i-genomic Plant DNA Extraction Mini Kit

The Instruction Manual for Genomic DNA Extraction from Plant Tissues using Silica Membrane.

RUO *Research Use Only* REF *17371* Σ 50

DESCRIPTION

- i-genomic Plant DNA Extraction Mini Kit provides a fast and easy way to purify DNA from plant-like samples such as various leaf, stem, root, fruit, and seed. 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 Protocol List (Table 1). If you need some more information in selecting a protocol, please do not hesitate to contact our Technical Assist Teams.

CHARACTERISTICS

- **Speed** : Takes only 20 ~ 30 minutes to extract genomic DNA.
- **Smart** : High quality and quantity of DNA recovery
- **Steady** : Complete removal of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability.
- **Stage-up** : No need various DNA Extraction kit – vast applicability. The Kit is suitable to use various kinds of biological samples. Advanced GxN technology for rapid and efficient purification of DNA without ethanol precipitation.

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[2]	Washing Buffer B (add 40 ml of EtOH)	10 ml
Buffer PE[3]	Elution Buffer	20 ml
Enhancer Solution[4]	Binding enhancer buffer	0.5 ml
Spin Columns (Green color O-ring)	Inserted into the collection tubes. (2.0ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	100 tubes
RNase A[5]	Dissolve in 0.3ml of DW	3 mg
Proteinase K[6]	Dissolve in 1.1ml of DW	22 mg

1. Contains a chaotropic salt. Carefully handle.
2. Buffer PWB are supplied as concentrates. Add 40ml of ethanol (96~100%) according to the bottle label before use.
3. Buffer PE is finally 10mM Tris-HCl (pH 8.0). You may use your lab's buffer.
4. After receiving, the Binding enhancer solution should be stored at 2~8℃.
5. Lyophilized RNase A : Dissolve the RNase A in 0.3 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance.
6. Lyophilized Proteinase K : Dissolve the Proteinase K in 1.1 ml of pure D.W. to each vial. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance.

STORAGE

i-genomic Plant DNA Extraction Mini Kit should be stored dry at room temperature (15–25°C). Under these conditions, the kit can be stored for up to 24 months without showing any reduction in performance and quality. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized enzyme can only be dissolved in D.W.; dissolved enzyme should be immediately stored at -20℃. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

APPLICATIONS

- Plant genomic research
- Pathogen detection study
- Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, microarray
- GMO, LMO related study

PROTOCOL LIST

Table 1. Protocols according to the Plant Sample Groups (7 Protocols)

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

NOTICE BEFORE USE

i-genomic Plant DNA Extraction Mini Kit provides almost all reagents for extracting DNA, including lyophilized enzyme. 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.

• Common equipment and reagents

- Equipment for disruption and homogenization, including Grinding Jar Set (mortar)
- Pipettes and pipette tips
- Water bath or heating block
- Vortexer
- Absolute ethanol (EtOH, 96~100%)
- Liquid nitrogen
- Microcentrifuge with rotor for 2.0 ml tubes
- Microcentrifuge tubes (1.5 ml)
- Ice
- Other general lab equipments

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. 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.

CAUTION:
DO NOT add bleach or acidic solutions directly to the sample preparation waste.

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.

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.

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of i-genomic Plant DNA Extraction Mini Kit is tested against predetermined specifications to ensure consistent product quality. The quality of the isolated genomic DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- i-genomic spin column control : The DNA binding capacity was tested by determining the recovery with 10 ~ 15 μg of genomic DNA from 1 x 10^6 cells.
- RNase A / Proteinase K : In case of RNase A, the activity was determined 20K ~ 25K unit per mg of protein using tolura yeast RNA hydration test. In case of Proteinase K, the activity was determined from cleavage of the substrate releasing p-nitroaniline which can be measured spectrophotometrically at 410nm.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below

Table 2. Quality control criteria of each components

Buffer	Conductivity	pH
Buffer PG	50 ~ 56 mS/cm	7.4 ~ 8.2
Buffer PPT	127 ~ 142 mS/cm	7.7 ~ 8.4
Buffer PB	57 ~ 64 mS/cm	6.9 ~ 7.7
Buffer PWA	63 ~ 70 mS/cm	6.7 ~ 7.4
Buffer PWB	10 ~ 12 mS/cm	7.0 ~ 8.0
Buffer PE	500 ~ 700 μS/cm	7.2 ~ 8.2

COLUMN INFORMATION

Table 3. Column information of i-genomic Series

Column Membrane [1]	Silica-based membrane
Spin Column [1]	Individually, is inserted in a 2.0 ml collection tube [2].
Loading Capacity	Maximum 800μl
DNA Binding Capacity	Maximum 45μg
Recovery	85 ~ 95% depending on the elution volume
Elution Volume	Generally, eluted with 20 ~ 200 ml of elution buffer or water.

1. After use, seal the pack containing spin columns tightly without getting dry. Then, the spin columns are stable for over 2 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 POINTS BEFORE STARTING

- **Buffer PWB (Washing Buffer)**
  Buffer PWB is supplied as concentrates. Before using for the first time, be sure to add 40 ml of absolute ethanol (96 ~ 100% EtOH) to obtain a working solution.
- **Lyophilized enzyme**
  Dissolve the lyophilized enzyme in appropriate volume of pure D.W.
  - Lyophilized RNase A : Dissolve the RNase A in 0.3 ml of pure D.W.
  - Lyophilized Proteinase K : Dissolve the Proteinase K in 1.1 ml of pure D.W.
  The lyophilized enzyme can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized enzyme can only be dissolved in D.W.; dissolved enzyme should be immediately stored at -20℃. The enzyme solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date
- **Enhancer Solution**
  We recommend keeping the Enhancer Solution at 2 ~ 8 C upon arrival, and then is stable for 1 year. The Enhancer Solution is a yellow color solution with precipitates upon storage.
- **Preheat a water bath or heating block to 65°C.**
- **Centrifugation**
  All centrifugation steps are carried out at RT (15 ~ 25°C) in a microcentrifuge.
- **Choosing the Right Protocol according to Plant Sample**
  Seven kinds of different protocols in this instructions provide detailed protocols to use i-genomic plant Kit for purifying genomic DNA from various plant samples as seen in Table 1. These protocols are optimized for use. Especially, with iNtRON's sixty five samples in more than five categories, we have verified practically by several experiments to ensure the quality and the application of the Kit. We recommend to choose the right protocol according to your plant sample. For more information, please contact iNtRON Technical Assist Teams.
- **Collection and Storage of Plant Tissues**
  Generally, for higher quality of genomic DNA, it is preferable to collect young plant materials. The fresh plant tissues contain more 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℃. When grind plant tissues, use liquid nitrogen as in detailed manual instructions. Ground plant tissue powder can also be stored at -80 ℃. 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 Buffer PG without using liquid nitrogen, but this may cause shearing of high molecular-weight DNA. We do not recommend disrupting frozen material 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. Disruption for more than 1 minute may lead to shearing of genomic DNA.

NOTES FOR SAMPLE SIZING

we recommend to measure the amount of starting material after disrupting plant tissues in i-genomic Plant 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 to measure conveniently the amount of starting material.

Table 4. Recommended Volume of Starting Material according to Plant samples.

Sample	Amount
Dried or Lyophilized Leaf	~ 5 mg
Fresh Leaf	~ 50 mg
Stem	~ 50 mg
Root	50 ~ 100 mg
Fruit	100 mg
Seed (except Gramineae)	50 mg
Seed (Gramineae)	10 mg

PROTOCOL A (for Lyophilized leaf)

1. Prepare lyophilized leaf sample.
Note : Plant tissue can be lyophilized after harvesting to allow storage at room temperature (15~20℃). 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 need not any special disruption & homogenization steps. Furthermore, when using lyophilized tissue, the samples do not need to be frozen in liquid nitrogen.

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

Note : It's difficult to handle to measure the fine powder sample due to its static electricity. It can be inhibited by previously chilling 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.

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

4. Incubate the lysate for 30 min at 65℃.
Note : For complete lysis, mix 5 or 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.

5. Add 100 ㎕ Buffer PPT to the lysate, mix well, and incubate for 5 min on ice.
Note : This step precipitates detergent, proteins, and polysaccharides. During incubation on ice, please mix 5 to 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.

6. Centrifuge the lysate for 5 min at 13,000 rpm at room temperature.
Note : 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.

7. Transfer carefully 200 ㎕ of supernatant from step 6 into a new 1.5 ml tube.
Note : 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.

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

9. Pipette 650 ㎕ of the mixture from step 8, including any precipitate that may have formed, into the spin column inserted in a 2.0 ml collection tube. Centrifuge for 1 min 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.

10. Repeat step 9 with remaining sample (maximum 200 ㎕). Discard flow-through and collection tube altogether.
Note : If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

11. 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.

12. 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 additionally 1 min to dry the membrane. Discard the flow-through and collection tube altogether.
Note : 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.

13. 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.
Note : 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.

PROTOCOL B (for Leaf)

1. Prepare leaf sample.
Note : If possible, it is preferable to collect young fresh leaf since they contain more 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℃ after harvesting. It is good for disruption and homogenization if the sample is sliced off when store at -20℃ or -80℃.

2. Slice off the prepared sample to small pieces by the scalpel or scissor.
Note : To reduce disruption and homogenization time, we recommend to slice off it.

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 to step 4.
Note : 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.

4. Measure 10 ~ 50 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.
Note : To prevent thaw the frozen sample during transfer it, previously 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.

5. Follow the Protocol A (for Lyophilized leaf) from Step 3.

PROTOCOL C (for Stem)

1. Prepare stem sample.
Note : 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 due to it absorbs all of lysis buffer. Also, plant stem sample is very tough and thick, therefore it is difficult to disrupt and homogenize. Alike other plant sample, 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 frozen or kept in plastic bag containing a wet paper towel after harvesting. It is good for disruption and homogenization if the sample is sliced off when store.

2. Slice off the prepared sample to small pieces by the scalpel or scissor.
Note : 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 off it. Some stem sample is very tough and thick, to cut off it by the saw.

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 to step 4.
Note : 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.

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

Note : To prevent thaw the frozen sample during transfer it, previously 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.

5. Follow the Protocol A (for Lyophilized leaf) from Step 3.

PROTOCOL D (for Root)

1. Prepare root sample.
Note : 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. Alike stem, root sample does not keep wet condition , therefore we recommend to frozen condition (-20℃ or -80℃) 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 store. In frozen root, 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.

2. Cut off the prepared sample to small pieces by the blade or scissor.
Note : To reduce disruption and homogenization time, we recommend to cut off it. 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.
Note : 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.

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

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

5. Follow the Protocol A (for Lyophilized leaf) from Step 3.

PROTOCOL E (for Fruit)

1. Prepare fresh fruit sample.
Note : While other plant's organ contains a few water, fruit organ contains water about 80 ~ 95%, therefore increase the amount of starting material when 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℃ or lyophilize it and store at room temperature (15℃ ~ 25℃) after harvesting. Before freezing, peel the shell the fruit sample and slice off it to suitable size. When extract 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.

2. Slice off the prepared sample to small pieces by the blade or scissor.
Note : To reduce disruption and homogenization time, we recommend to slice off it.

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.
Note : 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.

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

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

5. Follow the Protocol A (for Lyophilized leaf) from Step 3.

PROTOCOL F (for Seed - Other)

1. Prepare dried or fresh seed sample.
Note : 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 use 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 freezing-thawing.

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.
Note : 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.

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

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

4. Follow the Protocol A (for Lyophilized leaf) from Step 3.

PROTOCOL G (for Seed – gramineae)

1. Prepare dried or fresh gramineae seed sample.
Note : It is hardly difficult to get good result more than other seed sample as gramineae seed sample has too much secondary product and polyphenolic compounds. Even if seed keep at room temperature (15℃ ~ 25℃) after dry, there is no problem when use later. If not, store at -20℃ or -80℃ 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 freezing-thawing.

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.
Note : 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 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.

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

Note : 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 thaw the frozen sample during transfer it, previously chill the spatula and 1.5ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA

4. Follow the Protocol A (for Lyophilized leaf) from Step 3.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low flow rate in column	Clogged spin column by particulate material	Completely perform the Disruption & Homogenization step
		Increase the incubation time at 65°C in Lysis step.
	Problem in centrifugation	Check your centrifuge, and then speed up or increase the centrifugation time.
Low DNA yield	Inadequate lysis	Reduce the amounts of starting material.
		Increase the incubation time at 65°C in Lysis step.
	Error in DNA binding	Check that the amount of Buffer PB is added correctly to the supernatant.
	Incorrect Washing step	Check again that the amount of ethanol (EtOH) is added correctly to Washing buffer.
	Insufficient DNA elution	Increase the volume of Buffer PE to 100 µl.
		Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.
DNA sheared	Vigorously vortex	Do not vortex the mixture after adding Buffer MB as described in protocol.
Problems in down-stream experiments	Ethanol contamination	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.
		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	Check again to add EtOH previously into Buffer BW.
		Store Buffer BW at room temperature (15 ~ 20°C).
	Amount of DNA used in experiments.	Optimize the amount of DNA used in your downstream experiments.