DNA extraction kit from plants, yeasts and bacteria

ISOPLANT II

Manual (Third edition)

Code No. 310-04151　For 100 extractions

Code No. 316-04153　For 20 extractions

NIPPON GENE CO., LTD.

Table of contents

I Product description ---------- **1**

II Contents of kit ---------- **1**

III Storage ---------- **2**

IV Precautions ---------- **2**

V Protocol ---------- **3**

1. Plant ---------- 3
2. Yeasts and bacteria ---------- 6

VI Troubleshooting ---------- **8**

VII Data collection ---------- **9**

1. RAPD analysis using DNA extracted from plant leaves ---------- 9
2. Restriction enzyme reaction of DNA extracted from plant leaves ---------- 9
3. Restriction enzyme reaction of DNA extracted from bacteria and yeasts ----10
4. Comparison of purity between ISOPLANT II and a conventional product (ISOPLANT) ----------10

VIII References ---------- **11**

I Product description

ISOPLANT II is a kit for extracting DNA from plants, yeasts and bacteria in a short period of time.
Plant tissues contain more of polysaccharides and polyphenols compared to animal tissues, and these may contaminate extracted DNA. These substances inhibit restriction enzyme reactions and PCR making the quantitative determination of DNA difficult.
ISOPLANT II disrupts cell walls, cell membranes, nuclear membranes and the like with benzyl chloride, which is the main component of the Solution II of the ISOPLANT II as well as the conventional ISOPLANT, and solubilizes in the presence of a surface-active agent. Further, in the ISOPLANT II, the reagents and protocol have been improved so that the inhibitors such as polysaccharides and polyphenols are effectively removed. Also, DNA can be extracted not only from herbaceous plants but also from woody plants from which DNA extraction has been difficult until now, and the obtained DNA can be used for PCR and restriction enzyme reactions.

II Contents of kit

		(For 100 extractions)	(For 20 extractions)
Wash Buffer	:	100 ml	20 ml
Solution I *1	:	30 ml	6 ml
Solution II *2	:	15 ml	3 ml
Solution III-A *3	:	10 ml	2 ml
Solution III-B	:	12 ml	2.5 ml
TE (pH8.0)	:	10 ml	2 ml
RNase A (1 mg/ml)	:	100 µl	20 µl
Manual			

*1 White precipitates may appear in the Solution I but this does not affect the quality. In such cases, use the solution after completely dissolving the crystals in a water bath at about 50°C. Also, handle the solution with care because the Solution I contains a protein denaturant and the like. If the Solution I enters eye or contacts skin, immediately wash well with large amounts of water. Further, if there are other abnormal symptoms, immediately consult a physician.

*2 Benzyl chloride is the main component of the Solution II. Benzyl chloride is a designated hazardous material under the Fire Service Act (group 4 hazardous material, group 2 petroleum, hazard degree III, flammable, keep away from flames). When handling, make sure to read the precautions in p. 2, Section IV.

*3 The Solution III-A is a white suspension. When the Solution III-A is evaporated to dryness and only the white solid phase remains, dissolve the solid phase using sterilized distilled water (for details, see the troubleshooting instructions in p. 8, Section VI).

Note) When DNA is extracted from plant samples, 2-Mercaptoethanol (designated hazardous material under the Fire Service Act) and in some cases $NaBH_4$ (designated hazardous material under the Fire Service Act) are required separately.

III Storage

Chilled storage (4°C)

All the reagents except Wash Buffer and RNase A can be stored at room temperature.

Further, when the kit is not to be used for a long period of time, store RNase A frozen (-20°C).

IV Precautions

○ This product is a reagent for research and cannot be used for other purposes such as for pharmaceutical purposes. Also, this product should be handled only by persons who have basic knowledge of reagents.

○ The main component of the Solution II included in this product is benzyl chloride. Benzyl chloride is a designated hazardous material under the Fire Service Act (group 4 hazardous material, group 2 petroleum, hazard degree III, flammable, keep away from flames). Handle with care. See the following for hazardous effects and handling precautions.

· Hazardous effects…

This product has extremely strong irritant properties and tearing properties to skin and mucous membranes, and may cause chemical burn on contact. Inhaling vapor at high concentrations may cause symptoms such as coughing, headaches, burning sensations, hypoesthesia, consciousness loss, tremor of limbs and paralysis.

· Handling precautions…

Keep away from flames and avoid contact with strong oxidants.

Install a local ventilating apparatus in the area where the reagent is handled, and close the lid of the container tightly after use.

Wear proper protective gears so as not to inhale or allow contact to eye, skin and clothing.

If the eye, skin or the like makes contact with the reagent, perform the following procedures.

If the reagent enters eye :

Immediately wash with a large amount of water for not less than 15 min.

Skin contact :

Wash with a large amount of water using soap.

Inhalation :

Rest and stay warm in a well-ventilated area.

Accidental ingestion :

Rinse out the mouth, drink activated charcoal suspended in water and consult a physician. Since the mucous membrane in the stomach and the like is damaged, do not force vomiting.

Also, if there are other abnormal symptoms, immediately consult a physician.
For storage, avoid direct sunlight, keep in a cool dark place and avoid contact with air by hermetically sealing the product. Further, if a minute amount of a metal such as iron is mixed in the container, a strong degradation reaction may occur, causing break-up of the container.

○ Handle this product in accordance with the descriptions in the manual. We cannot take responsibility for problems caused if the product is not handled according to the descriptions in the manual.

V Protocol

1. Plant

■ Reagent preparation: prepare the following reagents (A, B, C) when extracting DNA from a plant sample.

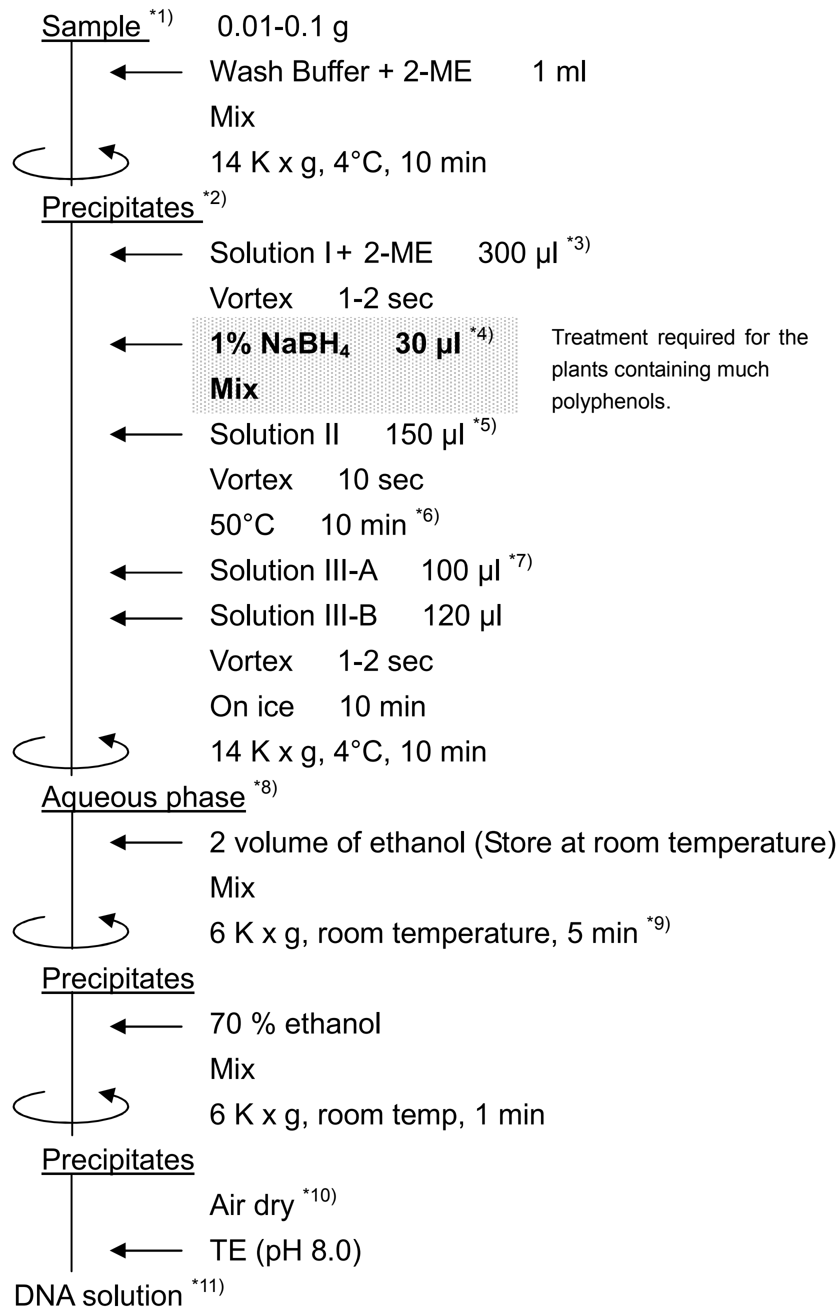
Prepare reagents (A, B, C) immediately before use in the amount required for extraction. Use up all of the reagents because storage of the reagent may cause decrease in yield and contamination of the enzyme reaction inhibitors.

A. Wash Buffer + 2-Mercaptoethanol (2-ME)
Add 2-Mercaptoethanol (required separately) to attached Wash Buffer to a final concentration of 0.5 %.

B. Solution I + 2-Mercaptoethanol (2-ME)
Add 2-Mercaptoethanol (required separately) to attached Solution I to a final concentration of 1 %.
(Note) 2-Mercaptoethanol is a designated hazardous material under the Fire Service Act. When handling, check the precautions.

Example: Plants with high polyphenol content
C. 1% $NaBH_4$
Dissolve $NaBH_4$ (Sodium tetrahydroborate: required separately) with sterilized water to 1 % (w/v).
(Note) Sodium tetrahydroborate is a designated hazardous material under the Fire Service Act. When handling, check the precautions.

■ Protocol



*1) Mince 0.01 g-0.1 g of the plants to 1 mm cubes with a cutter, or freeze/pulverize with liquid nitrogen. The yield is higher when freezing/pulverization is performed. Especially for woody plants, the yield can be very low without freezing/pulverization. Immerse the plant sample in the Wash Buffer immediately after freezing/pulverization. If left in the air for a long period of time, a browning reaction takes place due to oxidation of polyphenols causing problems for DNA extraction.

*2) Remove as much of the supernatant as possible. If the minced sample is used, the sample floats even if centrifugation is performed and removing the supernatant may be difficult. Dip the end of a pipette tip in the solution and suck out so that the remaining amount of the solution is as small as possible.

*3) White crystals may appear in the Solution I. In such cases, dissolve the crystals completely in a water bath at about 50°C, stir so that the content becomes homogeneous and then use it. Also, since the Solution I contains protein denaturant and the like, handle with care. If the Solution I enters eye or contacts skin, immediately wash well with a large amount of water.

*4） $NaBH_4$ (sodium tetrahydroborate: designated as a hazardous material under the Fire Service Act) is a strong reducing agent and used for suppressing oxidation of polyphenols contained in the plant tissues. When $NaBH_4$ is added, bubbles are formed. Take care not to allow the bubbles to leak from the tube and do not let the contents expand out from the tube when opening the cap.
These dangers can be avoided by quenching bubbles by lightly spinning down after adding $NaBH_4$ and before and after the 50°C incubation.

*5） The main component of the Solution II included in this product is benzyl chloride. Benzyl chloride is a designated hazardous material under the Fire Service Act. Handle with care. (See p. 2, *IV Handling precautions.*)
If eye, skin or the like make contact with the reagent, perform the following procedures immediately.

If the reagent enters eye :
Immediately wash with a large amount of water for not less than 15 min.

Skin contact :
Wash with a large amount of water using soap.

Inhalation :
Rest and stay warm in a well-ventilated area.

Accidental ingestion :
Rinse out the mouth, drink activated charcoal suspended in water and consult a physician. Since the mucous membrane in the stomach and the like is damaged, do not force vomiting.

Also, if there are other abnormal symptoms, immediately consult a physician.

*6） Cell walls, cell membranes, nuclear membranes and the like are disrupted by benzyl chloride, which is the main component of the Solution II, and DNA is dissolved into aqueous phase. For plant samples, the morphological changes are normally not visible.

*7） Shake the Solution III-A well to homogeneity and suck out with a pipette tip with a cut edge.

*8） Avoid taking substances other than the aqueous phase, such as benzyl chloride in the organic phase, the solid white substance found after the centrifugation and floating pieces of the plant, as much as possible. If substances other than the aqueous phase are mixed in, centrifuge again and collect only the aqueous phase. When the aqueous phase is cloudy white, add Solution III-A and B again, mix, centrifuge (14 K x g, 4°C, 10 min) and use the aqueous phase. (See p. 8, *VI Troubleshooting.*)

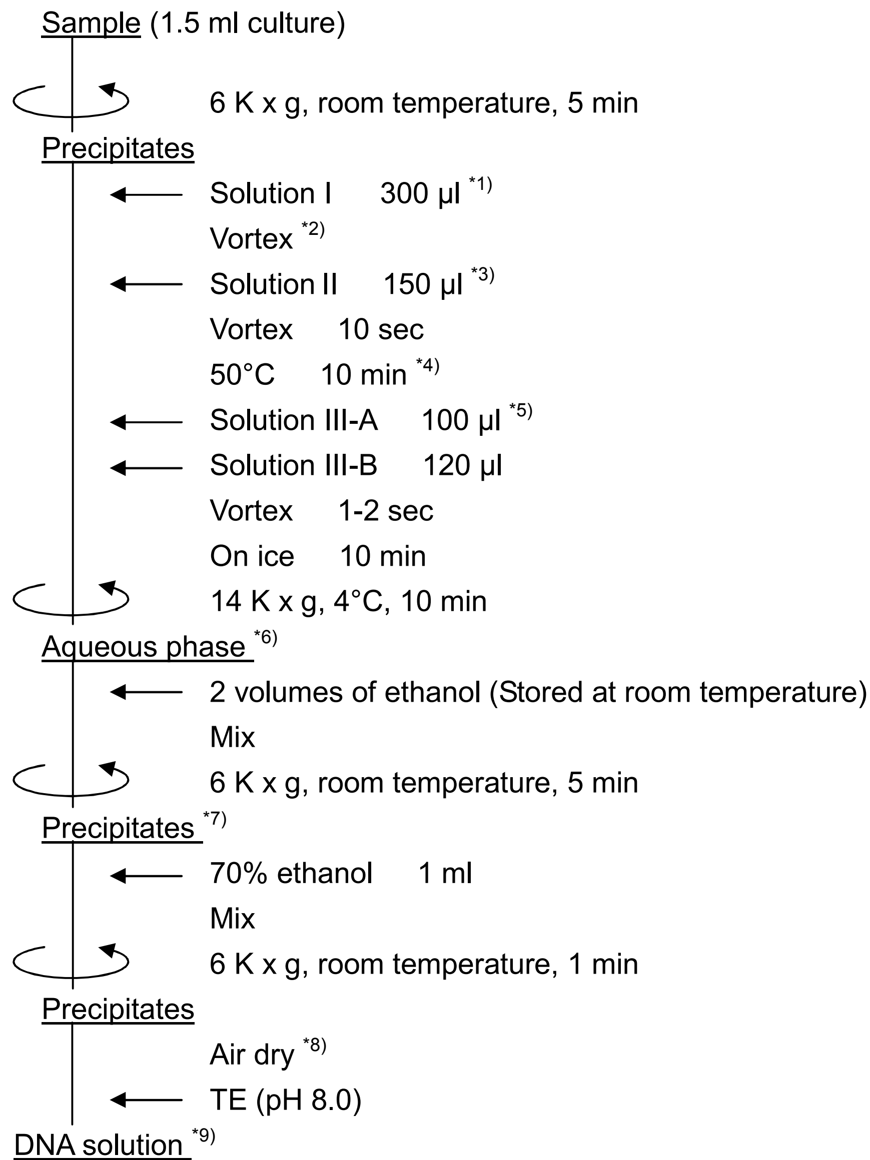
*9） Centrifuge immediately after adding ethanol stored at room temperature. If left standing at a low temperature such as -20°C, contaminants may be mixed in.

*10） Since completely dried precipitates become very difficult to dissolve, make sure not to dry too much.

*11） When insoluble substances exist in the DNA solution, dissolve DNA by standing on ice for a while, centrifuge lightly and use the supernatant. When RNA in the DNA solution is to be removed, add attached RNase A to a final concentration of 10-20 µg/ml and react at 37°C for 30 min. If necessary, perform phenol/chloroform treatment after the reaction. (See p. 8, *VI Troubleshooting.*)

2. Yeasts and bacteria

■ Protocol



*1） White crystals may appear in the Solution I. In such cases, dissolve the crystals completely in a water bath at about 50°C, stir so that the content becomes homogeneous and then use it. Also, since the Solution I contains protein denaturant and the like, handle with care. If the Solution I enters eye or contacts skin, immediately wash well with a large amount of water.

*2） Suspend the bacteria body well.

*3） The main component of the Solution II included in this product is benzyl chloride. Benzyl chloride is a designated hazardous material under the Fire Service Act. Handle with care. (See p. 2, *IV Handling precautions*.)
If eye, skin or the like make contact with the reagent, perform the following procedures immediately.

If the reagent enters eye :
Immediately wash with a large amount of water for not less than 15 min.

Skin contact :
Wash with a large amount of water using soap.

Inhalation :
Rest and stay warm in a well-ventilated area.

Accidental ingestion :
Rinse out the mouth, drink activated charcoal suspended in water and consult a physician. Since the mucous membrane in the stomach and the like is damaged, do not force vomiting.

Also, if there are other abnormal symptoms, immediately consult a physician.

*4） Cell walls, cell membranes, nuclear membranes and the like are disrupted by benzyl chloride, which is the main component of the Solution II, and DNA is dissolved into aqueous phase.

*5） Shake well the Solution III-A to homogeneity and suck out with a pipette tip with a cut edge.

*6） Avoid taking substances other than the aqueous phase, such as benzyl chloride in the organic phase and the solid white substance found after the centrifugation, as much as possible. If substances other than the aqueous phase are mixed in, centrifuge again and collect only the aqueous phase.

*7） DNA precipitates may not be visible.

*8） Since completely dried precipitates become very difficult to dissolve, make sure not to dry too much.

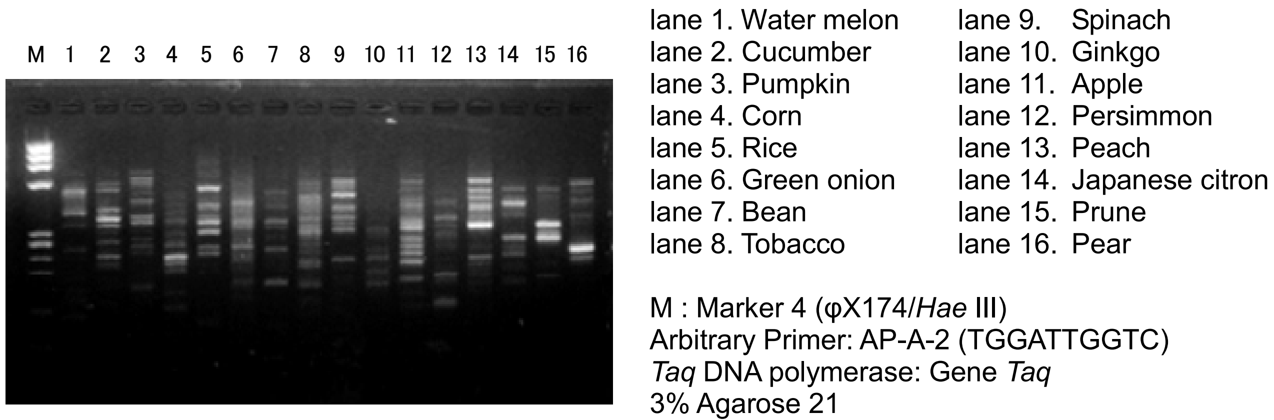
*9） When insoluble substances exist in the DNA solution, dissolve DNA by standing on ice for a while, centrifuge lightly and use the supernatant. When RNA in the DNA solution is to be removed, add attached RNase A to a final concentration of 10-20 μg/ml and react at 37°C for 30 min. If necessary, perform phenol/chloroform treatment after the reaction. (See p. 8, *VI Troubleshooting*.)

VI Troubleshooting

Trouble	Countermeasure
White crystals appear in the Solution I.	Dissolve crystals completely in a water bath at about 50°C, stir so that the content becomes homogeneous and then use it.
The Solution III-A is evaporated to dryness leaving only white solid phase.	Dissolve using sterilized distilled water. (1) Add H_2O until the entire solid phase is immersed. (2) Since the solid phase has water absorbing property, wait for a while. (3) When the surface is dried, add H_2O again. (4) Repeat H_2O addition and water absorption until entire surface of the solid phase is covered by H_2O.
It is difficult to collect the supernatant because the plant sample is floating after the centrifugation after adding the Wash Buffer + 2-ME.	Depending on the plant species, some sample may not be precipitated by the centrifugation. In such cases, dip the end of a pipette tip into the solution and suck out. Sometimes pieces of the plant may be sucked out together but this is unavoidable.
The supernatant after adding the Solution III-A, B and centrifuging is turbid or colored.	After transferring the supernatant to another tube, add 100 µl of the Solution III-A and 120 µl of the Solution III-B again, mix and centrifuge (14 K x g, 4°C, 10 min), and use the supernatant. (▼)
DNA is brown after ethanol precipitation and centrifuging.	If the precipitates are found to have brown coloring, perform re-extraction. In such cases, try the following treatments in the extraction procedure. (1) Perform $NaBH_4$ treatment. (See p. 3.) (2) If 2-Mercaptoethanol was not added to the Wash Buffer and Solution I, then add 2-Mercaptoethanol. (See p. 3.) (3) Incubate at 50°C for 10 min after adding the Wash Buffer + 2-ME to the plant sample. (4) Perform (▼) described above.
White precipitates other than DNA, are formed.	・ When the precipitates are insoluble in TE, centrifuge and use the supernatant. (▲) ・ Try (▼) described above in the extraction procedure.
DNA precipitate is not dissolved.	Leave standing at 4°C overnight or at 50°C for 1 hr. Pipetting may be performed but the obtained DNA may be broken.
Yield is low.	・ Samples should be as fresh as possible. ・ Use frozen pulverized sample. If freezing and pulverization cannot be performed, mince the sample as finely as possible. ・ Scale up. ・ Make the Solution II treating time longer. Keep in mind that this may also increase contamination by foreign matter.
A large amount of RNA is contaminating the obtained DNA solution.	Add the attached RNase A to the obtained DNA solution to a final concentration of 10-20 µg/ml and react at 37°C for 30 min. If necessary, perform phenol/chloroform treatment after the reaction.
No amplification is found when PCR is performed using the obtained DNA as a template.	・ When the DNA solution is colored, try (▼) described above. If white precipitates, which are different from DNA, are obtained, try (▲) described above. ・ Inhibition of PCR can be reduced by decreasing the amount of the template. (1/10 dilution etc.)
Obtained DNA is broken down.	Use a pipette tip with a cut edge to prevent physical breakdown of DNA during the extraction process.

[RAPD analysis using DNA extracted from plant leaves]

RAPD analyses were performed using 1/100 amount of DNA extracted from 0.1 g of respective frozen pulverized leaves.



lane 1. Water melon
lane 2. Cucumber
lane 3. Pumpkin
lane 4. Corn
lane 5. Rice
lane 6. Green onion
lane 7. Bean
lane 8. Tobacco
lane 9. Spinach
lane 10. Ginkgo
lane 11. Apple
lane 12. Persimmon
lane 13. Peach
lane 14. Japanese citron
lane 15. Prune
lane 16. Pear

M : Marker 4 (φX174/*Hae* III)
Arbitrary Primer: AP-A-2 (TGGATTGGTC)
Taq DNA polymerase: Gene *Taq*
3% Agarose 21

[RAPD-PCR]

・PCR Mixture

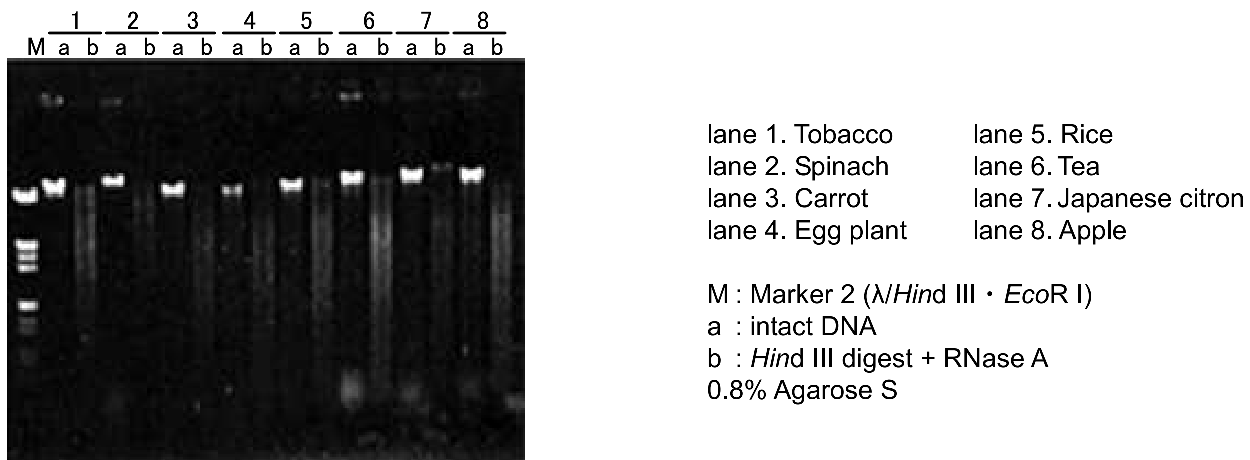
Template DNA	1 μl
10×Gene *Taq* Universal Buffer	5 μl
dNTP Mixture (2.5 mM each)	4 μl
AP-A-2 primer (10 μM)	1 μl
Gene *Taq* (5 units/μl)	0.25 μl
H_2O	38.75 μl
	50 μl

・PCR Condition

95°C	3 min.	1 cycle
40°C	5 min.	
72°C	1 min.	
↓		
95°C	15 sec.	25 cycles
40°C	2 min.	
72°C	1 min.	
↓		
72°C	5 min.	

[Restriction enzyme reaction of DNA extracted from plant leaves]

Restriction enzyme reactions (5-20 units) are performed using 1/20 of the amount of DNA extracted from 0.1 g of respective frozen pulverized leaves.



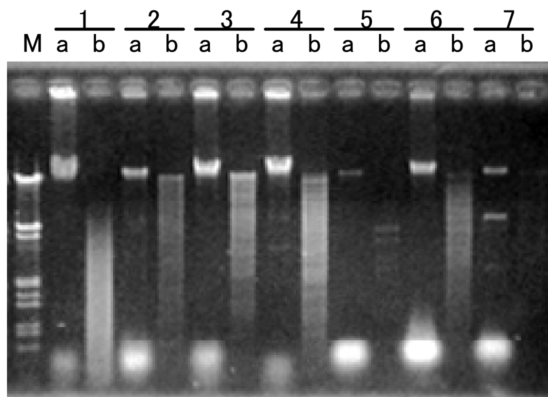
lane 1. Tobacco
lane 2. Spinach
lane 3. Carrot
lane 4. Egg plant
lane 5. Rice
lane 6. Tea
lane 7. Japanese citron
lane 8. Apple

M : Marker 2 (λ/*Hin*d III・*Eco*R I)
a : intact DNA
b : *Hin*d III digest + RNase A
0.8% Agarose S

(Note) The above data were obtained using DNA treated with $NaBH_4$ when extracted.

[Restriction enzyme reaction of DNA extracted from bacteria and yeasts]

Restriction enzyme reactions (5-20 units) are performed using 1/20 amount of DNA extracted from 1.5 ml of respective liquid cultures.



lane 1. *Serratia marcescens*
lane 2. *Moraxella bovis*
lane 3. *Haemophilus influenzae c*
lane 4. *Proteus vulgaris*
lane 5. *Shizosaccharomyces pombe*
lane 6. *Pichia pastoris*
lane 7. *Saccaromyces cerevisiae*

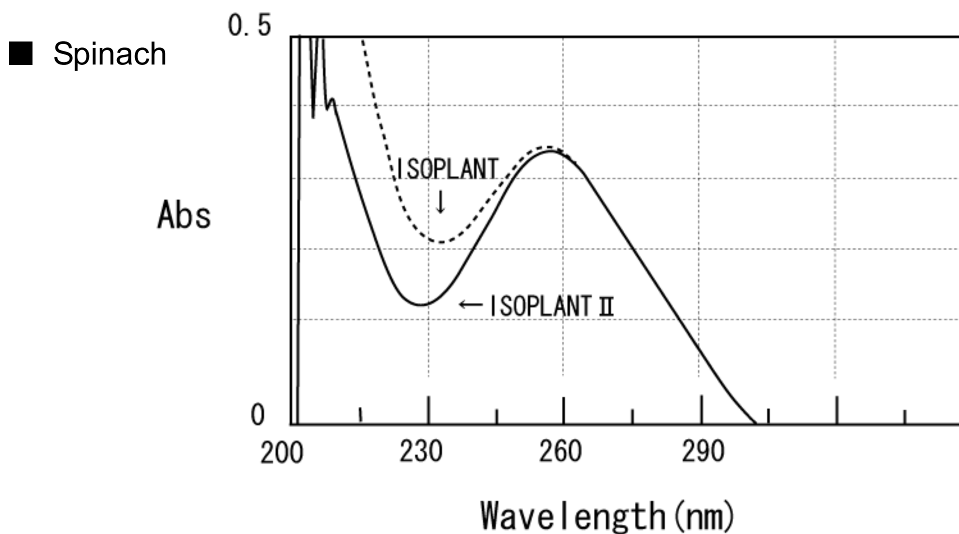
M : Marker 2 (λ/*Hin*d III・*Eco*R I)
a : intact DNA
b : *Hin*d III digest + RNase A
0.8% Agarose S

[Comparison of purity between ISOPLANT II and a conventional product (ISOPLANT)]

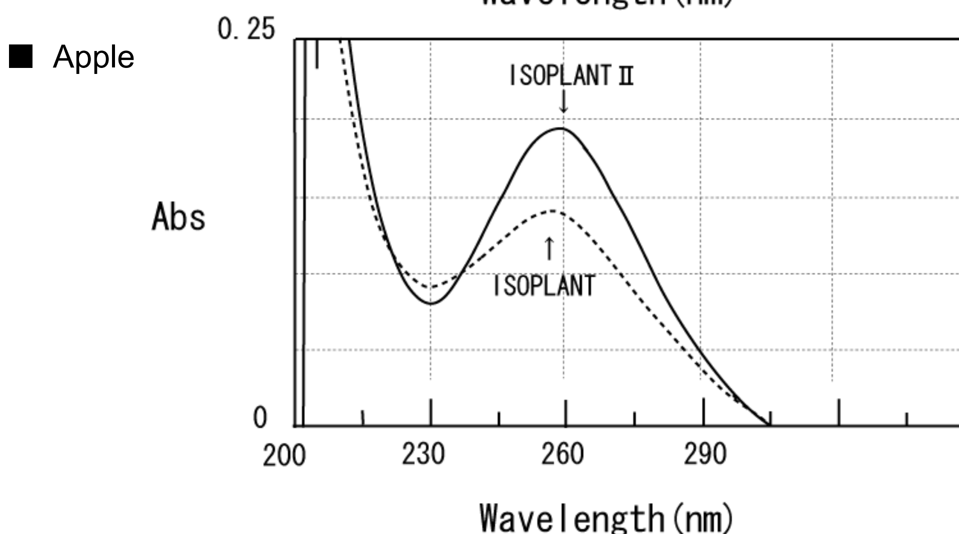
DNA was extracted from the same amount of samples of spinach and apple leaves using ISOPLANT II and the conventional product (ISOPLANT), and the absorbance (200-300 nm) was measured.

DNA extracted with ISOPLANT II demonstrates a smooth mound shape having a peak at 260 nm, indicating that these are highly purified DNA preparations from which contaminants such as polysaccharides and polyphenols have been removed.

■ Spinach



■ Apple



VIII References

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2. C. S. Kim, C. H. Lee, J. S. Shin, Y. S. Chung and N. I. Hyung:
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