Handbook v1.0

The Best Way to GENOMIC DNA

Never Before ! 4-28-260 !
4 kinds of Products divided !
28 Protocols provided !
260 Samples adjusted !
i-genomic series

PART IV

i-genomic Plant
DNA Extraction Mini Kit Handbook

Leaf

Type A Protocol
Type B Protocol

Stem

Type C Protocol

Root

Type D Protocol

Fruit

Type E Protocol

Seed

Type F Protocol
Type G Protocol

www.interchim.com

interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

i-genomic Plant DNA Extraction Mini Kit

Plant

iNtRON Biotechnology, Inc.

Contents

i-genomic Plant DNA Mini Kit Contents

i-genomic Plant DNA Extraction Mini Kit
(Cat. No. 17371, 50 columns)

Table 1. Kit Contents

Label	Description	Contain
Buffer PG[1]	Lysis Buffer	30 ml
Buffer PPT	Precipitation buffer	7 ml
Buffer PB	Binding Buffer	50 ml
Buffer PWA	Washing Buffer A	40 ml
Buffer PWB(concentrate)[2]	Washing Buffer B	10 ml (add 40 ml of EtOH)
Buffer PE [3]	Elution Buffer	20 ml
Enhancer Solution	Binding enhancer buffer	0.5 ml
Spin Columns (Green color O-ring)	Inserted into the collection tubes. (2.0 ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	100 tubes
RNase A Solution [4]	20 mg/ml (store at -20°C)	0.3 ml
Proteinase K Solution [5]	20 mg/ml (store at -20°C)	1.2 ml

[1] Contains a chaotropic salt. Carefully handle. See page 7 for safety information.

[2] Buffer PWB is supplied as concentrate. Add 40 ml of ethanol (96 ~ 100%) according to the bottle label before use.

[3] Buffer PE is finally 10 mM Tris-HCl (pH 8.0). You may use your lab's buffer.

[4] Store at -20°C. The RNase A solution is completely free of DNase activity.

[5] Store at -20°C. After thawing, freshly use. We recommend to aliquot to small volume of Proteinase K. Use carefully according to the instruction manual (page 23).

Figure 1. i-genomic Plant DNA Extraction Mini Kit

Storage

We recommend that all components of i-genomic Plant DNA Extraction Mini Kit is stored dry at room temperature (15 ~ 25℃). However, two components, including RNase A, and Proteinase K as a stock solution, should be stored at -20℃, and are stable for 1 year under these conditions.

Product Use Limitations

All i-genomic series Kits are developed, designed and sold for research purpose only.
They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

Precautions and Safety Information

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant.
Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

Product Warranty and Satisfaction Guarantee

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

Quality Control

As iNtRON quality control program, the performance of iNtRON's products are monitored routinely on a lot-to-lot basis. The genomic DNA yield of i-genomic series Genomic DNA Mini Kit is tested by preparing various samples and assaying the genomic DNA yield spectrophotometrically. The quality of isolated genomic DNA is checked by restriction digestion, PCR, agarose gel electrophoresis, and spectrophotometry. The i-genomic Plant DNA Mini Kit is tested to ensure the absence of DNase contamination. All buffers are each incubated with 1mg pUC18 DNA for 6 hours at 37℃. The DNA is then visualized by electrophoresis on an agarose gel and compared to a positive control to determine if any linear or nicked plasmid DNA is visible.

Safety Information

When working with chemicals, always should wear a suitable lab coat, disposable gloves and protective goggles. For more information, please request the appropriate material safety data sheets (MSDS). Do not add bleach or acidic solutions directly to the waste.
Buffer PG contains a chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water

Technical Assistance

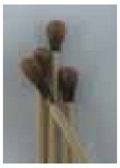
Our Technical Assistance Team is staffed by experienced researchers with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the i-genomic series Genomic DNA Extraction Mini Kits or other products in general, please do not hesitate to contact us. Your information and questions are helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about products' performances or new applications and techniques. For technical assistance and more information, please call or send e-mail.

Hot Line e-mail : intronbio@intronbio.com, info@intronbio.com

i-genomic series
Genomic DNA Extraction Mini Kits

i-genomic series Genomic DNA Extraction Mini Kits provide four kinds of kits according to the type of samples as seen in Table 2. These i-genomic series Kits provide a fast and easy way to purify DNA from various samples. The kits procedures provide pure genomic DNA for reliable PCR performance Southern blotting less than 1~2 hours. Purification require no phenol or chloroform extraction or alchol precipitation. Pure DNA extracted by i-genomic series Kit is eluted in low-salt buffer or water, ready to use in downstream applications, including PCR, RAPD analysis, AFLP analysis, RFLP analysis, Southern blotting, microsatellite analysis, SNP-genotyping and quantitative real-time PCR. Purified DNA get an $A_{260/280}$ ratio of 1.7 ~ 1.9, indicating high purity of the DNA.

Table 2. Four Kinds of i-genomic series Genomic DNA Extraction Mini Kits

Product Name	Samples	Examples
CAT. NO. 17341 **i-genomic CTB** **DNA Extraction Mini Kit**	Cells Tissues Gram(-) Bacteria	Mouse / Guinea pig / Rabbit / Chicken / Zebra fish / Shrimp / Pig / Human cultured cells / Mouse cultured cells / Insect / Animal hair / Worm / Stool / Buccal swab / Gram(-) bacteria / Others
CAT. NO. 17351 **i-genomic Blood** **DNA Extraction Mini Kit**	Blood	Whole blood / Buffy Coat / Dried Spot / Blood Swab / Plasma / Serum / Others
Whole blood  ***Buffy Coat*** 	***Dried Spot***  ***Blood Swab*** 	***Plasma***  ***Serum*** 
CAT. NO. 17361 **i-genomic BYF** **DNA Extraction Mini Kit**	Gram(+) Bacteria Yeast Fungi	*Azotobacter sp.* *Staphylococcus sp.* *Saccharomyces sp.* *Aspergillus sp.* Others
CAT. NO. 17371 **i-genomic Plant** **DNA Extraction Mini Kit**	Plant	Leaf / Root / Stem / Fruit / Seed /Others

i-genomic series Protocol Table

Table 3. Protocol Table (28 kinds of protocols)

i-genomic series Kits provide 28 of different protocols for each sample, and therefore are compatible with almost all samples. Just select optimal protocol type according to your sample, and follow the procedure. Refer to each kit's instruction manual.

Protocol Name		CTB										
Protocol Type		A	B	C	D	E	F	G	H	I	J	K
Sample Treatment step	Preparation step	■	■	■	■	■	■	■	■	■	■	■
	Disrupt. & Homogen.	□	■	■	■	■	■	■	□	■	□	□
	Sample Sizing step	■	■	■	■	■	■	■	■	■	■	■
	Pre-Treating step	□	□	□	■	■	□	□	■	□	■	□
DNA Extraction Step	Pre-Lysis step	□	□	□	□	□	□	□	□	□	□	□
	Lysis step	■	■	■	■	■	■	■	■	■	■	■
	Precipitation step	□	□	□	□	□	□	□	□	□	□	□
	DNA Binding step	■	■	■	■	■	■	■	■	■	■	■
	Washing step A	□	□	□	□	□	□	□	□	□	□	□
	Washing step B	■	■	■	■	■	■	■	■	■	■	■
	Elution step	■	■	■	■	■	■	■	■	■	■	■
		Cultured Cell	Animal Tissue	Rodent Tail	Formalin Fixed Tissue	Paraffin Embedded Tissue	Animal Hairs	Insect / Worm	Stool	Bone	Buccal Swab	Gram(-) Bacteria
		Total : 11 Types										

Protocol Name		Blood						BYF			
Protocol Type		A	B	C	D	E	F	A	B	C	D
Sample Treatment step	Preparation step	■	■	■	■	■	■	■	■	■	■
	Disrupt. & Homogen.	□	□	□	□	□	□	□	□	■	□
	Sample Sizing step	■	■	■	■	■	■	■	■	■	■
	Pre-Treating step	□	□	■	■	□	□	□	□	□	■
DNA Extraction Step	Pre-Lysis step	□	□	□	□	□	□	■	■	■	■
	Lysis step	■	■	■	■	■	■	■	■	■	■
	Precipitation step	□	□	□	□	□	□	□	□	□	□
	DNA Binding step	■	■	■	■	■	■	■	■	■	■
	Washing step A	■	■	■	■	■	■	□	□	□	□
	Washing step B	■	■	■	■	■	■	■	■	■	■
	Elution step	■	■	■	■	■	■	■	■	■	■
		Whole Blood	Buffy Coat	Dried Spot	Blood Swab	Plasma	Serum	Gram(+) Bacteria	Yeast	Fungal Tissue	Fungi
		Total : 6 Types						Total : 4 Types			

Protocol Name		Plant						
Protocol Type		A	B	C	D	E	F	G
Sample Treatment step	Preparation step	■	■	■	■	■	■	■
	Disrupt. & Homogen.	□	■	■	■	■	■	■
	Sample Sizing step	■	■	■	■	■	■	■
	Pre-Treating step	□	□	□	□	□	□	□
DNA Extraction Step	Pre-Lysis step	□	□	□	□	□	□	□
	Lysis step	■	■	■	■	■	■	■
	Precipitation step	■	■	■	■	■	■	■
	DNA Binding step	■	■	■	■	■	■	■
	Washing step A	■	■	■	■	■	■	■
	Washing step B	■	■	■	■	■	■	■
	Elution step	■	■	■	■	■	■	■
		Lyophilized Leaf	Leaf	Stem	Root	Fruit	Seed (Others)	Seed (Gramineae)
		Total : 7 Types						

■ Perform
□ Do Not Perform

Sample Treatment Step

Preparation step : To prepare and to pick sample
Disrupt. & Homogen. : To disrupt or homogenize sample
Sample Sizing step : To take correct quantity of sample
Pre-Treating step : To treat PBS solution before lysis

DNA Extraction Step

Pre-Lysis & Lysis : To pre-lyse or lyse sample
Precipitation step : To precipitate protein, polysaccharides, etc.
DNA Binding step : To bind DNA to the membrane
Washing step A & B : To wash the columns
Elution step : To elute genomic DNA from the column

i-genomic Plant DNA Extraction Mini Kit

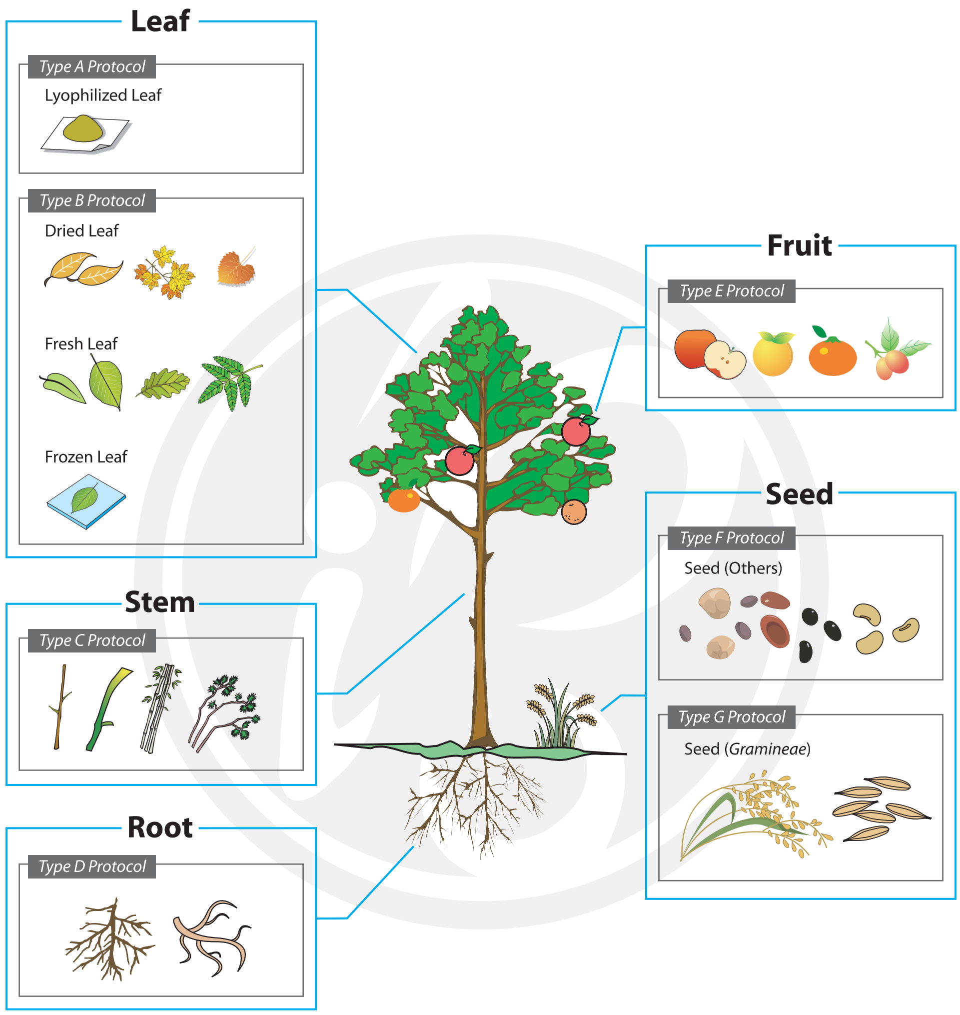
i-genomic Plant DNA Extraction Mini Kit provides a fast and easy way to purify DNA from plant-like samples such as various leaves, stems, roots, fruits, and seeds.
Furthermore, we have tested i-genomic Plant DNA Mini Kit to get more practical data with 104 plant samples. You can see vast sample photos, vast samples and vast practical data.

i-genomic Plant DNA Mini Kit provides 7 kinds of protocols, Type A, Type B, Type C, Type D, Type E, Type F and Type G. You can also extract genomic DNA from various plant samples in addition to 75 plant samples by selecting an appropriate protocol. When you choose a protocol, please refer to Plant Sample List (Figure 2). If you need some more information for selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

Table 4. Seven Kinds of Protocols according to Plant Sample

Plant Samples	Protocol Type
Leaf	
Lyophilized leaf	Type A Protocol
Fresh, Dried, or Frozen leaf	Type B Protocol
Stem	
Stem	Type C Protocol
Root	
Root	Type D Protocol
Fruit	
Fruit	Type E Protocol
Seed	
All seeds w/o *Gramineae*	Type F Protocol
Gramineae	Type G Protocol

(Ex) Seed samples



Leaf
Type A Protocol
Lyophilized Leaf
Type B Protocol
Dried Leaf
Fresh Leaf
Frozen Leaf
Stem
Type C Protocol
Root
Type D Protocol
Fruit
Type E Protocol
Seed
Type F Protocol
Seed (Others)
Type G Protocol
Seed (Gramineae)

Plant Samples Grouping according to Protocols

i-genomic Plant DNA Mini Kit provides seven kinds of protocols according to plant samples. We recommend to select an appropriate protocol for your samples. The samples show 75 samples tested with i-genomic Plant DNA Mini Kit (see Figure 2). You can extract efficiently genomic DNAs from various plant samples.

iNtRON customers are a major source of information regarding advanced or specialized use of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance, new applications or techniques.

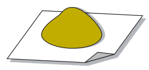
See next pages.

Figure 2. Plant Samples Grouping (Samples tested by iNtRON)

LEAF

For Lyophilized Leaf

Lyophilized Leaf

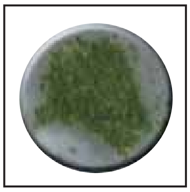


Type A Protocol

Persimmon
(*Diopyros kaki*)

Millet (Hog Millet)
(*Panicum millacerm*)

Mung Bean
(*Phasedus radiutus*)



Green Perilla
(*Perilla fru tescens*)

Paddy
(*Oryza sativa*)

Ginger
(*Zingiber officinale*)

Amaranth
(*Amarantus*)

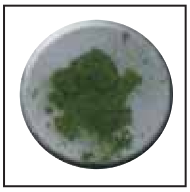
Corn
(*Zea mays*)

Leek
(Allium tuberosum)

Italian Millet
(Setania italica)

Adlay
(Coix lachrymajobi)

Ginseng
(Panax ginseng)



LEAF

For Fresh, Dried, or Frozen Leaf

Fresh Leaf **Dried Leaf** **Frozen Leaf**

Type B Protocol

Geranium
(Pelargonium inquinans)

Lettuce
(*Lactuca sarita*)

Peanut
(Arachis hypogaea)

Buckwheat
(*Fagopyrum esculentum*)

Green Pepper
(Capsicum annuum)

African Millet
(*Sorghum bicolor*)

Spinach
(Spinacia oleracea)

Rhododendron
(*Rhododendron schlippenbachii*)

Arabidopsis
(Arabidopsis)

Cabbage
(*Brassica olecrea*)

Cabbage
(Brassica campestris)

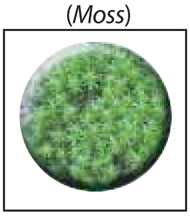
Radish (Turnip)
(*Brassica rapa*)

Sesame
(Sesamum indicum)

Black Bean
(*Glycine max*)

Fallen Leaf
(Fallen leaves)

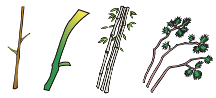
Moss
(*Moss*)



STEM

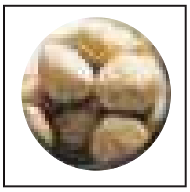
For Stem

Stem



Type C Protocol

Potato
(*Solanum tuberosum*)



Onion
(Allium cepa)

Elm
(*Ulmus davidiana*)

Radish
(Raphanus sativus)

Geranium
(*Pelargonium inquinans*)

Rhododendron
(Rhododendron schlippenbachii)

Sesame
(*Sesamum indicum*)

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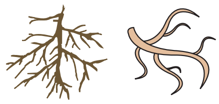
ROOT

For Root

Root



Type D Protocol

Sweet Potato
(*Ipomoea batatas*)

Carrot
(*Daucus carota var sativa*)

Platycodon
(*Platycodon grandiflorum*)

Radish (Turnip)
(*Brassica rapa*)

Sesame
(Sesamum indicum)

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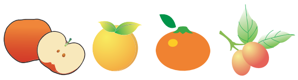
FRUIT

For Fruit

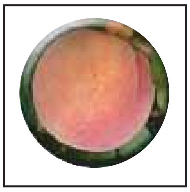
Fruit



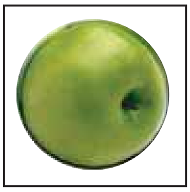
Type E Protocol

Melon
(*Cucumis melo*)

Peach
(Prunus persica)

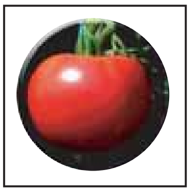


Apple
(*Malus pumila*)



Muscat
(Vitis spp)

Tomato
(*Lycopersicon esculentum*)



Pine Apple
(Ananas comosus)

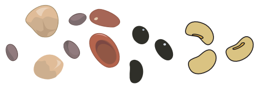
Grape
(*Vitis vinifera L.)*

iNtRON Waits For Your Data.

SEED

For All Seeds w/o Gramineae

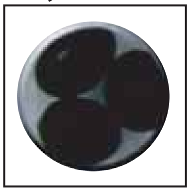
Seed (Others)



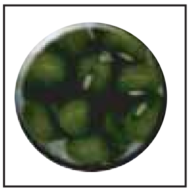
Type F Protocol

Leguminosae

Black Bean (*Glycine max*)

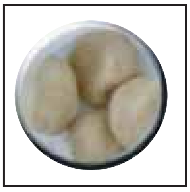


Mung Bean (*Phaseolus radiatus*)



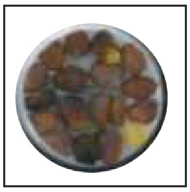
Yellow Bean (*Glycine max*)

Pea (*Pisum sativum*)

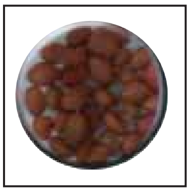


Cruciferae

Radish Type 1 (*Raphanus sativus*)



Radish Type 2 (*Raphanus sativus*)



Radish Type 3 (*Brassica rapa*)

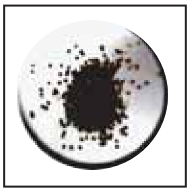
iNtRON Waits For Your Data.

Solanaceae

Green Pepper (*Capsicum annuum*)

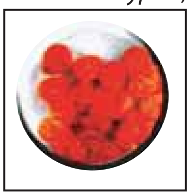
Paprika (*Capsicum annuum*)

Petunia (*Petunia hybrida*)



Tomato Type 1 (*Lycopersicon esculentum Type 1*)

Tomato Type 2 (*Lycopersicon esculentum Type 2*)



Tomato Type JPN (*Lycopersicon esculentum Type JPN*)

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Seed (Others)

SEED

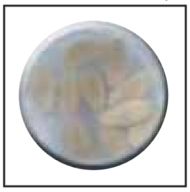
For All Seeds w/o Gramineae

Type F Protocol

Cucurbitaceae

Pumpkin (*Cucurbita moschata duchesne*)

Melon (*Cucumis melo var. makuwa*)



Cucumber (*Cucumis sativus*)

Water Melon (*Citrullus vulgaris*)

Gourd (*Lagenaria leucantha*)

Melon (*Cucumis melo*)

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Others

Carrot (*Daucus carota var. sativa*)

Spinach (*Spinach oleracea*)

Sesame (*Sesamum indicum*)

Sunflower (*Helianthus annuus*)

SEED

For Gramineae

Seed *(Gramineae)*

Type G Protocol

Grass
(*Zoysia japonica*)

Rice
(*Oryza sativa*)

African Millet
(*Sorghum bicolor*)

Wheat
(*Triticum aestivum vulgare*)

Barley
(*Hordeum vulgare var. hexastichon*)

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Equipments and Reagents to Be Supplied by User

i-genomic Plant DNA Mini Kit provides almost all reagents for extracting DNA, including RNase A and Proteinase K stock solutions. However, you should prepare some equipments and reagents as follows for a fast and easy extraction. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- ✓ Equipment for disruption and homogenization, including grinding jar set (mortar)
- ✓ Pipettes and pipette tips
- ✓ Water bath or heating block
- ✓ Vortex mixer
- ✓ Microcentrifuge with rotor for 2.0 ml tubes.
- ✓ Microcentrifuge tubes (1.5 ml)
- ✓ Liquid nitrogen
- ✓ Absolute ethanol (EtOH, 96 ~ 100%)
- ✓ Ice
- ✓ Other general lab equipments

Notice Before Use

Important Points Before Starting

- **Buffer PWB (Washing Buffer B)**
  : Buffer PWB is supplied as concentrate. Before using for the first time, be sure to add 40 ml of absolute ethanol (96 ~ 100% EtOH) to obtain a working solution

- **Enhancer Solution**
  : We recommend keeping the Enhancer Solution at 2 ~ 8°C upon arrival, and then it is stable for 1 year. The Enhancer Solution is a yellow color solution with precipitates upon storage. Before use, warm it to 65°C to redissolve. After use, store at 2 ~ 8°C.

- **Proteinase K Solution (20 mg/ml)**
  : Proteinase K possesses a high specific activity which remains stable over a wide range of temperature and pH values with substantially increased activity at higher temperatures. Proteinase K Solution shows a milk-white color, since it is supplied as concentrate. After thawing, freshly use. DO NOT heat to redissolve. We recommend to aliquot to small volume of Proteinase K Solution.

- **Pre-heat a water bath or a heating block to 65°C.**

- **Centrifugation**
  : All centrifugation steps are carried out at RT (15 ~ 25°C) in a microcentrifuge.

Column Information

- **i-genomic series Spin Column**

Column Membrane [1]	Silica-based membrane
Spin Column [1]	Individually, is inserted in a 2.0 ml collection tube [2].
Loading Capacity	Maximum 800 μl
DNA Binding Capacity	Maximum 45 μg
Recovery	85 ~ 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 ~ 200 μl of elution buffer

[1] After use, seal the pack containing spin columns tightly without getting dry. Then, the spin columns are stable for over 1 years under these conditions. It's not good for DNA binding to be dried completely.
[2] Additional collection tubes (100 ea) are also supplied for your convenient handling.

Important Notes

Choosing the Right Protocol according to Plant Sample

Seven kinds of different protocols in this handbook provide detailed instructions to use i-genomic Plant DNA Mini Kit for purifying genomic DNA from various plant samples (see Figure 2). These protocols are optimized for use. Especially, with iNtRON's 75 samples more than five categories, we have verified practically by several experiments to ensure the quality and the application of i-genomic Plant DNA Mini Kit. We recommend you will choose the right protocol according to your plant sample. For more information, please contact iNtRON Technical Assistance Team.

Collection and Storage of Plant Samples

Generally, for higher quality of genomic DNA, it is preferable to collect from young plant materials. The fresh plant tissues contain more viable cells per weight and therefore result in higher yields and purity of genomic DNA. If plant tissue will not be used freshly, after harvesting, we recommend it should be quickly frozen in liquid nitrogen, and then stored at -80^{o}C. When grind plant tissues, use liquid nitrogen as in detailed instruction manual. Ground plant tissue powder can also be stored at -80^{o}C.

Alternatively, plant tissues can be dried or lyophilized after harvesting to allow storage at room temperature. To ensure high quality of genomic DNA, plant tissues should be completely dried within 24 hours of collection.

Disruption and Homogenization

Almost all samples can be disrupted on a mortar after freezing in liquid nitrogen without Buffer PG. Namely, disruption can be performed without lysis buffer by keeping the sample submerged in liquid nitrogen before and during disruption on a mortar. Especially hard tissues, such as roots or seeds, are relatively difficult to be disrupted, and therefore be careful to use a mortar in liquid nitrogen. Alternatively, fresh leaf can be directly disrupted in lysis buffer (Buffer PG) without using liquid nitrogen, but this may cause shearing of high molecular-weight DNA. We do not recommend frozen materials to disrupt in lysis buffer as this can result in low yields and degraded DNA.

In case of dried or lyophilized leaf, this disruption step is omitted, and therefore do not add liquid nitrogen. After Sample Sizing step, directly treat lysis buffer (Buffer PG) to the sample for lysis step.

For optimal results, we recommend to keep the disruption time as short as possible.

When disruption for more than 1 minute it may lead to shearing of genomic DNA.

Notes for Sample Sizing

Measuring the Sample Amount after Disruption Step

We recommend to measure the amount of starting material after disrupting plant tissues in i-genomic Plant DNA Mini Kit, since it brings a loss of starting material. If the plant samples are disrupted on a mortar submerged in liquid nitrogen, the samples will turn into powder-like form.
It makes them to measure conveniently the amount of starting material.

Table 5 shows a recommended amount of starting material after disruption step according to plant samples. Please follow the manual instruction not to be over the required amounts.

Sample Volume

i-genomic Plant DNA Mini Kit procedures are optimized for 5 mg ~ 100 mg of wet-weight starting material. Table 5 provides guidelines according to the plant tissues.
Exceeding the recommended amount of starting material will result in inefficient lysis, resulting in low yield and purity. In the large, DNA yields and purity will be varied by depending on genome size, sample viscosity and age of sample.

Table 5. Recommended Volume of Starting Material according to Plant Sample

▪ **Type A Protocol : Lyophilized Leaf**

Plant Tissue	Amount
Persimmon *(Diopyros kaki)*	5 mg
Millet, Hog Millet *(Panicum millacerm)*	5 mg
Mung Bean *(Phasedus radiutus)*	5 mg
Perilla *(Perilla fru tescens)*	5 mg
Paddy *(Oryza sativa)*	5 mg
Ginger *(Zingiber officinale)*	5 mg

Plant Tissue	Amount
Amaranth *(Amarantus)*	5 mg
Corn *(Zea mays)*	5 mg
Leek *(Allium tuberosum)*	5 mg
Italian Millet *(Setania italica)*	5 mg
Adlay *(Coix lachrymajobi)*	5 mg
Ginseng *(Panax ginseng)*	5 mg

▪ **Type B Protocol : Leaf**

Fresh, Dried, or Frozen Leaf

Plant Tissue	Amount
Geranium *(Pelargonium inquinans)*	50 mg
Lettuce *(Lactuca sarita)*	50 mg
Peanut *(Arachis hypogaea)*	50 mg
Buckwheat *(Fagopyrum esculentum)*	50 mg
Green Pepper *(Capsicum annuum)*	50 mg
African Millet *(Sorghum bicolor)*	50 mg
Spinach *(Spinacia oleracea)*	50 mg
Rhododendron *(Rhododendron schlippenbanchii)*	50 mg

Plant Tissue	Amount
Arabidopsis (Arabidopsis)	50 mg
Cabbage *(Brassica olecrea)*	50 mg
Cabbage *(Brassica campestris)*	50 mg
Radish,Turnip *(Brassica rapa)*	50 mg
Sesame *(Sesamum indicum)*	50 mg
Black Bean Leaves *(Glycine max)*	50 mg
Fallen Leaf *(Fallen leaves)*	50 mg
Moss *(Moss)*	50 mg

▪ **Type C Protocol : Stem**

Plant Tissue	Amount
Potato *(Solanum tuberosum)*	50 mg
Onion *(Allium cepa)*	50 mg
Elm *(Ulmus davidiana)*	50 mg
Radish *(Raphanus sativus)*	50 mg

Plant Tissue	Amount
Geranium *(Pelargonium inquinans)*	50 mg
Rhododendron *(Rhododendron schlippenbanchii)*	50 mg
Sesame *(Sesamum indicum)*	50 mg

▪ **Type D Protocol : Root**

Plant Tissue	Amount
Sweet Potato *(Ipomoea batatas)*	50 mg
Carrot *(Daucus carota var.sativa)*	50 mg
Platycodon *(Platycodon grandiflorum)*	50 mg

Plant Tissue	Amount
Radish, Turnip *(Brassica rapa)*	50 mg
Sesame *(Sesamum indicum)*	50 mg

▪ **Type E Protocol : Fruit**

Plant Tissue	Amount
Melon *(Cucumis melo)*	100 mg
Peach *(Prunus persica)*	100 mg
Apple *(Malus pumila)*	100 mg
Muscat *(Vitis spp)*	100 mg

Plant Tissue	Amount
Tomato *(Lycopersicon esculentum)*	100 mg
Pine Apple *(Ananas comosus)*	100 mg
Grape *(Vitis vinifera L.)*	100 mg

▪ **Type F Protocol : Seed**

Leguminosae

Plant Tissue	Amount
Black Bean *(Glycine max)*	50 mg
Mung Bean *(Phaseolus radiatus)*	50 mg
Yellow Bean *(Glycine max)*	50 mg
Pea *(Pisum sativum)*	50 mg

Cruciferae

Plant Tissue	Amount
Radish Type 1 *(Raphanus sativus)*	50 mg
Radish Type 2 *(Raphanus sativus)*	50 mg
Radish Type 3 *(Brassica rapa)*	50 mg

Solanaceae

Plant Tissue	Amount
Green Pepper *(Capsicum annuum)*	50 mg
Paprika *(Capsicum annuum)*	50 mg
Petunia *(Petunia hybrida)*	50 mg

Plant Tissue	Amount
Tomato Type 1 *(Lycopersicon esculentum Type 1)*	50 mg
Tomato Type 2 *(Lycopersicon esculentum Type 2)*	50 mg
Tomato JPN *(Lycopersicon esculentum JPN)*	50 mg

Cucurbitaceae

Plant Tissue	Amount
Pumpkin *(Cucurbita moschata duchesne)*	50 mg
Melon *(Cucumis melo var. makuwa)*	50 mg
Cucumber *(Cucumis sativus)*	50 mg

Plant Tissue	Amount
Water Melon *(Citrullus vulgaris)*	50 mg
Gourd *(Lagenaria leucantha)*	50 mg
Melon *(Cucumis melo)*	50 mg

Others

Plant Tissue	Amount
Umbellifeae	
Carrot *(Daucus carota var. sativa)*	50 mg
Chenopodiaceae	
Spinach *(Spinacia oleracea)*	50 mg

Plant Tissue	Amount
Pedaliaceae	
Sesame *(Sesamum indicum)*	50 mg
Compositae	
Sunflower *(Helianthus annuus)*	50 mg

- **Type G Protocol : Seed**

Gramineae

Plant Tissue	Amount
Grass *(Zoysia japonica)*	100 mg
Rice *(Oryza sativa)*	10 mg
African Millet *(Sorghum bicolor)*	10 mg

Plant Tissue	Amount
Wheat *(Triticum aestivum vulgare)*	10 mg
Barley *(Hordeum vulgare var. hexastichon)*	10 mg

Standard Protocols

Plant
Type A
Bench Protocol

Type A Protocol
For Lyophilized Leaf

A-1. Sample Treatment Step

▪ ***Lyophilized Leaf***

■ I. Preparation step ☐ II. Disrupt. & Homogen. ■ III. Sample Sizing step ☐ IV. Pre-Treating step

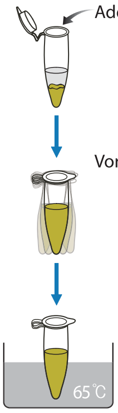
A-2. DNA Extraction Step

▪ ***Lyophilized Leaf***

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

II. Lysis step

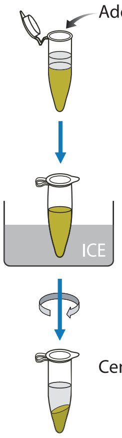


1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

2 Incubate the lysate at 65℃ for 30min
(invert 5~6 times for more lysis)

III. Precipitation step

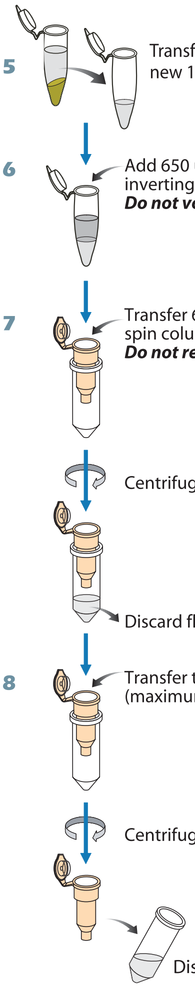


3 Add 100 ul of Buffer PPT and mix well

Incubation on ice for 5 min

4 Centrifuge at 13,000 rpm(RT) for 5 min

IV. DNA Binding step



5 Transfer 200 ul of supernatant to new 1.5 ml tube

6 Add 650 ul of Buffer PB and mix by inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1 min

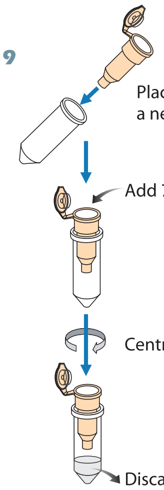
Discard flow-through

8 Transfer the rest of mixture (maximum 200 ul) to spin column

Centrifuge at 13,000 rpm for 1 min

Discard

V. Washing setp A



9

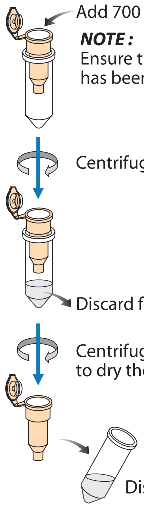
Place spin column into a new 2.0 ml collection tube

Add 700 ul of Buffer PWA

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

VI. Washing step B



10

Add 700 ul Buffer PWB to spin column

NOTE :
Ensure that 40 ml of ethanol(EtOH) has been added to Buffer PWB

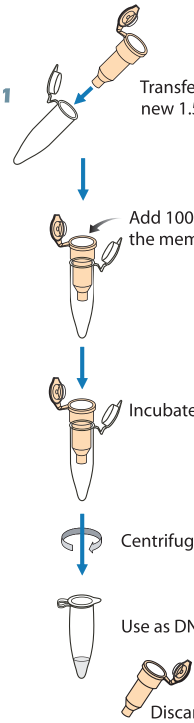
Centrifuge at 13,000 rpm for 1 min

Discard flow-through

Centrifuge at 13,000 rpm for 1 min to dry the membrane

Discard

VII. Elution step



11

Transfer spin column to new 1.5 ml tube

Add 100 ul of Buffer PE directly onto the membrane

Incubate at RT for 1 min

Centrifuge at 13,000 rpm for 1 min

Use as DNA template

Discard

A-1. Sample Treatment Step

Lyophilized Leaf

■ I. Preparation step ☐ II. Disrupt.& Homogen. ■ III. Sample Sizing step ☐ IV. Pre-Treating step

I. Preparation step

1. Prepare lyophilized leaf sample.

Plant tissue can be lyophilized after harvesting to allow storage at room temperature (15 ~ 20°C). To ensure DNA quality, we recommend that samples should be completely lyophilized within 24 hours of collection. Generally, lyophilized leaves are fine powder form, and therefore don't need to have any special disruption & homogenization steps. Furthermore, when using lyophilized tissue, the samples do not need to be frozen in liquid nitrogen. Directly, you can perform the Sample Sizing Step.

III. Sample Sizing step

2. Measure 5 mg of lyophilized leaf, and then transfer into 1.5 ml tube using a spatula.

It's difficult to handle to measure the fine powder sample due to its static electricity.
It can be inhibited by previously chill the spatula and 1.5 ml tube in liquid nitrogen.
Exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity.

A-2. DNA Extraction Step

Lyophilized Leaf

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

Equilibrate samples at room temperature (15 ~ 25°C).
Heat a water bath or heating block to 65°C for use in step 2.
All centrifugation steps should be carried out at room temperature.

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

With lyophilized leaf it absorbs lysis buffer, and becomes swollen. It may be difficult to handle plant tissue due to its viscosity. Always keep the recommended amount of starting material. Furthermore, vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65℃ for 30 min.

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended sample amount.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min.

Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step.

IV. DNA Binding step

5. Transfer carefully 200 ㎕ of supernatant from step 4 into a new 1.5 ml tube.

Although the supernatant is typically 350 ~ 400 ㎕, we recommend to recover only 200 ㎕ of lysate. More lysate can results in shearing of the DNA and contaminating the next step with impurities. When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 650 ㎕ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or by pipetting. DO NOT vortex.

This step is an equilibration step for binding genomic DNA to column membrane.
A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA Mini Kit proce ure.

7. Pipette 650 ㎕ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm (RT) for 1 min , and discard the flow-through. Reuse the collection tube in step 8.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

8. Repeat step 7 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer PWA, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 ㎕ Buffer PWB to the spin column, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.

It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.

NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

11. Place the spin column into a new 1.5 ml tube (not supplied), and 100 ㎕ Buffer PE directly onto the membrane. Incubate at room temperature for 1 min, and then centrifuge at 13,000 rpm for 1 min to elute.

Elution with 50 ㎕ (instead of 100 ㎕) increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 ㎕ increases generally overall DNA yield.

NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

Alternatively, the tube can be reused for the second elution step to combine the eluates.

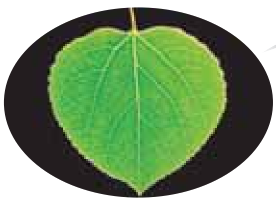
Plant
Type B
Bench Protocol

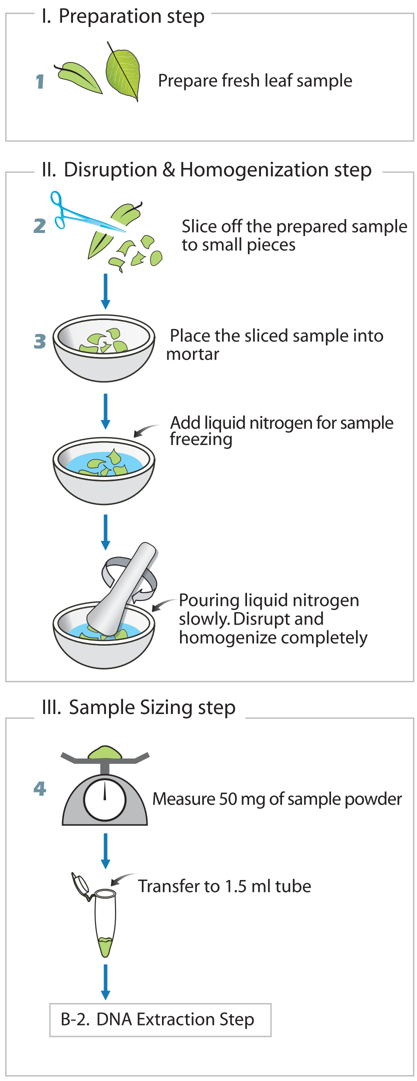
Type B Protocol
For Fresh, Dried, or Frozen Leaf

B-1. Sample Treatment Step

- ***Fresh Leaf***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

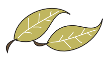




▪ ***Dried Leaf***

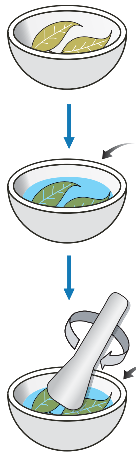
■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step ☐ IV. Pre-Treating step

I. Preparation step



1 Prepare dried leaf sample

II. Disruption & Homogenization step

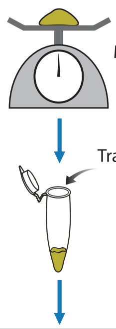


2 Place the sample into mortar

Add liquid nitrogen for sample freezing

Pouring liquid nitrogen slowly, Disrupt and homogenize completely

III. Sample Sizing step



3 Measure 50 mg of sample powder

Transfer to 1.5 ml tube

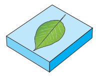
B-2. DNA Extraction Step

Type B Protocol
Leaf

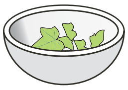
▪ ***Frozen Leaf***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step ☐ IV. Pre-Treating step

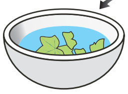
I. Preparation step

1  Prepare from frozen leaf sample

II. Disruption & Homogenization step

2  Place the sliced or whole sample into mortar

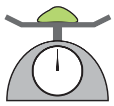
↓

 Add liquid nitrogen for sample freezing

↓

 Pouring liquid nitrogen slowly. Disrupt and homogenize completely

III. Sample Sizing step

3  Measure 50 mg of sample powder

↓

 Transfer to 1.5 ml tube

↓

B-2. DNA Extraction Step

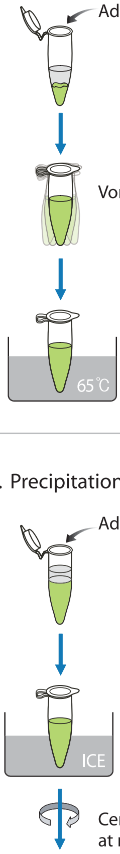
B-2. DNA Extraction Step

▪ ***Fresh, Dried, or Frozen Leaf***

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

II. Lysis step



1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

2 Incubate the lysate at 65℃ for 30 min
(invert 5 ~ 6 times for more lysis)

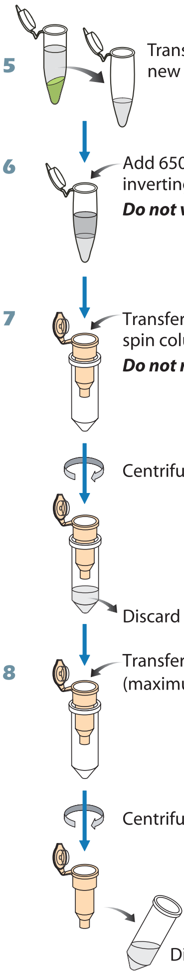
III. Precipitation step

3 Add 100 ul of Buffer PPT and mix well

Incubation on ice for 5 min

4 Centrifuge at 13,000 rpm for 5 min
at room temperature

IV. DNA Binding step



5 Transfer 200 ul of supernatant to
new 1.5 ml tube

6 Add 650 ul of Buffer PB and mix by
inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to
spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1min

Discard flow-through

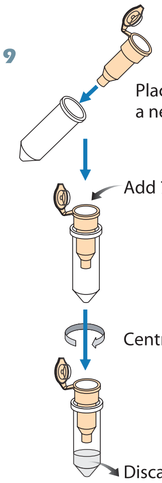
8 Transfer the rest of mixture
(maximum 200 ul) to spin column

Centrifuge at 13,000 rpm for 1 min

Discard

Type B Protocol
Leaf

V. Washing setp A



9

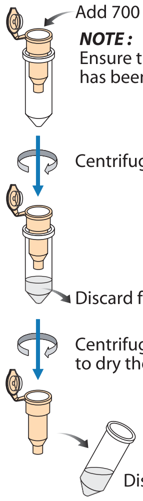
Place spin column into a new 2.0 ml collection tube

Add 700 ul of Buffer PWA

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

VI. Washing step B



10

Add 700 ul Buffer PWB to spin column

NOTE :
Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB

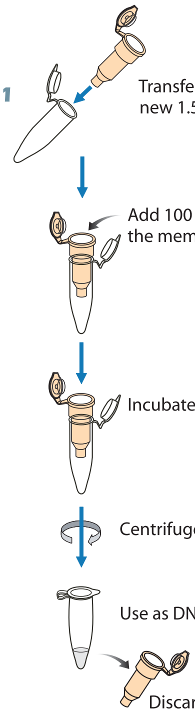
Centrifuge at 13,000 rpm for 1 min

Discard flow-through

Centrifuge at 13,000 rpm for 1 min to dry the membrane

Discard

VII. Elution step



11

Transfer spin column to new 1.5 ml tube

Add 100 ul of Buffer PE directly onto the membrane

Incubate at RT for 1 min

Centrifuge at 13,000 rpm for 1 min

Use as DNA template

Discard

B-1. Sample Treatment Step

▪ ***Fresh Leaf***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare fresh leaf sample.

If possible, it is preferable to collect young fresh leaf since they contain more viable cells per weight and therefore result in higher yields. In addition, young fresh leaves contain smaller amounts of polysaccharides and polyphenolics. Therefore, they are easier to handle than other plants. For storage of harvested fresh leaf, in general, when genomic DNA is to be isolated, plant leaves from most species should be frozen and kept at -80°C after harvesting. It is good for disruption and homogenization if the sample is sliced off when it stores at -20°C or -80°C.

II. Disruption & Homogenization step

2. Slice off the prepared sample to small pieces by the scalpel or scissor.

To reduce disruption and homogenization time, we recommend to slice it off.

3. Place the sliced sample material into grinding mortar and add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately step 4.

Disruption and homogenization time depends on the leaf samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of fresh leaf sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen.

Generally, it is a fine powder form after disruption and homogenization.

III. Sample Sizing step

4. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and 1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation.

And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity.

Type B Protocol
Leaf

▪ ***Dried Leaf***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare dried leaf sample.

If possible, it is preferable to collect young leaves since they contain more viable cells per weight and therefore result in higher yields. Alternatively, leaf can be also dried after harvesting to allow storage at room temperature (15 ~ 20°C).

II. Disruption & Homogenization step

2. Place the sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately step 3.

Disruption and homogenization time depend on the leaf samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of dried sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Generally, it is a fine powder form after disruption and homogenization.

III. Sample Sizing step

3. Measure 20 ~ 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

If too much dry, it absorbs all of Buffer PG (lysis buffer) to difficult to handle to lysis. And in that time, some dried sample becomes swollen. To prevent it, reduce the amount of starting sample material if it is. If it is, we recommended below 30 mg of amount starting material. Especially, the ocean dried grass organ. In case of organ, normally use 10 mg for amount of starting material. But in case of fallen leaves, use 50 mg for extracting DNA.

Exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and 1.5 ml tube in liquid nitrogen.

The freezing-thawing repetition of frozen sample will result in the DNA

▪ ***Frozen Leaf***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare frozen leaf sample.

It is important to keep the frozen leaf sample frozen in liquid nitrogen during all of sample treatment step to inhibit low DNA yields and degraded DNA. More long-term storage of the sample, it should be frozen and kept at -80°C after harvesting. It is good for disruption and homogenization if the sample is sliced off when store at -20°C or -80°C.

Young fresh leaf is good for extraction of DNA, if possible, we recommend to collect young fresh leaf since they contain more viable cells per weight and therefore result in higher yields.

II. Disruption & Homogenization step

2. Place the sliced or whole sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 3.

Disruption and homogenization time depends on the leaf samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of fresh leaf sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Generally, it is a fine powder form after disruption and homogenization.

III. Sample Sizing step

3. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfer it, previously pre-chill the spatula and 1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation.

And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity.

B-2. DNA Extraction Step

▪ ***Fresh, Dried or Frozen Leaf***

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

- Equilibrate samples to room temperature (15 ~ 25°C).
- Heat a water bath or heating block to 65°C for use in step 2.
- All centrifugation steps should be carried out at room temperature.

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNaseA Solution into sample tube, and vortex vigorously.

Like lyophilized or dried leaf, ground fresh and frozen leaf absorbs Buffer PG (lysis buffer), and becomes swollen. It may be difficult to handle plant tissue due to its viscosity. Always keep the recommended amount of starting material. Furthermore, vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65°C for 30 min .

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate on ice for 5 min .

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min .

Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step. Plant leaf contains chloroplast, including much have chlorophyl, therefore shows green color.
So, after centrifugation, the color of supernatant shows clear green color. But disappear after pass through washing process two times.

IV. DNA Binding step

5. Transfer carefully 200 ㎕ of supernatant from step 4 into a new 1.5 ml tube.
Although the supernatant is typically 400 ~ 450 ㎕, we recommend to recover only 200 ㎕ of lysate. More lysate can results in shearing of the DNA and contaminating the next step with impurities. When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 650 ㎕ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or pipetting. DO NOT vortex.
This step is an equilibration step for binding genomic DNA to column membrane. A precip itate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA Mini Kit procedure.

7. Pipette 650 ㎕ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm (RT) for 1 min, and discard the flow-through. Reuse the collection tube in step 8.
If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

8. Repeat step 7 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.
If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer PWA, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 ㎕ Buffer PWB to the spin column, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.
It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.
NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

11. Place the spin column into the a new 1.5 ml tube (not supplied), and 100 $\mu\ell$ Buffer PE directly onto the membrane. Incubate at room temperature for 1 min, and then centrifuge at 13,000 rpm for 1 min to elute.

With 50 $\mu\ell$ of Buffer PE increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 $\mu\ell$ increases generally overall DNA yield.

NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

Alternatively, the tube can be reused for the second elution step to combine the eluates.

Plant

Type C

Type C Protocol

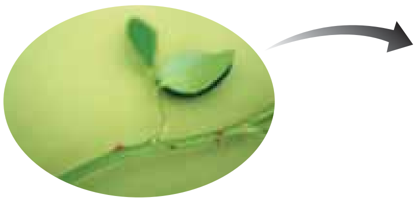
For Stem

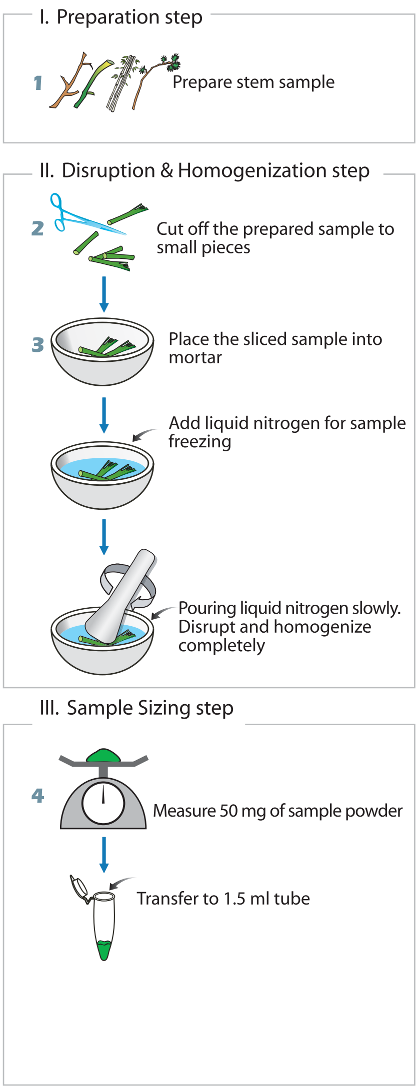
Bench Protocol

C-1. Sample Treatment Step

▪ ***Stem***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step





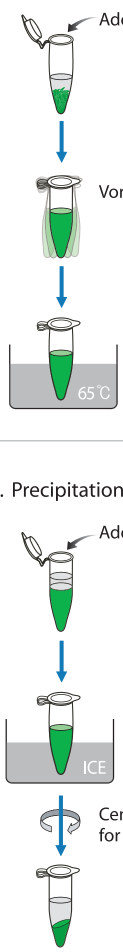
Type C Protocol
Stem

C-2. DNA Extraction Step

▪ *Stem*

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step



II. Lysis step

1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

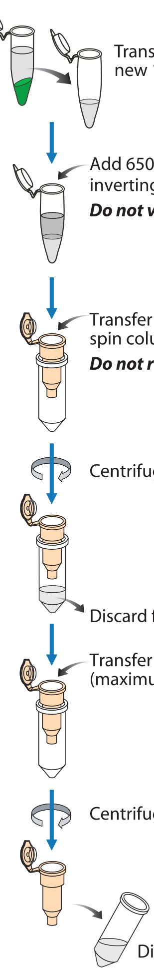
2 Incubate the lysate at 65℃ for 30 min
(invert 5 ~ 6 times for more lysis)

III. Precipitation step

3 Add 100 ul of Buffer PPT and mix well

Incubation on ice for 5 min

4 Centrifuge at 13,000 rpm (RT)
for 5min



IV. DNA Binding step

5 Transfer 200 ul of supernatant to
new 1.5 ml tube

6 Add 650 ul of Buffer PB and mix by
inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to
spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

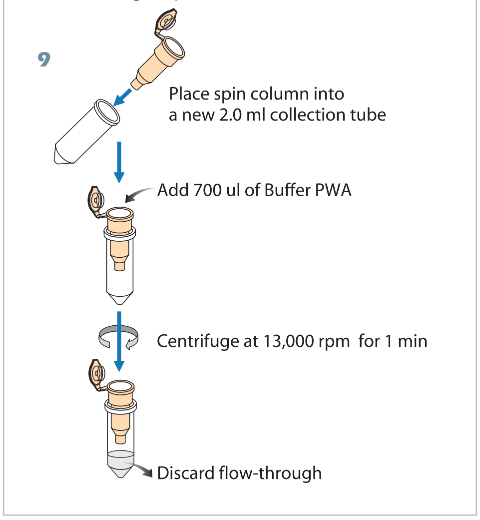
8 Transfer the rest of mixture
(maximum 200 ul) to spin column

Centrifuge at 13,000 rpm for 1 min

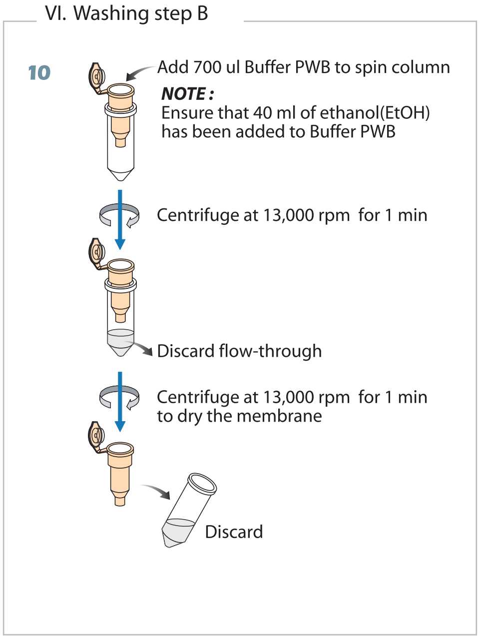
Discard

Type C Protocol
Stem

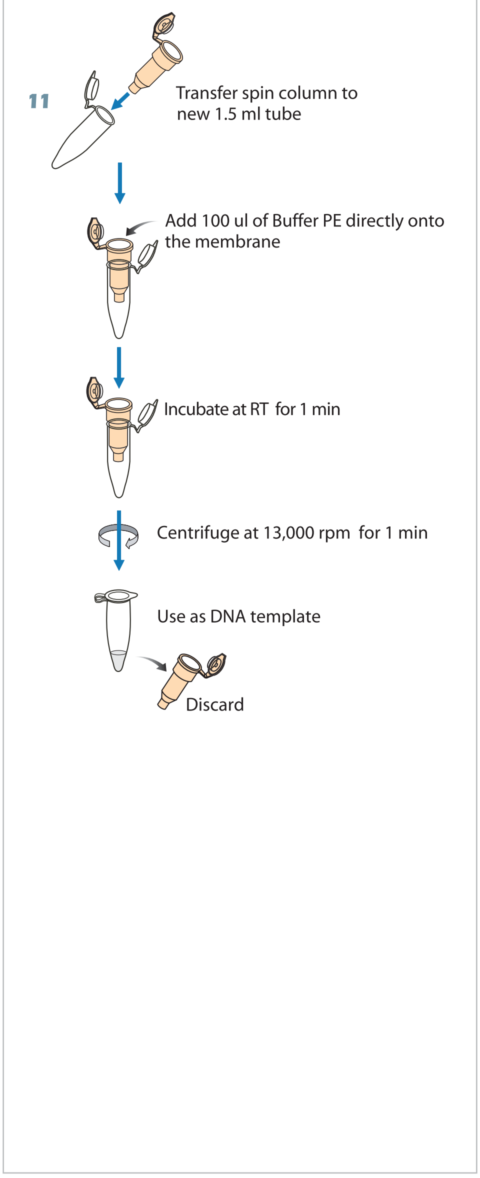
V. Washing setp A



VI. Washing step B



VII. Elution step



C-1. Sample Treatment Step

▪ ***Stem***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

Type C Protocol
Stem

I. Preparation step

1. Prepare the stem sample.

We recommend to collect the fresh stem, but If it is impossible, it is preferable to collect non dried stem sample. The more stem sample is dried, the more it is difficult to lysis becuase it absorbs all of lysis buffer. Also, plant stem sample is very tough and thick, therefore it is difficult to disrupt and homogenize. Same as other plant samples, although it's organization is similar to leaf's organization, it is so hard plant tissue. For storage of harvested stem sample, In general, when genomic DNA is to be isolated, to be not dried, we recommend to freeze or keep in plastic bag containing a wet paper towel after harvesting.
It is good for disruption and homogenization if the sample is sliced off when it store.

II. Disruption & Homogenization step

2. Cut off the prepared sample to small pieces by the blade or scissor.

To disruption and homogenization perfectly in trunk of tree, peel the shell of trunk. If not, the shell powder is floated above the supernatant after the precipitation step. It has an effect on low DNA yield and purity.
To reduce disruption and homogenization time, we recommend to cut if off. Some stem sample is very tough and thick, to cut off it by the saw.

3. Place the sliced sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately step 4.

In stem sample, it is difficult to disrupt and homogenize to the fine powder using liquid nitrogen with mortar and pestle. But, we recommend to be disrupted completely until no tissue clumps are not visible. Clumps of stem sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen.

III. Sample Sizing step

4. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and 1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation. And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity.

C-2. DNA Extraction Step

▪ ***Stem***

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step
■ V. Washing step A	■ VI. Washing step B	■ VII. Elution step	

▪ Equilibrate samples to room temperature (15 ~ 25°C).
▪ Heat a water bath or heating block to 65°C for use in step 2.
▪ All centrifugation steps should be carried out at room temperature.

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

Although ground stem sample is not fine powder, it absorbs Buffer PG (lysis buffer). Especially, dried stem sample absorbs all of Buffer PG. To prevent this case, always keep the recommended amount of starting material. Be careful. This step is cell lysis step. After adding them, immediately vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65°C for 30 min .

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min .

Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step.
Due to the nature color of stem, sometimes supernatant shows green or dark brown color. Nevertheless, it will disappearea be after washing step.

Type C Protocol
Stem

IV. DNA Binding step

5. Transfer carefully 200 ㎕ of supernatant from step 4 into a new 1.5 ml tube.

In trunk of tree, if not peel the shell when the disruption and homogenization step, you can see the shell in above supernatant. In this situation, the shell is attached the external of yellow tip, while taking the supernatant with yellow tip by pipette. Be careful of taking the supernatant. Although the supernatant is typically 400 ~ 450 ㎕, too much dried stem sample supernatant is 100 ~ 200 ㎕. If it is, reduce the amount of starting material. Always keep the recommended amount of starting material.

Normally, we recommend to recover only 200 ㎕ of lysate in stem sample. More lysate can result in shearing of the DNA and contaminate the next step with impurities.

When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 650 ㎕ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or by pipetting. DO NOT vortex.

This step is an equilibration step for binding genomic DNA to column membrane. A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA mini Kit procedure.

7. Pipette 650 ㎕ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm (RT) for 1min, and discard the flow-through. Reuse the collection tube in step 8.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm. After centrifugation, sometimes spin column membrane becomes light brown or light green color. Do not worry about that. It will be disappeared after washing step.

8. Repeat step 7 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer PWA, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 ㎕ Buffer PWB to the spin column, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.

It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.

NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

1. **Place the spin column into a new 1.5 ml tube (not supplied), and 100 ㎕ Buffer PE directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge for 1 min at 13,000 rpm to elute.**
   Elution with 50 ㎕ (instead of 100 ㎕) increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 ㎕ increases generally overall DNA yield.
   NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.
   Alternatively, the tube can be reused for the second elution step to combine the eluates.

Plant

Type D

Bench Protocol

Type D Protocol

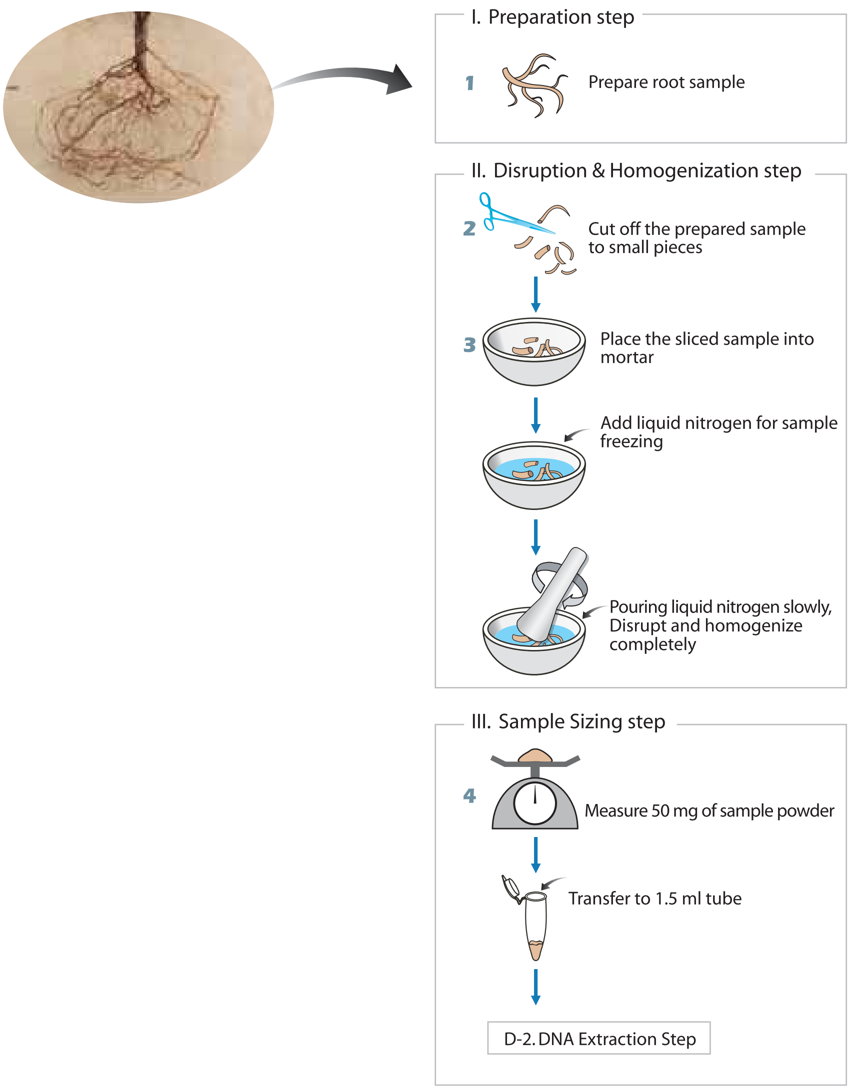
For Root

Type D Protocol
Root

D-1. Sample Treatment Step

▪ ***Root***

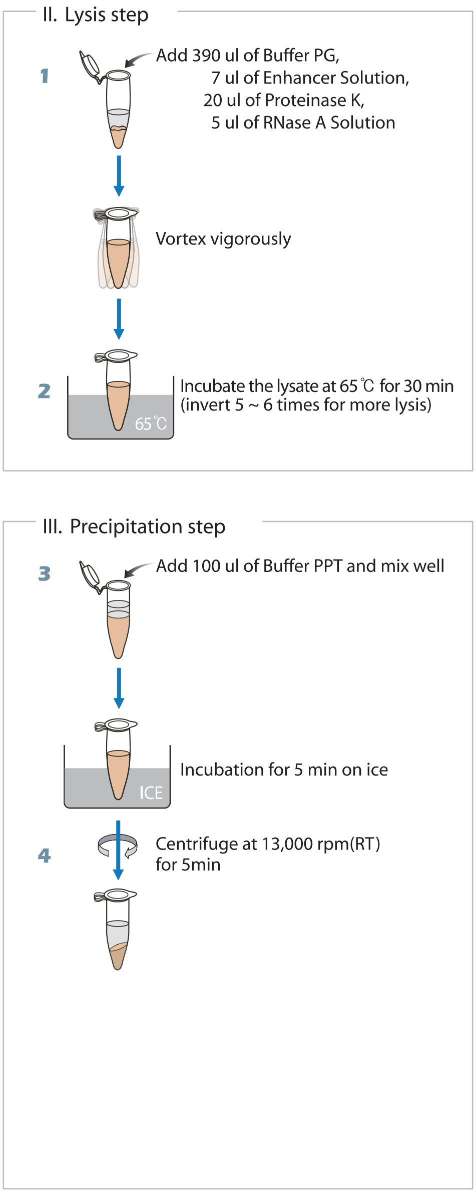
■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

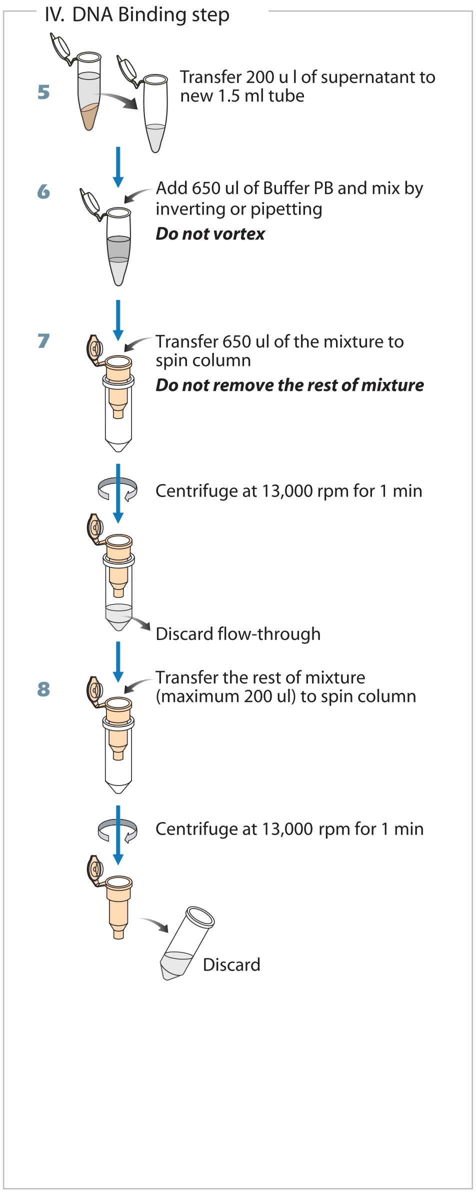


D-2. DNA Extraction Step

▪ ***Root***

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step
■ V. Washing step A	■ VI. Washing step B	■ VII. Elution step	

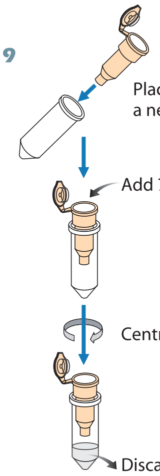




Type D Protocol
Root

Type D Protocol
Root

V. Washing setp A



9

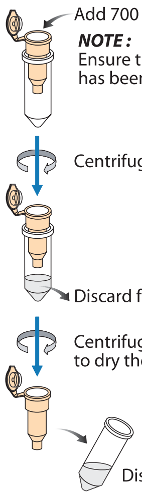
Place spin column into a new 2.0 ml collection tube

Add 700 ul of Buffer PWA

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

VI. Washing step B



10

Add 700 ul Buffer PWB to spin column

NOTE :
Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB

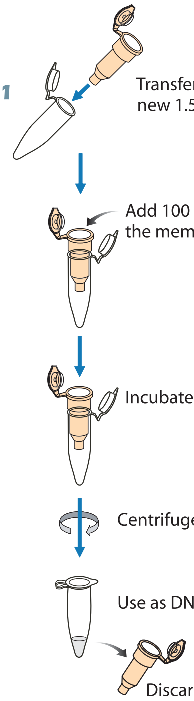
Centrifuge at 13,000 rpm for 1 min

Discard flow-through

Centrifuge at 13,000 rpm for 1 min to dry the membrane

Discard

VII. Elution step



11

Transfer spin column to new 1.5 ml tube

Add 100 ul of Buffer PE directly onto the membrane

Incubate at RT for 1 min

Centrifuge at 13,000 rpm for 1 min

Use as DNA template

Discard

D-1. Sample Treatment Step

▪ ***Root***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

Type D Protocol
Root

I. Preparation step

1. Prepare root sample.

In root sample, we recommend to collect same condition of the fresh stem sample. Although root is very tough and thick as stem, it is not difficult to disrupt and homogenize. Same as stem, root sample does not keep wet condition , therefore we recommend to frozen condition (-20°C or -80°C) for storage of harvested of root sample. Before freezing, remove impurities on surface of root sample that are inhibited DNA extraction by washing. And then, store at the frozen condition after removing the wetness. As ever the other plant sample, it is good for disruption and homogenization if the sample is sliced off when it stores.

In frozen root, it is important to keep frozen state in liquid nitrogen during all of sample treatments step to inhibit low DNA yields and degraded DNA. Do not repeat freezing-thawing.

II. Disruption & Homogenization step

2. Cut off the prepared sample to small pieces by the blade or scissor.

To reduce disruption and homogenization time, we recommend to cut it off.
Some root sample is very tough and thick, to cut off it by the saw.

3. Place the sliced sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely.
Allow the liquid nitrogen to evaporate, and proceed immediately to step 4.

We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of sample will not lyse properly and will be resulted in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Generally, it is a fine powder form after disruption and homogenization. Be careful to handle liquid nitrogen.

III. Sample Sizing step

4. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and 1.5ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation.

And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis,resulting in low DNA yield and purity.

D-2. DNA Extraction Step

▪ ***Root***

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step
■ V. Washing step A	■ VI. Washing step B	■ VII. Elution step	

- Equilibrate samples to room temperature (15 ~ 25°C).
- Heat a water bath or heating block to 65°C for use in step 2.
- All centrifugation steps should be carried out at room temperature.

Type D Protocol
Root

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

In ground root sample, It is properly fine powder form. Like ground fresh leaf, it absorbs Buffer PG (lysis buffer) after adding them. It is not difficult to mix until any root clumps are not visible if only keep the recommended amount of starting material and immediately vortex or pipette them vigorously. If not, it may be difficult to handle it due to its viscosity. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA.
A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.
During lysis step, the lysate color express to mix the cloudy and the nature color of root sample.

2. Incubate the lysate at 65°C for 30 min.

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate.
Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min .

Plant materials can create very viscous lysates and large amounts of precipitates during this step.
If you keep our recommended amounts of starting material, optimal results are obtained.
If not, you should perform one more centrifugation step.

IV. DNA Binding step

5. Transfer carefully 200 ㎕ of supernatant from step 4 into a new 1.5 ml tube.

Although the supernatant is typically 350 ~ 400 ㎕, we recommend to recover only 200 ㎕ of lysate.
More lysate can results in shearing of the DNA and contaminating the next step with impurities.
When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 650 μℓ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or by pipetting. DO NOT vortex.

This step is an equilibration step for binding genomic DNA to column membrane.
A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA mini Kit procedure.

7. Pipette 650 μℓ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge for 1min at 13,000 rpm (RT), and discard the flow-through. Reuse the collection tube in step 8.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

8. Repeat step 7 with remaining sample (maximum 200 μℓ). Discard flow-through and collection tube altogether.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 μℓ Buffer PWA, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 μℓ Buffer PWB to the spin column, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.

It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.

NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

11. Place the spin column into a new 1.5 ml tube (not supplied), and 100 μℓ Buffer PE directly onto the membrane. Incubate at room temperature for 1 min, and then centrifuge at 13,000 rpm for 1 min to elute.

Elution with 50 μℓ(instead of 100 μℓ) increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 μℓ increases generally overall DNA yield.

NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

Alternatively, the tube can be reused for the second elution step to combine the eluates.

Plant

Type E

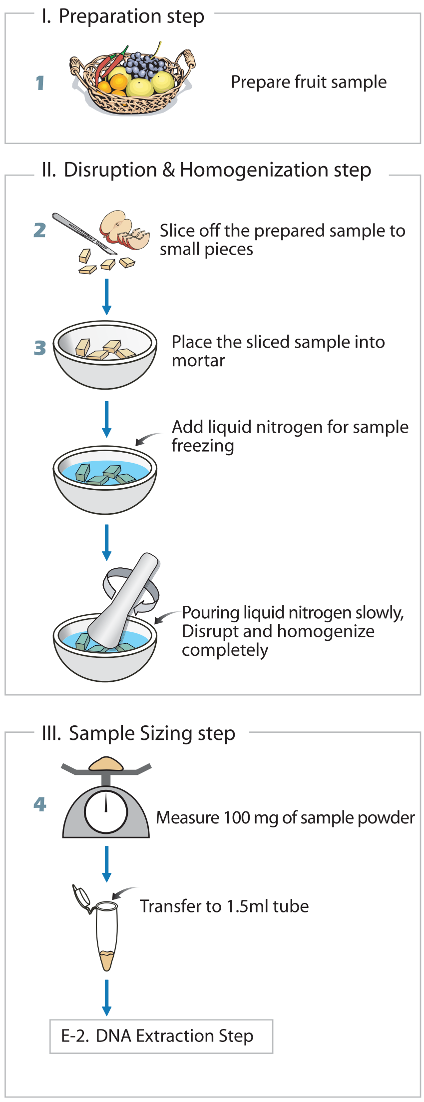
Bench Protocol

Type E Protocol
For Fruit

E-1. Sample Treatment Step

▪ ***Fruit***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step ☐ IV. Pre-Treating step



Type E Protocol
Fruit

E-2. DNA Extraction Step

▪ ***Fruit***

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

II. Lysis step

1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

2 Incubate the lysate at 65℃ for 30 min
(invert 5 ~ 6 times for more lysis)

III. Precipitation step

3 Add 100 ul of Buffer PPT and mix well

Incubation for 5min on ice

4 Centrifuge at 13,000 rpm (RT)
for 5 min

IV. DNA Binding step

5 Transfer 300 ul of supernatant to new 1.5 ml tube

6 Add 975 ul of Buffer PB and mix by inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

8 Transfer the rest of mixture (maximum 625 ul) to spin column

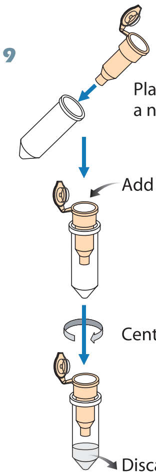
Centrifuge at 13,000 rpm for 1 min

Discard

Type E Protocol
Fruit

V. Washing setp A

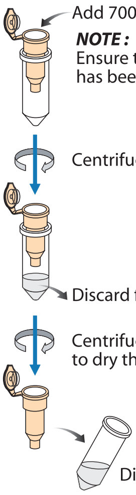
9



- Place spin column into a new 2.0 ml collection tube
- Add 700 ul of Buffer PWA
- Centrifuge at 13,000 rpm for 1 min
- Discard flow-through

VI. Washing step B

10

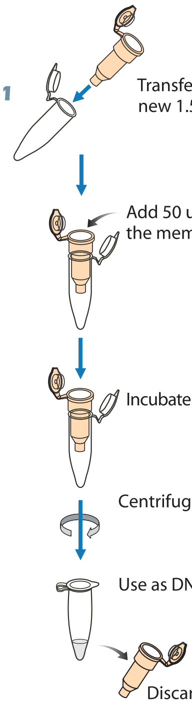


- Add 700 ul Buffer PWB to spin column

  NOTE :
  Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB
- Centrifuge at 13,000 rpm for 1 min
- Discard flow-through
- Centrifuge at 13,000 rpm for 1min to dry the mem brane
- Discard

VII. Elution step

11



- Transfer spin column to new 1.5 ml tube
- Add 50 ul of Buffer PE directly onto the membrane
- Incubate at RT for 1 min
- Centrifuge at 13,000 rpm for 1min
- Use as DNA template
- Discard

E-1. Sample Treatment Step

▪ ***Fruit***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare fresh fruit sample.

While other plant's organ contains a little water, fruit organ contains water about 80 ~ 95%, therefore increase the amount of starting material during the experiment. When keep fruit sample long at RT, It should denaturalize easily and will therefore result in a lower yield of DNA. For storage of fruit, we recommend to frozen and keep at -80°C or lyophilize it and store at room temperature (15°C ~ 25°C) after harvesting. Before freezing, peel the shell the fruit sample and slice off it to suitable size. When extracting DNA from fruit, generally use the flesh of fruit.

In frozen fruit, it is important to keep frozen state in liquid nitrogen during all of sample treatment step to inhibit low DNA yields and degraded DNA. Do not repeat freezing-thawing.

II. Disruption & Homogenization step

2. Slice off the prepared sample to small pieces by the blade or scissor.

To reduce disruption and homogenization time, we recommend to slice if off.

3. Place the sliced sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 4.

We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Generally, it is a fine powder form after disruption and homogenization.

III. Sample Sizing step

4. Measure 100 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and 1.5ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation. Because fruit organ contains water about 80 ~ 95%, increase the amount of starting material. But exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity.

E-2. DNA Extraction Step

▪ ***Fruit***

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step
■ V. Washing step A	■ VI. Washing step B	■ VII. Elution step	

- Equilibrate samples to room temperature (15 ~ 25°C).
- Heat a water bath or heating block to 65°C for use in step 2.
- All centrifugation steps should be carried out at room temperature.

Type E Protocol
Fruit

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

With fruit sample absorbs lysis buffer, and becomes swollen. When apply exceeding the recommended amount of starting material, it may be difficult to handle plant tissue due to its viscosity. Therefore, always keep the recommended amount of starting material. Furthermore, vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65°C for 30 min .

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min.

Because plant fruit sample contain a few amount of fat, protein and other material, fruit sample create a small size and few amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step.

5. Transfer carefully 300 μℓ of supernatant from step 4 into a new 1.5 ml tube.

Although the fruit supernatant is typically over 450 ~ 500 μℓ ,we recommend to recover onl y300 μℓ of lysate. Generally other plant sample take 200 μℓ of supernatant. But, take more quantitative supernatant than other plant sample, because fruit sample contains an amount of water. When pipette the supernatant , please be careful without disturbing the cell-debris pellet. If much pellet is transferred with the supernatant, try one more centrifugation with the transferred supernatant. More lysate can results in shearing of the DNA and contaminating the next step with impurities. When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 975 μℓ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or by pipetting. DO NOT vortex.

This step is an equilibration step for binding genomic DNA to column membrane.
A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA Mini Kit procedure.

7. Pipette 650 μℓ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm(RT) for 1min and discard the flow-through. Reuse the collection tube in step 8.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.
After centrifugation, sometimes spin column membrane becomes light brown or light green color. Do not worry about that. Disappear after washing step.

8. Repeat step 7 with remaining sample (maximum 625 μℓ). Discard flow-through and collection tube altogether.

If a small amount will not pass through, please centrifuge again at 13,000 rpm for 1 min.

Type E Protocol
Fruit

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 μℓ Buffer PWA, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 μℓ Buffer PWB to the spin column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.

It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.

NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

11. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ㎕ Buffer PE directly onto the membrane. Incubate at room temperature for 1 min , and then centrifuge for 1 min at 13,000 rpm to elute.

Elution with 50 ㎕(instead of 100 ㎕) increases the final DNA concentration, but it will be reduced overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 ㎕ increases generally overall DNA yield.

NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

Alternatively, the tube can be reused for the second elution step to combine the eluates.

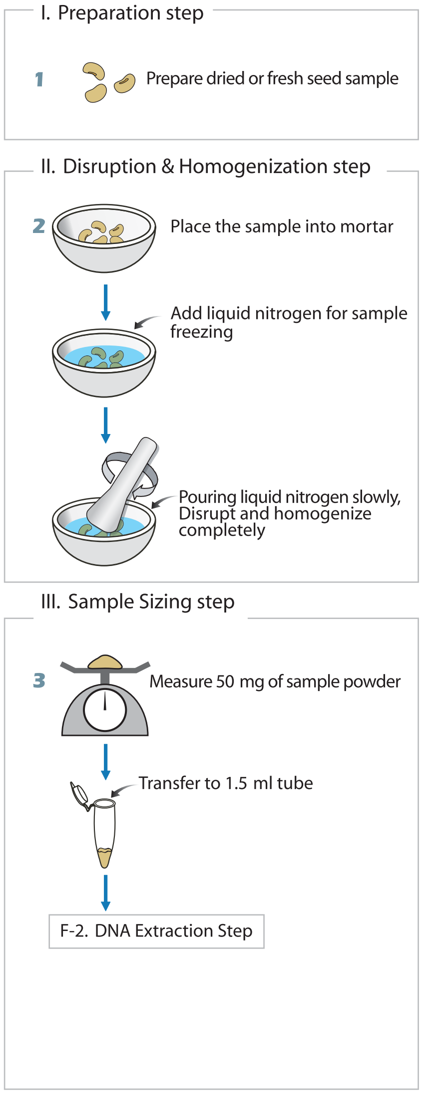
Plant
Type F
Bench Protocol

Type F Protocol
For Seed(Others)

F-1. Sample Treatment Step

▪ ***Seed (Others)***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step



F-2. DNA Extraction Step

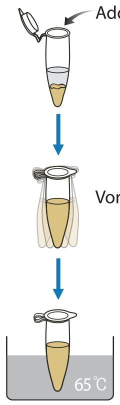
▪ *Seed (Others)*

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

Type F Protocol
Seed (Other)

II. Lysis step

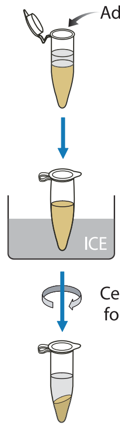


1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

2 Incubate the lysate at 65℃ for 30 min
(invert 5 ~ 6 times for more lysis)

III. Precipitation step

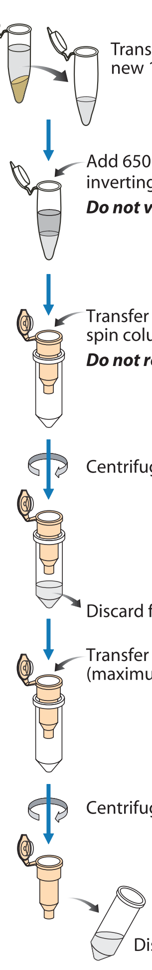


3 Add 100 ul of Buffer PPT and mix well

Incubation on ice for 5min

4 Centrifuge at 13,000 rpm (RT)
for 5 min

IV. DNA Binding step



5 Transfer 200 ul of supernatant to
new 1.5 ml tube

6 Add 650 ul of Buffer PB and mix by
inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to
spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1 min

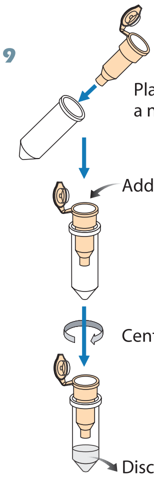
Discard flow-through

8 Transfer the rest of mixture
(maximum 200 ul) to spin column

Centrifuge at 13,000rpm for 1 min

Discard

V. Washing setp A



9

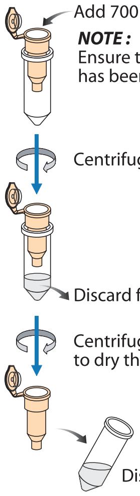
Place spin column into a new 2.0 ml collection tube

Add 700 ul of Buffer PWA

Centrifuge at 13,000 rpm for 1 min

Discard flow-through

VI. Washing step B



10

Add 700 ul Buffer PWB to spin column

NOTE :
Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB

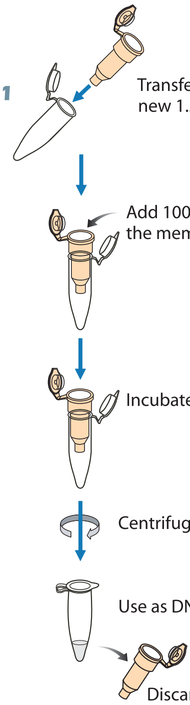
Centrifuge for 1 min at 13,000 rpm

Discard flow-through

Centrifuge at 13,000 rpm for 1 min to dry the membrane

Discard

VII. Elution step



11

Transfer spin column to new 1.5 ml tube

Add 100 ul of Buffer PE directly onto the membrane

Incubate at RT for 1 min

Centrifuge at 13,000 rpm for 1 min

Use as DNA template

Discard

F-1. Sample Treatment Step

▪ ***Seed (Others)***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare dried or fresh seed sample.

Plant seed sample corresponds to dormancy state at cycle of plant and consists embryo and albumen. Seed is kept in dry well in dry state, for albumen consist starch or fat. Even if seed keep at room temperature (15℃ ~ 25℃) after dry, there is no problem when using later. If not, store at -20℃ or -80℃ for long term storage. Before storage, to isolate pure DNA, wash the surface of the sample with distilled water.

If frozen seed sample, it is important to keep frozen state in liquid nitrogen during all of sample treatment step to inhibit low DNA yields and degraded DNA. Do not repeat freeze-thaw.

II. Disruption & Homogenization step

2. Place the sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 3.

In seed sample, break outer shell of seed due to the hard outer shell after putting one, two or more dried or fresh seed sample in the mortar. It is difficult to disrupt and homogenize. Slowly pouring the liquid nitrogen and pressing the power, repeat again repeat again. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Finally, It is a fine powder form after disruption and homogenization.

III. Sample Sizing step

4. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

To prevent thawing the frozen sample during transfering it, previously pre-chill the spatula and1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation. And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis,resulting in low DNA yield and purity.

F-2. DNA Extraction Step

Seed (Others)

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step

- Equilibrate samples to room temperature (15 ~ 25°C).
- Heat a water bath or heating block to 65°C for use in step 2.
- All centrifugation steps should be carried out at room temperature.

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

With seed sample absorbs lysis buffer, and becomes swollen. It may be difficult to handle plant tissue due to its viscosity. Always keep the recommended amount of starting material. Furthermore, vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and therefore it will result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65°C for 30 min.

For complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased.

Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min at room temperature.

Seed sample is contain much fat, so let you see thick fat layer on supernatant. Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step.

IV. DNA Binding step

5. Transfer carefully 200 ㎕ of supernatant from step 4 into a new 1.5 ml tube.

As seed sample is containing much fats and it forms fatty layer, so it is difficult to take the supernatant in fatty layer. When pipetting the supernatant, must observe so that much fat should not stick to tip and do not take pellet together. If much pellet and fat are transferred with the supernatant together carelessly, debris prevent binding the column membrane. Although the supernatant is typically over 250 ~ 300 ㎕, we recommend to recover only 200 ㎕ of lysate. More lysate can results in shearing of the DNA and contaminating the next step with impurities. When pipetting, please be careful without disturbing the cell-debris pellet.

6. Add 650 ㎕ Buffer PB to the lysate, and mix well by gently inverting 5 to 6 times or by pipetting. DO NOT vortex.

This step is an equilibration step for binding genomic DNA to column membrane.
A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA Mini Kit procedure.

7. Pipette 650 ㎕ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge for 1min at 13,000 rpm (RT), and discard the flow-through. Reuse the collection tube in step 8.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm. After centrifugation, sometimes spin column membrane becomes light brown or light green color. Do not worry about that. It will be disappeared after washing step.

8. Repeat step 7 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.

If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer PWA, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the collection tube in step 10.

VI. Washing step B

10. Add 700 ㎕ Buffer PWB to the spin column, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.

It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.
NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

1. **Place the spin column into a new 1.5 ml tube (not supplied), and 100 μℓ Buffer PE directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge for 1 min at 13,000 rpm to elute.**

   Elution with 50 μℓ (instead of 100 μℓ) increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 μℓ increases generally overall DNA yield.

   NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

   Alternatively, the tube can be reused for the second elution step to combine the eluates.

Plant
Type G
Bench Protocol

Type G Protocol
For Seed (Gramineae)

G-1. Sample Treatment Step

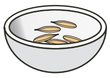
▪ ***Seed (Gramineae)***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

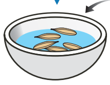
I. Preparation step

1  Prepare dried or fresh *gramineae* seed sample

II. Disruption & Homogenization step

2  Place the sample into mortar

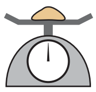
↓

 Add liquid nitrogen for sample freezing

↓

 Pouring liquid nitrogen slowly, Disrupt and homogenize completely

III. Sample Sizing step

3  Measure 10 mg of sample powder (cf, 100 mg of grass species sample powder)

↓

 Transfer to 1.5 ml tube

↓

G-2. DNA Extraction Step

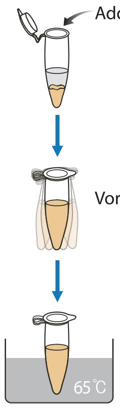
G-2. DNA Extraction Step

▪ *Seed (Gramineae)*

☐ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

II. Lysis step

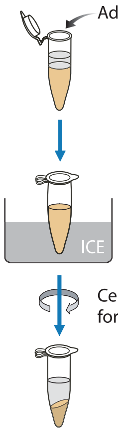


1 Add 390 ul of Buffer PG,
7 ul of Enhancer Solution,
20 ul of Proteinase K,
5 ul of RNase A Solution

Vortex vigorously

2 Incubate the lysate at 65℃ for 30 min (invert 5 ~ 6 times for more lysis)

III. Precipitation step

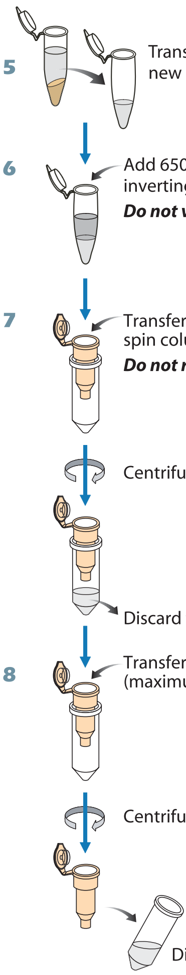


3 Add 100 ul of Buffer PPT and mix well

Incubation for 5 min on ice

4 Centrifuge at 13,000 rpm (RT) for 5 min

IV. DNA Binding step



5 Transfer 200 ul of supernatant to new 1.5 ml tube

6 Add 650 ul of Buffer PB and mix by inverting or pipetting
Do not vortex

7 Transfer 650 ul of the mixture to spin column
Do not remove the rest of mixture

Centrifuge at 13,000 rpm for 1 min

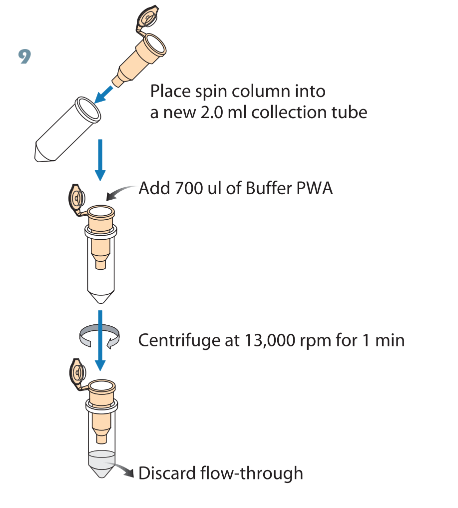
Discard flow-through

8 Transfer the rest of mixture (maximum 200 ul) to spin column

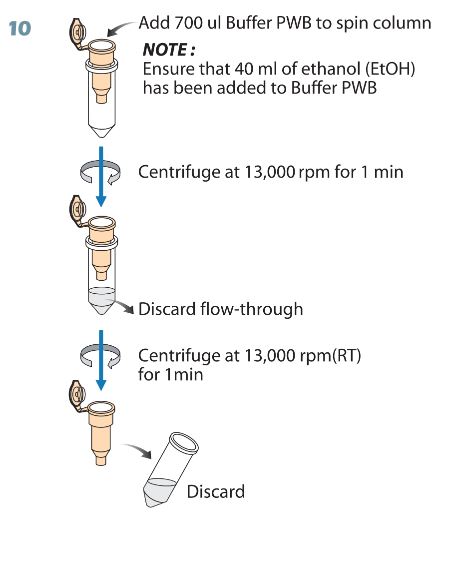
Centrifuge at 13,000 rpm for 1min

Discard

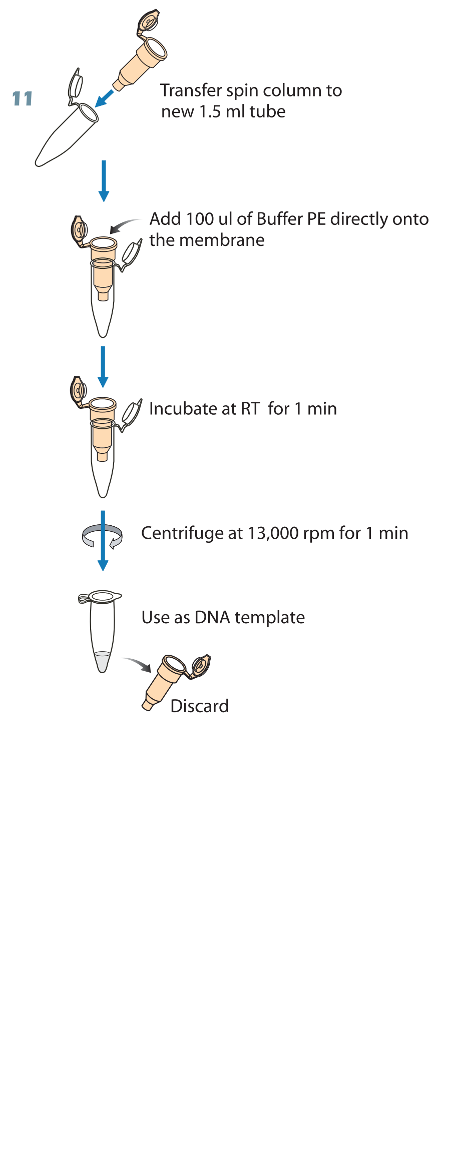
V. Washing setp A



VI. Washing step B



VII. Elution step



G-1. Sample Treatment Step

▪ ***Seed (Gramineae)***

■ I. Preparation step ■ II. Disrupt.& Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare dried or fresh gramineae seed sample.

It is hardly difficult to get good result more than other seed sample as gramineae seed sample, it has too much secondary product and polyphenolic compounds. Even if seed keep at room temperature(15°C ~ 25°C) after drying, there is no problem when use later. If not, store at -20°C or -80°C for long-term storage.

If frozen seed sample, it is important to keep frozen state in liquid nitrogen during all of sample treatment step to inhibit low DNA yields and degraded DNA. Do not repeat freeze-thaw.

II. Disruption & Homogenization step

2. Place the sample material into a grinding mortar. Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 3.

Because seed sample of gramineae is thinner than other seed sample, easily grind sample. It's very important to keep the sample frozen in liquid nitrogen during disruption and then homogenization step to inhibit low DNA yields and degraded DNA. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of sample will not lyse properly and will therefore result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen. Generally, It is a fine powder form after disruption and homogenization.

III. Sample Sizing step

3. Measure 10 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.

Although the thin seeds are easily ground, but rice, wheat etc are so thick to difficult to grind. Rice and wheat like this sample, when lysis incubation time, It absorbs all of Buffer PG (lysis buffer) to difficult to handle to lysis. And in that time, becomes swollen. To prevent it, reduce the amount of starting sample material. In gramineae seed sample, previously, use below 10 mg for amount of starting material. But, in case of grass sample, use 100 mg for amount of starting material. Exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. To prevent thawing the frozen sample during transfering it, previously pre-chilling the spatula and 1.5ml tube in liquid nitrogen.

The freezing-thawing repetition of frozen sample will result in the DNA

G-2. DNA Extraction Step

▪ ***Seed (Gramineae)***

☐ I. Pre-Lysis step	■ II. Lysis step	■ III. Precipitation step	■ IV. DNA Binding step
■ V. Washing step A	■ VI. Washing step B	■ VII. Elution step	

- Equilibrate samples to room temperature (15~25°C).
- Heat a water bath or heating block to 65°C for use in step 2.
- All centrifugation steps should be carried out at room temperature.

II. Lysis step

1. Add 390 ㎕ Buffer PG, 7 ㎕ Enhancer Solution, 20 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.

With seed sample absorbs lysis buffer, and becomes swollen. It may be difficult to handle plant tissue due to its viscosity. Always keep the recommended amount of starting material. Furthermore, vortex or pipette vigorously to remove any clumps until any plant tissue clumps are not visible. Clumps of plant tissue will not lyse adequately and will therefore result in a lower yield of DNA. A disposable micropestle may be used if clumps are not be removed by pipetting and vortexing.

2. Incubate the lysate at 65°C for 30 min.

Like rice, it becomes boiled rice when incubation for lysis. You must mix by tapping every 3 minutes for complete lysis. Be careful this. In case of grass sample, mix 5 ~ 6 times during incubation by inverting tube for complete lysis. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.

III. Precipitation step

3. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 ~ 6 times by inverting tube. The reaction makes clear lysate into opaque slushy lysate. Generally, plant tissues contain large amounts of polysaccharides and polyphenolics, and are therefore relatively not easier to handle. These impurities may be present in the purified DNA if the amount of starting material is increased. Always keep the recommended amounts of samples.

4. Centrifuge the lysate at 13,000 rpm (RT) for 5 min .

In case of rice, although rice becomes the boiled rice, you can see the clear supernatant after centrifugation if you use 10 mg for amount of starting material. Plant materials can create very viscous lysates and large amounts of precipitates during this step. If you keep our recommended amounts of starting material, optimal results are obtained. If not, you should perform one more centrifugation step.

IV. DNA Binding step

5. **Transfer carefully 300 ㎕ of supernatant from step 4 into a new 1.5 ml tube.**
   Although the supernatant is typically over 300 ~ 350 ㎕, we recommend to recover only 200 ㎕ of lysate. More lysate can results in shearing of the DNA and contaminating the next step with impurities. When pipetting, please be careful without disturbing the cell-debris pellet.
6. **Add 650 ㎕ Buffer PB to the lysate, and mix well by gently inverting 5 ~ 6 times or by pipetting. DO NOT vortex.**
   This step is an equilibration step for binding genomic DNA to column membrane.
   A precipitate may form after the addition of Buffer PB, but this will not affect the i-genomic Plant DNA Mini Kit procedure.
7. **Pipette 650 ㎕ of the mixture from step 6, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge for 1min at 13,000 rpm (RT), and discard the flow-through. Reuse the collection tube in step 8.**
   If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.
   After centrifugation, sometimes spin column membrane becomes light brown or light green color. Do not worry about that. Disappear after washing step.
8. **Repeat step 7 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.**
   If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

V. Washing step A

9. **Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer PWA, and centrifuge at 13,000 rpm for 1 min . Discard the flow-through and reuse the collection tube in step 10.**

VI. Washing step B

10. **Add 700 ㎕ Buffer PWB to the spin column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through, and again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and collection tube altogether.**
    It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.
    NOTE : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer PWB.

VII. Elution Step

11. **Place the spin column into a new 1.5 ml tube (not supplied), and 100 ㎕ Buffer PE directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge at 13,000 rpm for 1 min to elute.**

    Elution with 50 ㎕ (instead of 100 ㎕) increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 200 ㎕ increases generally overall DNA yield.

    NOTE : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

    Alternatively, the tube can be reused for the second elution step to combine the eluates.

Troubleshooting Guide

When working with i-genomic Plant DNA Extraction Mini Kit, always follow the description of each protocols. Nevertheless, if it causes problems upon extracting DNA, please refer to the following Troubleshooting Guide. This Troubleshooting Guide may be helpful in solving any problems that may arise. For more information, please contact our Technical Assistance Team. Our Technical Assistance Team is staffed by experienced researchers with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products.

Comments and Suggestions

Low flow rate in column

✓ Clogged spin column by particulate material
- (1) Completely perform the Disruption & Homogenization step.
- (2) Increase the incubation time at 65°C in Lysis step.
- (3) Ensure that no particulate material is transferred following precipitation step or when supernatants transferred to new 1.5 ml tube prior to addition of Buffer PB.

✓ High viscosity of Lysate
- (1) Reduce the amounts of starting material.
- (2) Increase the incubation time at 65°C in Lysis step.

✓ Problem in centrifugation
- (1) Check your centrifuge, and then speed up or increase the centrifugation time.

Low DNA yield

✓ Not enough disruption & homogenization
- (1) Check again your Disruption & Homogenization step, and always follow the protocol. The complete disruption and homogenization of the starting material is very important to get high DNA yield.

✓ Inadequate lysis
- (1) Reduce the amounts of starting material.
- (2) Increase the incubation time at 65°C in Lysis step.

✓ Error in DNA binding
- (1) After adding Buffer PB in DNA Binding step, please mix well by gently inverting.
- (2) Check that the amount of Buffer PB is added correctly to the supernatant.

✓ Incorrect Washing step
- (1) Check again that the amount of ethanol (EtOH) is added correctly to Washing buffer.
- (2) When storing Washing Buffer, always keep a lid shut tightly without evaporation.

continued from ***Low DNA yield.***

✓ Insufficient DNA elution
(1) Increase the volume of Buffer PE or water to 200 ㎕.
(2) Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.

Low DNA concentration

✓ Excess addition of elution buffer
(1) Reduce the amount of Buffer PE.
(2) Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.

DNA sheared

✓ Incorrect storage of plant tissue
(1) When store frozen tissues, always keep the samples frozen below -80°C.
(2) If possible, it is preferable to use fresh plant tissues.

✓ Vigorously vortex
(1) Do not vortex the mixture after adding Buffer PB as described in protocol.
(2) Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.

✓ Debris of precipitates in lysate
(1) Perform the optional centrifugation step before loading a large amount of the lysate onto the spin column.
(2) Always use the recommended amounts of starting material.

Problems in downstream experiments

✓ Ethanol contamination
(1) Ensure that during Washing Step B, the column membrane should be dried completely. Please centrifuge at full speed for 5 ~ 10 min to dry the membrane.
(2) During Washing Step B, after centrifugation, remove carefully the spin column from the collection tubes without contacting with the flow-through. This careless contact will result in contamination of ethanol.

✓ Salt contamination
(1) Check again to add EtOH previously into Buffer PWB.
(2) Store Buffer PWB at room temperature (15 ~ 20°C).

✓ Amount of DNA used in experiments.
(1) Optimize the amount of DNA used in your downstream experiments.

For more questions, please contact us without hesitation.

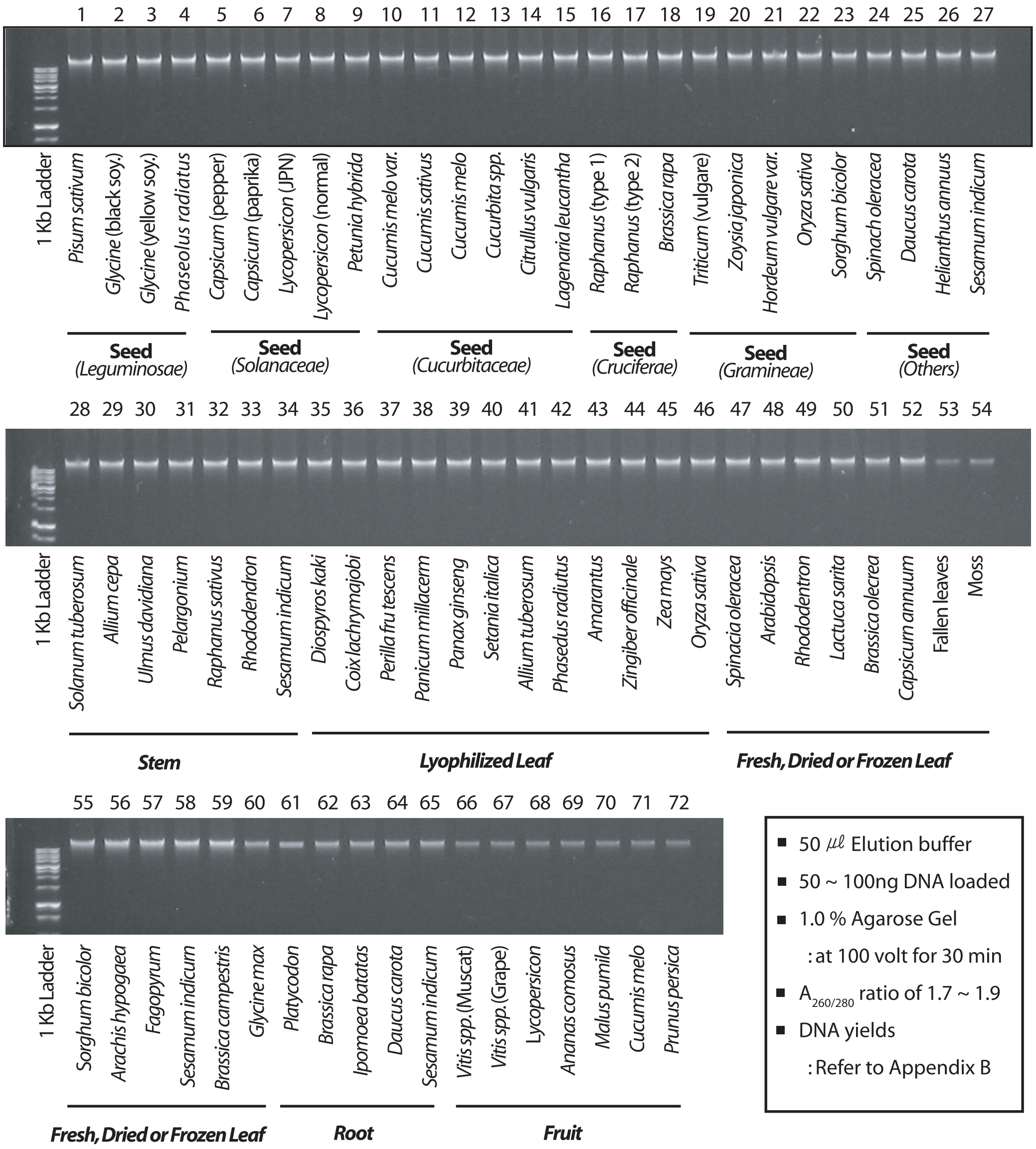
Data Information
Determination of Yield and Purity

Appendix A

Electrophoresis Results from Various Plant Samples

i-genomic Plant Mini Kit provides a reliable and practical method to purify efficiently genomic DNA from all kinds of plant tissues. DNA purified by i-genomic Plant Mini Kit is up to over 40 Kb, and has an $A_{260/280}$ ratio of 1.7 ~ 1.9, indicating high purity of the DNA. The following Figure 3 shows overall electrophoresis data from representative various samples in each plant groups.

Figure 3. Agarose Gel Electrophoresis of Eluted Genomic DNA (1.0 %)



Appendix B

Determination of Yield and Purity Data of DNA(Standard Protocol Applied)

- **Type A Protocol**
  : ***Lyophilized Leaf***

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Persimmon	5	3 ~ 5	1.73
2	Adray	5	2 ~ 4	1.83
3	Perilla	5	3 ~ 6	1.72
4	Millet (Hog mellet)	5	3 ~ 6	1.79
5	Ginseng	5	1.5 ~ 4	1.88
6	Millet	5	5 ~ 9	1.82
7	Mung bean	5	2 ~ 5	1.81
8	Leak	5	2 ~ 5	1.85
9	Amaranth	5	2 ~ 5	1.77
10	Ginger	5	2 ~ 5	1.89
11	Corn	5	2 ~ 5	1.90
12	Paddy	5	2 ~ 5	1.88

(B) DNA Purification and Enzyme Digestion (RE)

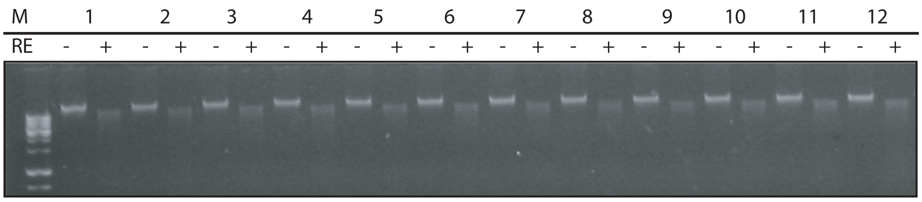


Fig 4-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

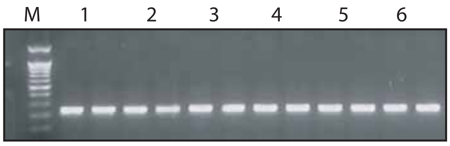


Fig 4-2. PCR Amplification
The housekeeping gene (18s, 222 bp)was amplified with the purified DNA as templates(10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ Type B Protocol
: *Fresh, Dried, or Frozen Leaf*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Spinach	50	8 ~ 9	1.85
2	Arabidopsis	50	8 ~ 11	1.81
3	Rhododendron	50	8 ~ 10	1.83
4	Lettuce	50	10 ~ 12	1.79
5	Cabbage (Brassica olecrea)	50	8 ~ 10	1.78
6	Green pepper	50	8 ~ 12	1.83
7	African millet	50	8 ~ 10	1.81
8	Peanut	50	8 ~ 12	1.81
9	Buckwheat	50	10 ~ 12	1.83
10	Sesame	50	8 ~ 10	1.77
11	Cabbage (Brassica cam pestris)	50	8 ~ 10	1.85
12	Radish	50	10 ~ 12	1.79
13	Black Bean leaves	50	8 ~ 10	1.75
14	Geranium	50	8 ~ 10	1.77
15	Fallen leaves	50	2 ~ 5	1.82
16	Moss	50	2 ~ 4	1.85

(B) DNA Purification and Enzyme Digestion (RE)

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
RE - + - + - + - + - + - + - + - + - + - + - + - + - + - +

Fig 5-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig 5-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as templates (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.

Additional Data
(Type B protocol-based modification)

When we have been developing i-genomic Plant DNA Mini Kit, iNtRON customers requested to be tested previously in their various samples for DNA yield, purity and PCR amplification.
The additional data are included in the following section. We have performed to extract DNA from various plant leaves supplied by customers with Type B protocol (occasionally, we slightly have modified Type B protocol). You can show good results from various plant leaves.

Fig 5-3. Additional Data (sample morphology)

Paddy
(Oriza sativa)

Cucumber
(Cucumis sativus)

Chinese matrimony vine Type 1
(Lyticum chinense Type 1)

Chinese matrimony vine Type 2
(Lyticum chinense Type 2)

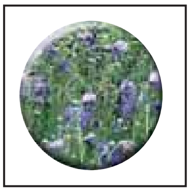
Japanese lady bell
(Aderophora triphylla)

Phyteuma
(Phyteuma japonicum)

Adenophora
(Adenophora remotiflora)

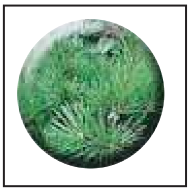
Bell flower
(*Campanula punctata*)

Platycodon
(platycodon)



Sea weed
(Ecklonia cara)

Pine needles
(Pinus densiflora)



Sweet potato
(Ipomoea batatas)

Ginkgo
(Ginkgo biloba)

Korean box tree
(Buxus microphylla)

Rhododendron
(Rhododendron schlippenbachii)

Cabbage
(Brassica olecrea)

Forsythia
(Forsythia korean)

iNtRON Waits For Your Data.

iNtRON Waits For Your Data.

iNtRON Waits For Your Data.

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (㎍)	$A_{260/280}$
1	Paddy	50	4 ~ 6	1.79
2	Cucumber	50	10 ~ 11	1.87
3	Chinese matrimony vine (type 1)	50	8 ~ 9	1.82
4	Chinese matrimony vine (type 2)	50	10 ~ 11	1.79
5	Japanese lady bell	42.2	9 ~ 12	1.82
6	Phyteuma	10	3 ~ 4.2	1.79
7	Adenophora	10	5 ~ 6	1.75
8	Bell flower	5	2 ~ 4	1.75
9	Platycodon	80	7 ~ 9	1.77
10	Sea weed	50	8 ~ 10	1.78
11	Pine needles	100	12 ~ 15	1.70
12	Sweet potato	50	12 ~ 13	1.87
13	Ginko	100	7 ~ 8	1.75
14	Korea box tree	100	9 ~ 11	1.77
15	Rhododendron	300	8 ~ 9	1.76
16	Cabbage (Brassica olecrea)	100	10 ~ 12	1.74
17	Forsythia	50	11 ~ 13	1.88

(B) Genomic DNA PCR

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

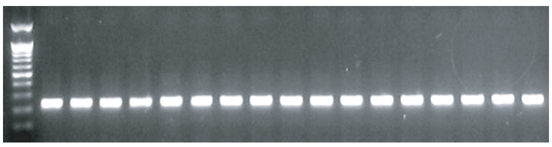


Fig 5-4. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as templates (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ Type C Protocol
: *Stem*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (㎍)	$A_{260/280}$
1	Potato	50	6 ~ 8	1.80
2	Onion	50	4 ~ 6	1.81
3	Elm	50	4 ~ 6	1.74
4	Geranium	50	4 ~ 5	1.78
5	Radish	50	5 ~ 6	1.78
6	Rhododendron	50	4 ~ 6	1.85
7	Sesame	50	4 ~ 7	1.80

(B) DNA Purification and Enzyme Digestion (RE)

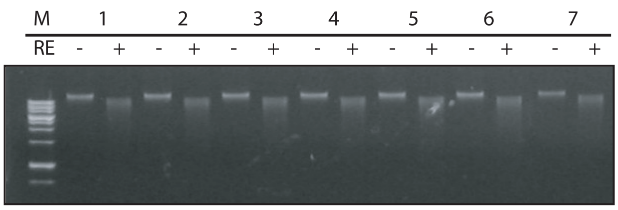


Fig 6-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 ㎕ Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

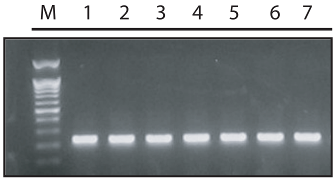


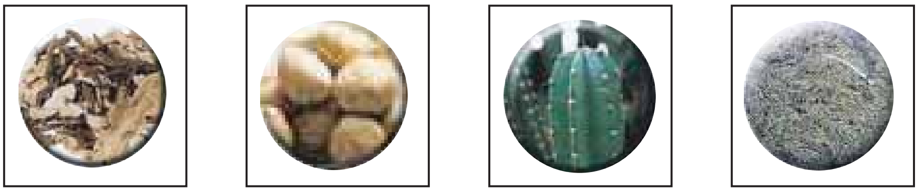
Fig 6-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

Additional Data
(Type C protocol-based modification)

When we have been developing i-genomic Plant DNA Mini Kit, iNtRON customers requested to be tested previously in their various samples for DNA yield, purity and PCR amplification.
The additional data are included in the following section. We have performed to extract DNA from various plant stems supplied by customers with Type C protocol (occasionally, we slightly have modified Type C protocol). You can show good results from various plant stems.

Fig 6-3. Additional Data (sample morphology)

Agelica *(Agelica gigas)* | Potato *(Solanum tuberosum)* | Cactus *(Opuntia ficus-indica)* | Sea weed fusiforme *(Hizikia tusiforme)*



(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (㎍)	$A_{260/280}$
1	Agelica	10	5 ~ 7	1.82
2	Potato	100	8 ~ 10	1.75
3	Cactus	100	4 ~ 6	1.75
4	Sea weed fusiforme	30	3 ~ 5	1.72

(B) Genomic DNA PCR

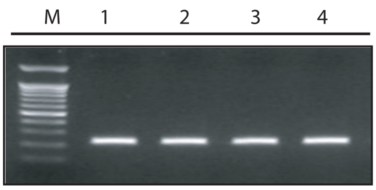


Fig 6-4. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as templates (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ Type D Protocol
: *Root*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Platycodon	50	5 ~ 7	1.83
2	Radish	50	6 ~ 10	1.79
3	Sweet potato	50	5 ~ 9	1.82
4	Carrot	50	8 ~ 10	1.85
5	Sesame	50	5 ~ 8	1.81

(B) DNA Purification and Enzyme Digestion (RE)

M 1 2 3 4 5
RE - + - + - + - + - +

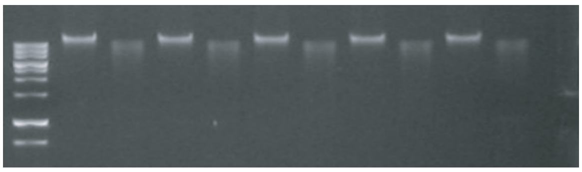


Fig 7-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

M 1 2 3 4 5

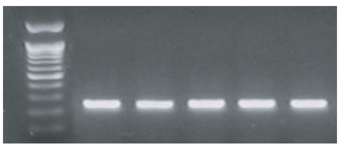


Fig 7-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

Additional Data
(Type D protocol-based modification)

When we have been developing i-genomic Plant DNA Mini Kit, iNtRON customers requested to be tested previously in their various samples for DNA yield, purity, and PCR amplification.
The additional data are included in the following section. We have performed to extract DNA from various plant roots supplied by customers with Type D protocol (occasionally, we slightly have modified Type D protocol). You can show good results from various plant roots.

Fig 7-3. Additional Data (sample morphology)

Ginseng Type 1 *(Panax ginseng Type 1)* | Ginseng Type 2 *(Panax ginseng Type 2)* | Ginseng Type 3 *(Panax ginseng Type 3)* | iNtRON Waits For Your Data.

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (µg)	$A_{260/280}$
1	Ginseng (Type 1)	50	5 ~ 7	1.78
2	Ginseng (Type 2)	50	3 ~ 4	1.80
3	Ginseng (Type 3)	50	4 ~ 6	1.81

(B) Genomic DNA PCR

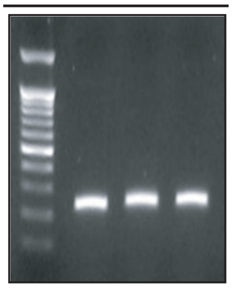


Fig 7-4. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as templates (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ Type E Protocol
: *Fruit*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (µg)	$A_{260/280}$
1	Muscat	100	3 ~ 4	1.87
2	Tomato	100	3 ~ 5	1.82
3	Pineapple	100	3 ~ 5	1.79
4	Apple	100	2 ~ 4	1.83
5	Melon	100	2 ~ 4	1.75
6	Peach	100	2 ~ 5	1.83
7	Grape	100	2 ~ 5	1.85

(B) DNA Purification and Enzyme Digestion (RE)

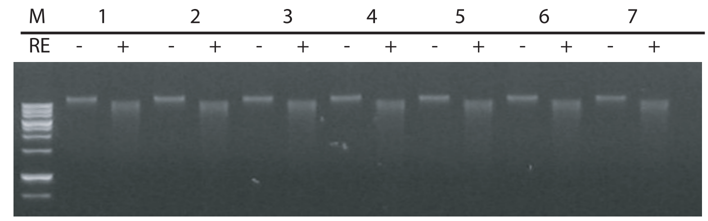


Fig 8-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 50 µl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

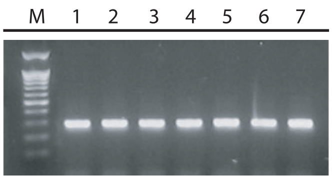


Fig 8-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

Additional Data
(Type E protocol-based modification)

When we have been developing i-genomic Plant DNA Mini Kit, iNtRON customers requested to be tested previously in their various samples for DNA yield, purity and PCR amplification.
The additional data are included in the following section. We have performed to extract DNA from various plant fruits supplied by customers with Type E protocol (occasionally, we slightly have modified Type E protocol). You can show good results from various fruits.

Fig 8-3. Additional Data (sample morphology)

Tomato *(Lycopersicon esculentum)* | Green pepper *(Capsicum annuum)* | iNtRON Waits For Your Data. | iNtRON Waits For Your Data.

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (㎍)	$A_{260/280}$
1	Tomato	50	2 ~ 3	1.83
2	Green pepper	50	2 ~ 4	1.85

(B) Genomic DNA PCR

M 1 2 3

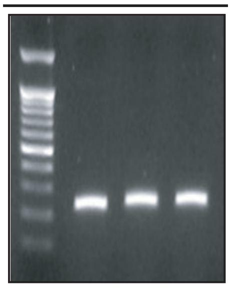


Fig 8-4. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as templates (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ **Type F Protocol**

[1] ***Seed (Leguminosae)***

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Pea	50	12 ~ 14	1.87
2	Black bean	50	10 ~ 14	1.88
3	Yellow bean	50	12 ~ 15	1.84
4	Mung bean	50	10 ~ 12	1.86

(B) DNA Purification and Enzyme Digestion (RE)

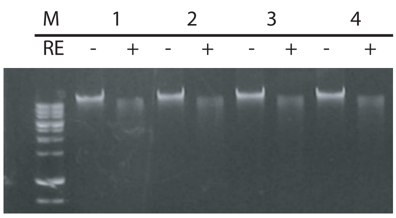


Fig 9-1. Results of DNA purification and enzyme digestion with *Eco*RI

After eluting genomic DNA with 100 μℓ Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

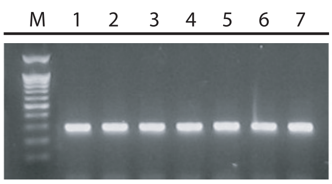


Fig 9-2. PCR Amplification

The housekeeping gene (18s, 222 bp) was amplified with the purified DNAs as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

[2] *Seed (Solanaceae)*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Green pepper	50	8 ~ 10	1.85
2	Paprika	50	10 ~ 12	1.85
3	Tomato type(normal)	50	9~ 12	1.78
4	Tomato JPN	50	8 ~ 12	1.77
5	Petunia	50	8 ~ 10	1.83

(B) DNA Purification and Enzyme Digestion (RE)

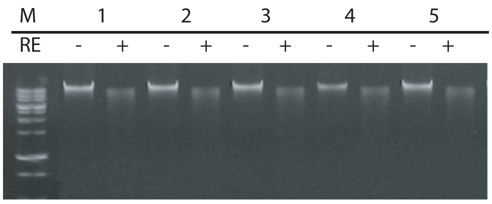


Fig 10-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

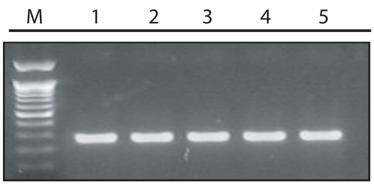


Fig 10-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng).
We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

[3] *Seed (Cruciferae)*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Radish type 1 *(Raphanus sativus)*	50	8 ~ 10	1.78
2	Radish type 2 *(Raphanus sativus)*	50	10 ~ 12	1.84
3	Radish type3 *(Brassica rapa)*	50	10 ~ 12	1.81

(B) DNA Purification and Enzyme Digestion (RE)

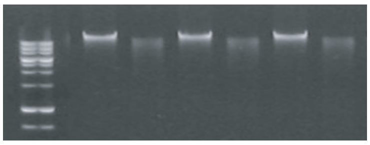


Fig 11-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μℓ Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

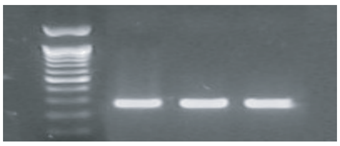


Fig 11-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

[4] ***Seed (Cucurbitaceae)***

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Green pepper	50	8 ~ 10	1.85
2	Paprika	50	10 ~ 12	1.85
3	Tomato type(normal)	50	9~ 12	1.78
4	Tomato JPN	50	8 ~ 12	1.77
5	Petunia	50	8 ~ 10	1.83

(B) DNA Purification and Enzyme Digestion (RE)

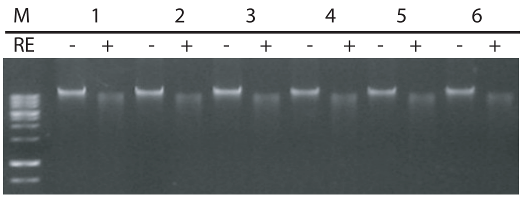


Fig 12-1. Results of DNA purification and enzyme digestion with ***Eco*****RI**

After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA marker, Cat. 24022)

(C) Genomic DNA PCR

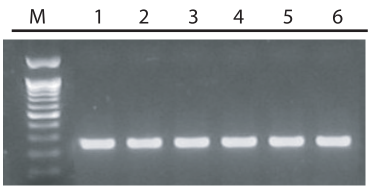


Fig 12-2. PCR Amplification

The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng).
We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

[5] *Other Seeds*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Spinach	50	8 ~ 10	1.74
2	Sunflower	50	8 ~ 10	1.75
3	Carrot	50	5 ~ 8	1.82
4	Sesame	50	10 ~ 12	1.77

(B) DNA Purification and Enzyme Digestion (RE)

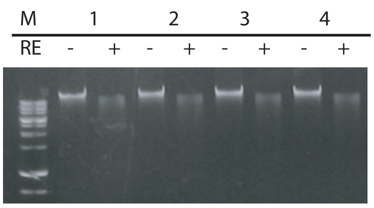


Fig 13-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 μl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

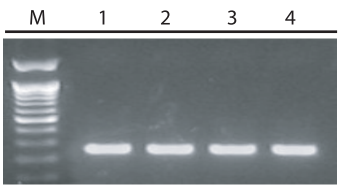


Fig 13-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

▪ Type G Protocol
: *Seed (Gramineae)*

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (µg)	$A_{260/280}$
1	Wheat	10	4 ~ 5	1.75
2	Grass	100	4 ~ 6	1.82
3	Barley	10	5 ~ 8	1.79
4	Rice	10	6 ~ 8	1.85
5	African millet	10	4 ~ 8	1.81

(B) DNA Purification and Enzyme Digestion (RE)

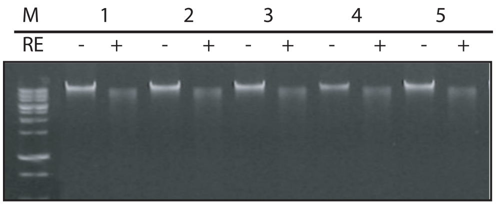


Fig 14-1. Results of DNA purification and enzyme digestion with *Eco*RI
After eluting genomic DNA with 100 µl Buffer PE, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37°C for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

(C) Genomic DNA PCR

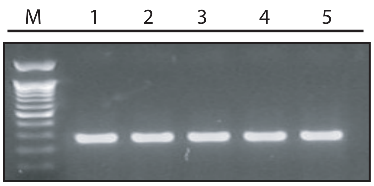


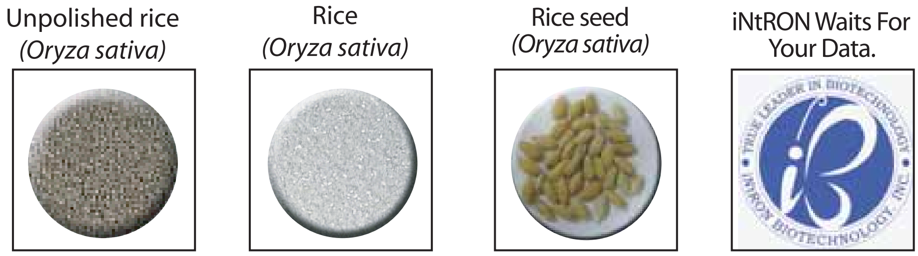
Fig 14-2. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNA as template DNA (10 ng).
We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction.
(Lane M : 100 bp DNA Ladder, Cat. 24012)

Additional Data

(Type G protocol-based modification)

When we have been developing i-genomic Plant DNA Mini Kit, iNtRON customers requested to be tested previously in their various samples for DNA yield, purity and PCR amplification. The additional data are included in the following section. We have performed to extract DNA from various plant seeds supplied by customers with Type G protocol (occasionally, we slightly have modified Type G protocol). You can show good results from various plant seeds.

Fig 10-3. Additional Data (sample morphology)



(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)	DNA Yield (μg)	$A_{260/280}$
1	Unpolished rice	50	3 ~ 5	1.73
2	Rice (1 of grain)	17.5	1~ 2.5	1.81
	Rice	50	2 ~ 3	1.75
3	Rice seed (1 of grain)	25.6	2 ~ 4	1.82
	Rice seed	250	2 ~ 4	1.71

(B) Genomic DNA PCR

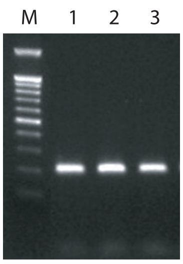


Fig 10-4. PCR Amplification
The housekeeping gene (18s, 222 bp) was amplified with the purified DNAs as templates(10 ng). We used Maxime PCR Premix Kit (i-StarTaq, Cat. 25165) for PCR amplification reaction. (Lane M : 100 bp DNA Ladder, Cat. 24012)

Appendix C : Source List of Genomic DNA

Scientific Name	Common Name			
	Korean	English	Japanese	Chinese
Gallus domesticus	닭	fowol	ニワトリ	鶏
Bos taurus	소	Cow	うし	牛
Canis familiaris	개	Dog	いぬ	犬
Drosophilidae	초파리	Drosophila	ショウジョウバエ	醯鶏
Lumbricidae	지렁이	Earth worm	ミミズ	蚓
Cavia porcellus	기니피그	Guinea pig	モルモット	天竺鼠
Homo sapiens	인간	Human	にんげん	人間
Gryllotalpa orientalis	땅강아지	Mole cricket	ケラ	东方蝼蛄
Culicidae	모기	Mosquito	か	蚊
Mus musculus	생쥐	Mouse	マウス	小家鼠
Sus domesticus	돼지	Pig	ブタ	豚
Oryctolagus cuniculus	토끼	Rabbit	ウサギ	兎
Rattus	쥐	Rat	ねずみ	鼠
Pandalus hypsinotus	도화새우	Humpback shrimp	ボタンエビ	牡丹蝦
Zebra fish	제브라피시	Zebra fish	ゼブラフィッシュ	狂放类型线
Bladder	방광	Bladder	ぼうこう	膀胱
Brain	뇌	Brain	のう	腦
Bronchus	기관지	Bronchus	きかんし	氣管支
Hair	털	Hair	へヤ	毛
Head	머리	Head	あたま	頭
Heart	심장	Heart	しんぞう	心臓
Kidney	신장	Kidney	じんぞう	腎臓
Liver	간	Liver	レバ	肝
Lung	폐	Lung	はい	肺
Muscle	근육	Muscle	きんにく	筋肉
Ovary	난소	Ovary	らんそう	卵巣
Pancreas	췌장	Pancreas	すいぞう	膵臓
Placenta	태반	Placenta	たいばん	胎盤
Skin	피부	Skin	ひふ	皮膚
Spleen	비장	Spleen	ひぞう	脾臓
Stomach	위	Stomach	い	胃
Subcutaneous fat	피하지방	Subcutaneous fat	ひかしぼう	皮下脂肪
Tail	꼬리	Tail	テール	尾
Thymus	흉선	Thymus	きょうせん	胸腺

Adenophora remotiflora	모시대	Adenophora	ソバナ	荠苨
Adenophora triphylla	잔대	Japanese lady bell	つりがねにんじん	山羊百科
Agelica gigas	당귀	Agelica	オニノダケ	當歸
Allium cepa	양파	Onion	タマネギ	洋葱
Allium tuberosum	부추	Korean leek	ニラ	韭
Amarantus	아마란스	Amaranth	ガンライコウ	反枝苋
Ananas comosus	파인애플	Pine apple	パイナップル	菠萝
Arabidopsis	애기장대	Arabidopsis	シロイヌナズナ	拟南芥
Arachis hypogaea	땅콩	Peanut	らっかせい	花生
Brassica campestris	배추	Cabbage	ハクサイ	菘
Brassica oleracea	양배추	Cabbage	タマナ	洋菘
Brassica rapa	순무	Radish (Turnip)	カブ	菁
Campanula punctata	초롱꽃	Bell flower	ホタルブクロ	山小菜
Capsicum annuum	고추	Green pepper	トウガラシ	苦椒
Capsicum annuum	파프리카	Paprika	ピーマン	甘唐芥子
Citrullus vulgaris	수박	Water melon	スイカ	水瓜
Coix Lachrymajobi	율무	Adlay	ハトムギ	薏
Cucumis melo	멜론	Melon	メロン	甜瓜
Cucumis melo var.makuwa	참외	Melon	マクワウリ	香瓜
Cucumis sativus	오이	Cucumber	キウリ	黃瓜
Cucurbita moschata	호박	Pumpkin	カボチャ	南瓜
Daucus carota var.sativa	홍당무	Carrot	ニンジン	紅蘿蔔
Diospyros kaki	감	Persimmon	かき	柿
Ecklonia cava	감태	Sea wood	カジメ	搗布
Fagopyrum esculentum	메밀	Buckwheat	ソバ	蕎
Fallen leves	낙엽	Fallen leves	らくよう	落葉
Forsythia korean	개나리	Forsythia	チョウセンレン	ギョウ
Ginkgo biloba	은행나무	Ginkgo	いちょう	銀杏–
Glycine max	검은콩	Black bean	くろまめ	黑大豆
Glycine max	메주콩	Yellow bean	ダイズ	大豆
Helianthus annuus	해바라기	Sunflower	ヒマワリ	向日花
Hizikia fusiforme	톳	Sea weed fusiforme	かいそう	海藻
Hordeum vulgare	보리	Barley	ムギ	麥
Ipomoea batatas	고구마	Sweet potato	サツマイモ	甘薯
Lactuca sativa var.capitata	양상추	Lettuce	ガーデンレタス	萵苣

Lagenaria leucantha	박	Gourd	ウリ科	扁蒲
Lycopersicon esculentum	토마토	Tomato	トマト	番茄
Lycopersicon esculentum JPN	토마토 JPN	Tomato JPN	ソバナ	番茄
Lyticum chinense	구기자	Chinese matrimony vine	クコ	枸杞子
Malus pumila	사과	Apple	リンゴ	沙果
Moss	이끼	Moss	こけ	苔
Opuntia ficus-indica	선인장	Cactus	サボテン	仙人掌
Oryza sativa	벼육	Paddy	いなほ	稻
Oryza sativa	벼	Rice	いね	米
Oryza sativa	현미	Unpolished rice	げん-まい	玄米
Panax ginseng	인삼	Ginseng	こうらいにんじん	人蔘
Panicum millacerm	기장	Millet (Hog millet)	キビ	粱
Pelargonium inquinans	제라늄	Geranium	ペラルゴニウム	小花天竺葵
Perilla frutescens	들깨	Green perilla	エゴマ	紫苏
Petunia hybrida	피튜니아	Petunia	サントリー「サフィニア	矮牽牛
Phasedus radiutus	녹두	Mung bean	リョクトウ	綠豆
Phyteuma japonicum	영아자	Phyteuma	キキョウ科 シデシャジン	牧根草属
Pinus densiflora	솔잎	Pine needles	まつば	松葉
Pisum sativum	완두콩	Pea	エンドウ	豌豆
Platycodon grandiflorum	도라지	Platycodon	キキョウ	桔梗
Prunus persica	복숭아	Peach	もも	桃
Raphanus sativus	무	Radish	だい-こん	萝卜
Rhododendron schlippenbachii	철쭉	Rhododendron	クロフネツツジ	灌木
Sesamum indicum	참깨	Sesame	ご-ま	胡麻
Setania italica	조	Italian millet	アワ	粟
Solanum tuberosum	감자	Potato	ジャガタライモ	马铃薯
Sorghum bicolor	수수	African millet	モロコシ	高粱
Spinacia oleracea	시금치	Spinach	ホウレンソウ	菠菜
Triticum aestivum (vulgare)	밀	Wheat	コムギ	小麥
Ulmus davidiana	느릅나무	Elm	ハルニレ	楡
Vitis vinifera L.	포도	Grape	ぶどう	葡萄
Vitis spp	청포도	Muscat	あおぶどう	青葡萄
Zea mays	옥수수	Corn	とうもろこし	玉米
Zingiber officinale	생강	Ginger	ショウガ	生薑
Zoysia japonica	잔디	Grass	しば	结缕草

Appendix D

Summarized Photo Procedure

This Summarized Photo Procedure is a representative figure for Type B Protocol in i-genomic Plant DNA Extraction Mini Kit Handbook v1.0. This figure is only reference for beginners, and therefore please follow your set-up method. For more detailed information, please contact us.

B-1. Sample Treatment Step

▪ ***Fresh Leaf***

■ I. Preparation step ■ II. Disrupt. & Homogen. ■ III. Sample Sizing step □ IV. Pre-Treating step

I. Preparation step

1. Prepare fresh leaf sample.

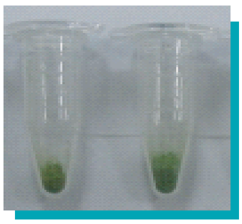
3. Add liquid nitrogen to the mortar and freeze. Disrupt and homogenize, pouring liquid nitrogen slowly.

II. Disruption & Homogenization step

2. Slice off the prepared sample to small pieces by the scalpel or scissor and place them the mortar.

III. Sample Sizing step

4. Measure 50 mg of sample powder, and then transfer into 1.5 ml tube.



See next page for DNA Extraction step.

B-2. DNA Extraction Step

□ I. Pre-Lysis step ■ II. Lysis step ■ III. Precipitation step ■ IV. DNA Binding step

■ V. Washing step A ■ VI. Washing step B ■ VII. Elution step

II. Lysis step

1. Add 390 ㎕ of Buffer PG,
   7 ㎕ of Enhancer Solution,
   20 ㎕ of Proteinase K,
   5 ㎕ of RNase A Solution.

Buffer PG

Enhancer Solution, Proteinase K, RNase A

Vortex vigorously.

2. Incubate the lysate at 65°C for 30 min.
   (invert 5 ~ 6 times for more lysis)

Heating block

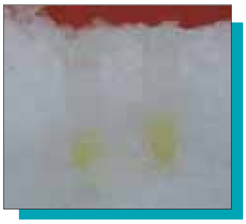
III. Precipitation step

3. Add 100 μℓ of Buffer PPT.

Buffer PPT

Then, mix well.

Incubation for 5 min on ice.



4. Centrifuge at 13,000 rpm at RT for 5 min.

IV. DNA Binding step

5. Transfer 200 μℓ of supernatant to a new 1.5 ml tube.

After centrifugation

See next page.

6. Add 650 µℓ of Buffer PB and mix by inverting or pipetting.
DO NOT vortex !

Buffer PB

7. Transfer 650 µℓ of the mixture to spin column.
Do not remove the rest of mixture.

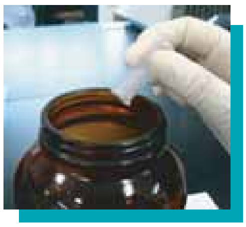
Centrifuge at 13,000 rpm (RT) for 1 min .

Discard flow-through.

8. Transfer the rest of mixture to spin column (maximum 200 µℓ).

Centrifuge at 13,000 rpm (RT) for 1 min.

Discard flow-through.



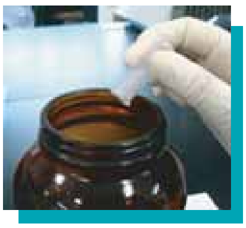
V. Washing step A

9. Place spin column into a new 2.0 ml collection tube.

Add 700 μℓ of Buffer PWA to the spin column.

Centrifuge at 13,000 rpm (RT) for 1 min .

Discard flow-through.

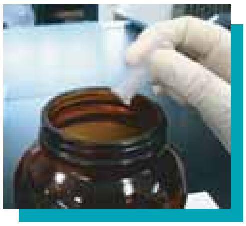


VI. Washing step B

10. Add 700 μℓ of Buffer PWB to column.
NOTE: Ensure that 40 ml of ethanol has been added to Buffer PWB.

Centrifuge at 13,000 rpm (RT) for 1 min .

Discard flow-through.



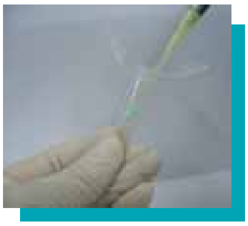
Centrifuge at 13,000 rpm (RT) for 1 min .

See next page.

VII. Elution step

11. Transfer the spin column to a new 1.5 ml tube.

Add 100 µℓ of Buffer PE directly onto the membrane.



Incubate at RT for 1 min .

Centrifuge at 13,000 rpm (RT) for 1 min .

Discard the spin column.

Finished DNA Extraction.

Ordering Information

Product Name	Samples	Examples
CAT. NO. 17341(50 Columns) i-genomic CTB DNA Extraction Mini Kit	Cells Tissues Gram(-) Bacteria	Human cultured cells / Mouse cultured cells / Mouse / Guinea pig / Rabbit / Chicken / Zebra fish / Shrimp / Pig / Insect / Animal hair / Worm / Stool / Buccal swab / Gram(-) bacteria / Others
CAT. NO. 17351(50 Columns) i-genomic Blood DNA Extraction Mini Kit	Blood	Whole blood / Buffy Coat / Dried Spot / Blood Swab /Plasma / Serum / Others
CAT. NO. 17361(50 Columns) i-genomic BYF DNA Extraction Mini Kit	Gram(+) Bacteria Yeast Fungi	*Azotobacter sp.* *Staphylococcus sp.* *Saccharomyces sp.* *Aspergillus sp.* Others
CAT. NO. 17371 (50 Columns) i-genomic Plant DNA Extraction Mini Kit	Plant	Leaf / Root / Stem / Fruit / Seed / Others

Larger kit sizes available; please inquire.

Related Products

Product Name	Cat. No.	Size
Maxime™ PCR PreMix (i-Taq)	25025 / 25026	*96 Tubes / 480 Tubes*
Maxime™ PCR PreMix (i-StarTaq)	25165 / 25167	*96 Tubes / 480 Tubes*
Maxime™ PCR PreMix (i-pfu)	25185	*96 Tubes*
Maxime™ PCR PreMix (i-MAX II)	25265	*96 Tubes*
i-Taq™ DNA Polymerase	25021 / 25022	*250 Units / 500 Units*
i-StarTaq™ DNA Polymerase	25161 / 25162	*250 Units / 500 Units*
i-MAX™ II DNA Polymerase	25261	*250 Units*
i-pfu DNA Polymerase	25181	*250 Units*
easy-Labeler™ Random Primed DNA Labeling PreMix for dCTP	16043	*30 Rxns*
PROBER™ Probe DNA Purifying System	17072	*100 Columns*
MicroPROBER™ Probe DNA Purifying System	17073	*50 Columns*

Global Distributors

Australia
Scientifix
Unit C3 Adamco Business Park 2A Westall Rd,Clayton, VIC
Phone : +61-3-9562-7488
Fax : +61-3-9548-7177
URL : www.scientifix.com.au

Belgium
Goffin Meyvis Analytical & Medical Systems
Park Rozendal Terhulpensesteenweg 6a 1560 Hoeilaart
Phone : +32-2-769-7474
Fax : +32-2-769-7499
URL : www.goffinmeyvis.com

China
Innovating Santaibio INC.
Room 322 Building A, Shengdi Mansion,
No. 29 Xueyuan Road,
Haidian District, Beijing 100083
Phone : +86-10-8232-3705, +86-10-8232-3706
Fax : +86-10-8232-3707
URL : www.santaibio.com

China
Chinagen INC.
FL1, Building one, 2nd Industrial District, Kefa Road,
Science&Technology Park Central,
Shenzhen 518057
Phone : +852-2646-5368
Fax : +852-2646-5037
URL : www.chinagen.com.cn

China
Tech Dragon Limited
Rm 808,Topsail Plaza,11 On Sum Street,
Shatin,N.T.,Hong Kong
Phone : +86-755-2601-4525
Fax : +86-632-418-5877
URL : www.techdragon.co.hk

Hungary
Kasztel-Med Ltd.
1118 Budapest Kelenhegyi 22
Phone : +36-1-385-3887
Fax : +36-1-381-0695
URL : www.kasztel.com

India
Biogene
B-41,IInd Floor,Moti Nagar, New Delhi-110015
Phone : +91-11-2592-0047/48
Fax : +91-11-2592- 0047
URL : www.biogene-india.com

Israel
Talron Biotech Ltd.
17 Hazait street Rehovot 76349
Phone : +97-2-8-947-2563
Fax : +97-2-8-947-1156
URL : www.talron.co.il

Indonesia
PT.Blue Sky Biotech
Blok J 3 No.46 Taman Tekno BSD
Serpong-Tangerang 15314
Phone : +62-21-7028-3685
Fax : +62-21-756-5165

Italy
Li StarFISH S.r.l
Via Silvo Pellico,3. 20061 Carugate (MI)
Phone : +39-02-9215-0794
Fax : +39-02-9215-7285
URL : www.listarfish.it

Japan
Cosmo Bio Co. Ltd.
Toyo-Ekimae Bldg, 2-20, Toyo 2-Chome, Koto-ku,
Tokyo, 135-0016
Phone : +81-3-5632-9610/9620
Fax : +81-3-5632-9619
URL : www.cosmobio.co.jp

Japan
Funakoshi Co. Ltd.
9-7, Hongo 2-Chome, Bunkyo-ku, Tokyo, 113-0033
Phone : +81-3-5684-1620
Fax : +81-3-5684-1775
URL : www.funakoshi.co.jp

Japan
Nacalai Tesque, Inc.
272 higashitsuchikawa-cho, Kuze Higashi, Minami-ku,
Kyoto, 601-8204
Phone : +81-75-251-1723
Fax : +81-75-251-1762
URL : www.nacalai.co.jp

Malaysia
NHK BIOSCIENCE SOLUTIONS SDN. BHD.
4 Lorong Checkor, 3rd mile off Jalan Kelang
Lama, 58000, Kuala Lumpur
Phone : +603-7987-8218
Fax : +603-7987-8213

Netherlands
Goffin Meyvis Analytical & Medical Systems
Ecustraat 11 4879 NP Etten-Leur
Phone : +31-76-508-6000
Fax : +31-76-508-6086
URL : www.goffinmeyvis.com

Pakistan
Lab Line
98, National Medicine Market, Arambagh Road,
Karachi 74200
Phone : +92-21-262-6097
Fax : +92-21-262-2194

Philippines
Hebborn Analytics INC.
A-7 Lester building,Quirino Highway, Lagro Quezon city
Phone : +632-461-7173
Fax : +632-418-5877

Singapore
ProGen Scientific
32 Maxwell Road #03-13, Whitehouse Singapore 069115
Phone : +65-9557-0665
Fax : +65-6363-4381

Spain
LABOTAQ, S.C
C1 Aviacion, 5 Poligono Industrial Calonge
41007 Seville
Phone : +34-954-31-7216
Fax : +34-954-31-7360
URL : www.labotaq.com

Thailand
Pacific Science Co. Ltd.
90 Soi Charansanitwong 49/1 Charansanitwong rd,
Bandbumru Bangplad, Bangkok 10700
Phone : +66-2-433-0068/9
Fax : +66-2-434-2609
URL : www.Pacificscience.co.th

Turkey
MedSanTek Ltd. Co.
Millet Cad. Findikzade sok. Emre Apt. No : 19/5
Findikzade, Istanbul
Phone : +90-212-635-8546
Fax : +90-212-635-8350
URL : www.Medsantek.com.tr

Taiwan
Hong-jing Co., Ltd.
5F, No. 174, CHUNG-SHAN Rd., Sec 1,
YOUNG-HO CITY, Taipei
Phone : +886-2-3233-8585
Fax : +886-2-3233-8686

U.A.E.
Emphor FZCO.
Shed No. MB-6, P.O. Box 61232
Jebel Ali Free Zone, Dubai
Phone : +971-4-883-0233
Fax : +971-4-883-0133

United Kingdom
Molecular Solutions Europe Ltd.
2nd floor LPL, 145-157 St. John Street,
London, EC1V 4PY?Phome : +44-0870-199-5067
Fax : +44-0207-253-9040
URL : http://www.mseu.co.uk

U. S. A.
Boca Scientific
1140 Holland Drive, Suite 11, Boca Raton, FL 33487
Phone : +1-561-995-5017
Fax : +1-561-995-5018
URL : www.bocascientific.com

U. S. A.
Lambda Biotech
3830 Washington Avenue, St. Louis, Mo 63108
Phone : +1-314-533-8650
Fax : +1-314-533-4007
URL : www.lambdabiotech.com

U. S. A.
Nacalai USA, Inc.
6640 Lusk Blvd,Suite A 200,
San Diego, CA 92121
Phone : +1-858-404-0403
Fax : +1-858-404-0408

Note

i-genomic BYF DNA Extraction Mini Kit

Plant

iNtRON Biotechnology, Inc.