling leaves, mung bean seedling leaves, or sunflower seedling leaves, respectively. The electrophoresis is in Figure 2.

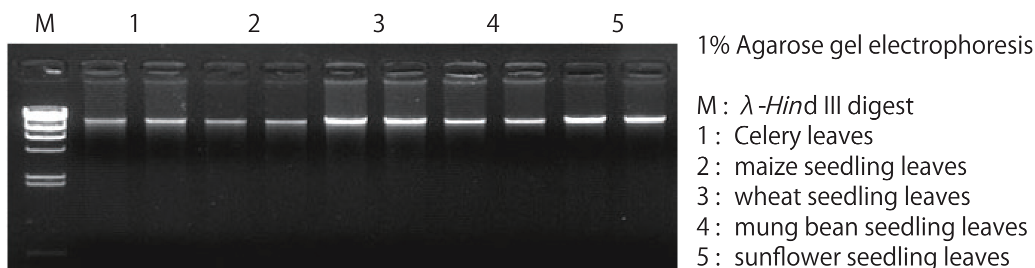

Figure 2. Electrophoresis of genomic DNA from general plant tissue

2. Purification of genomic DNA from plant tissues rich in polysaccharides, polyphenols, or fat
   Approximate 0.2 μg, 3 μg, 2 μg, 0.15 μg, 1.5 μg or 0.4 μg of highly purified genomic DNA was extracted from 100 mg of Aloe vera leaves, pine needles, anthurium leaves (flowers), mushroom fruiting bodies, peanuts seeds, or mushrooms mycelium, respectively. The electrophoresis is in Figure 3.

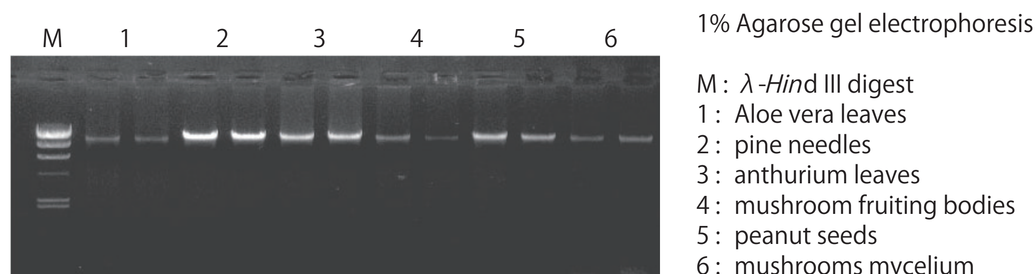

Figure 3. Electrophoresis of genomic DNA from plant tissues rich in polysaccharides, polyphenols , or fat

3. Purification of genomic DNA from sunflower seedling and pine needles and PCR analysis
   Approximate 3 μg of highly purified genomic DNA was extracted from sunflower seedling and pine needles. Using the genomic DNA as template, 5 kb ATP gene fragment was amplified by PCR. The electrophoresis are in Figure 4.

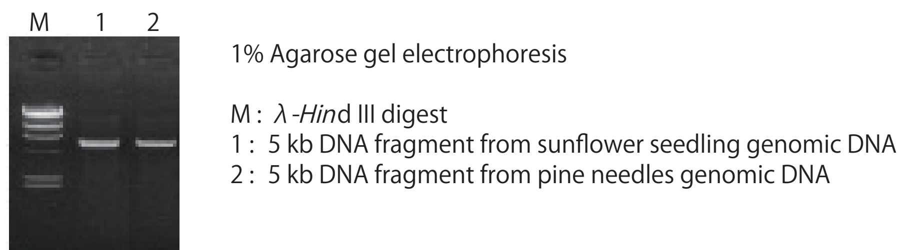

Figure 4. Electrophoresis of PCR fragments from genomic DNA from sunflower seedling and pine needles

VII. Extraction amount of the different plant tissues

Genomic DNA were extracted from the general plant tissues using Buffer HS I and 100 μl Elution Buffer. The starting amount and yields of DNA extracted are shown in Table 1.

Material	Starting amount	DNA yield
Spinach leaves	100 mg	3 - 6 μg
Rape leaves	100 mg	1 - 3 μg
Wheat seedling leaves	100 mg	3 - 10 μg
maize seedling leaves	100 mg	1 - 3 μg
Mung bean seedling blades	100 mg	1 - 3 μg
Radish seedling leaves	200 mg	1 - 3 μg
Sorghum seedling leaves	100 mg	1 - 3 μg
Sunflower seedling leaves	100 mg	1 - 3 μg
Buxus leaves	100 mg	1 - 3 μg
Celery leaves	100 mg	1 - 3 μg
Cabbage leaves	200 mg	1 - 3 μg
Arabidopsis leaves	200 mg	1 - 3 μg
Tobacco leaves	100 mg	1 - 3 μg

Table 1. The yields of DNA from the general plant

Genomic DNA were extracted from the plant tissues rich in polysaccharides, polyphenols, or fat, using Buffer HS II and 100 μl Elution Buffer. The starting amount of material and yields of DNA extracted are shown in Table 2.

Material	Starting amount	DNA yield
Apple fruits	500 mg	0.1 - 0.5 μg
Potato tubers	100 mg	1 - 3 μg
Tomato fruit	200 mg	1 - 3 μg
Banana pulp	200 - 500 mg	1 - 3 μg
Cypress leaves	100 mg	3 - 6 μg
Pine needles	100 mg	2 - 5 μg
Tea (frozen)	100 mg	3 - 10 μg
Cotton seed	100 mg	3 - 6 μg
Bolls shell	100 mg	1 - 3 μg
Grape shoot tips	200 mg	1 - 3 μg
Autumn Olive Fruit	200 mg	1 - 3 μg
Ginkgo biloba	100 mg	1 - 3 μg
Purple potato tubers	200 mg	0.2 - 1 μg
Keel blade (flowers)	100 mg	1 - 3 μg
Aloe vera leaves (flowers)	200 mg	0.2 - 1 μg
Peanut seeds	100 mg	1 - 2 μg
Banana seedling leaves	200 mg	0.2 - 2 μg
Anthurium leaves (flowers)	100 mg	1 - 3 μg
Rhodiola leaves (flowers)	200 mg	0.2 - 2 μg
Oyster mushroom fruiting bodies	200 mg	0.2 - 1 μg
Chlorophytum leaves (flowers)	200 mg	0.2 - 1 μg
Mushroom mycelium	100 mg	0.2 - 1 μg
Down roots leaves (flowers)	200 mg	0.2 - 1 μg
Corn seed	100 mg	1 - 3 μg
Orange peel	200 mg	0.1 - 0.5 μg
Rice seeds	100 mg	1 - 3 μg
Pear flesh	200 - 500 mg	0.1 - 1 μg

Table 2. The yields of DNA from plant rich in polysaccharides, polyphenols, or fat

VIII. Cautions

1. Use fresh experimental material to ensure the genomic DNA extracted not to be degraded.
2. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protection such as safety glasses, laboratory coat and gloves and try to operate at fume hood. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water and seek medical advice.
3. If the genomic DNA is stored for a long time, it is recommended to store in the Elution Buffer.
4. Yield and integrity of plant genomic DNA have a great relationship of the state and the growth of plants. If you want to get high-quality DNA, please use young plant material.
5. It is important to properly select the Lysis buffer depending on plant material. For general plant tissue, please use Buffer HS I, with 50X DTT Buffer. For fat, polyphenols, or polysaccharides rich tissues, use Buffer HS II. Handling improperly may result in reduced DNA yield, or even lack DNA.

X. Troubleshooting

Q1. How yield of genomic DNA?

A1. This kit is suitable for purification of genomic DNA from plant tissues. The yield of genomic DNA differs by starting material. Generally, 1 - 10 μg of genomic DNA can be extracted from 100 mg of plant tissue. DNA content in plant tissues is generally low, especially fruit plants contain more water content. Furthermore, DNA content of plants has a great relationship for growth stage. Using young plant material can obtain high quality DNA.

Q2. If the yield of genomic DNA is low or there is no yield.

A2. The following aspects can be considered:
(1) DNA content of experimental material is low, for example, only tens ng of genomic DNA can be extracted from 100 mg of apple fruit.
(2) Incomplete grinding plant tissue causes incomplete recovery of DNA. Grinding in liquid nitrogen fully is critical to obtain high quatity DNA.
(3) Incorrect estimation of DNA content in plant material.
(4) Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.
(5) Strictly follow the protocol.

Q3. The genomic DNA extracted is degraded.

A3. (1) The plant materials are not fresh enough or are not stored at low temperature after collecting. It is recommended that the materials should be stored at -80℃ and use dry ice during shipping.
(2) There is residue DNase on experimental materials. Wash with Buffer WA once more.
(3) If the genomic DNA is stored for a long time, it is recommended to store in the Elution Buffer.

Q4. There is contamination with RNA in genomic DNA extracted.

A4. (1) RNase A has not been used in operation. Strictly follow the protocol to use RNase A.
(2) RNase A may be inactivated. RNase A should be stored at -20℃. Activity of RNase A is stable and generally it is not easily inactivated.

Q5. When the genomic DNA extracted have low biological activity.

A5. (1) The salt concentration in genomic DNA extracted is too high. When washing the DNA preparation membrane with Buffer WA and Buffer WB, add the buffer along the tube wall of Spin Column and let it stand for 5 minutes at room temperature to wash off any residual salt and improve the washing effect.
(2) There is residual ethanol in Genomic DNA solution. Let the Spin Column stand for 2 minutes at room temperature before adding Elution Buffer to the Spin Column and it will improve the effect of elution.
(3) The Elution Buffer must be added in the center of membrane during DNA elution and be not residue on the tube wall of Spin Column.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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