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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.

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Instruction manual

i-genomic BYF DNA Extraction Mini Kit

The Instruction Manual for Genomic DNA Extraction from Bacteria, yeast and fungi samples using silica membrane.

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DESCRIPTION

- i-genomic BYF DNA Extraction Mini Kit provides a fast and easy way to purify DNA from microorganism samples such as various gram positive bacteria, yeast, fungal tissue and fungi. Furthermore, we have tested i-genomic BYF DNA Mini Kit to get more practical data with twenty of BYF samples. You can see vast sample photos, vast samples, and vast practical data.
- i-genomic BYF DNA Mini Kit provides 5 kinds of protocols, Type A, Type B, Type C, Type D and Type E. You can also extract genomic DNAs from various BYF samples in addition to 20 BYF samples by selecting an appropriate protocol. When you choose a protocol, please refer to BYF Sample Group (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
MYP Buffer	Yeast Pre-Lysis Buffer	12 ml
MP Buffer [1]	Pre-Lysis Buffer	7 ml
MG Buffer	Lysis Buffer	15 ml
MB Buffer	Binding Buffer	15 ml
MW Buffer (concentrate) [2, 3]	Washing Buffer (add 40 ml of EtOH)	10 ml
ME Buffer [3]	Elution Buffer	20 ml
Spin Columns (Blue 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[4]	Dissolve in 0.3 ml of DW	3 mg
Proteinase K[4]	Dissolve in 1.1 ml of DW	22 mg
Lysozyme[4]	Dissolve in 0.2 ml of DW	20 mg

1. This buffer contains chaotropic salt.

2. Buffer MW are supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.

3. Buffer MW and Buffer ME are finally 10 mM Tris-HCl (pH 8.0). You may use your lab's buffer.

4 The lyophilized enzyme 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.

STORAGE

i-genomic BYF DNA Extraction Mini Kit should be stored dry at room temperature (15–25°C). Under these conditions, i-genomic BYF DNA Extraction Mini Kit can be stored for up to 24 months without showing any reduction in performance and quality. The lyophilized enzyme can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance.

▪ Type E Protocol : Fungi

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)[1]	DNA Yield (μg)	$A_{260/280}$
1	*Aspergillus oryza*	2	5 ~ 7	1.81
2	*Aspergillus niger*	2	5 ~ 8	1.82
3	*Penicillium citrinum*	2	5 ~ 6	1.80

[1] The volume of cultured cell

(B) DNA Purification and Enzyme Digestion (RE)

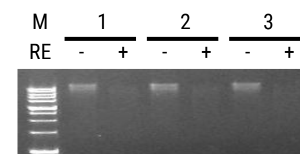


Figure 5. Results of DNA purification and enzyme digestion with *Eco*RI

After eluting genomic DNA with 50 μl Buffer ME, each 100 ng of DNAs 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.

▪ **Type D Protocol : Fungal Tissue**

(A) DNA Yield and Purity

Lane	Samples	Amounts (mg)[1]	DNA Yield (µg)	$A_{260/280}$
1	*Armillaria bulbosa*	50	9 ~ 13	1.84
2	*Tricholoma matsutake*	50	9 ~ 12	1.82
3	*Pleurotus ostreatus*	50	9 ~ 13	1.85
4	*Flammulina velutipes*	50	10 ~ 13	1.88

[1] The amount of ground fungal tissue powder

(B) DNA Purification and Enzyme Digestion (RE)

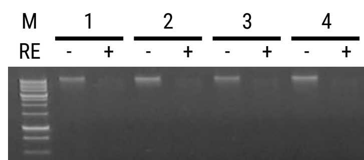


Figure 4. Results of DNA purification and enzyme digestion with *Eco*RI

After eluting genomic DNA with 50 µl Buffer ME, each 100 ng of DNAs 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.

CONSIDERATION BEFORE USE

▪ **Buffer MW (Washing Buffer)**

Buffer MW 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.
- Lyophilized Lysozyme : Dissolve the Lysozyme in 0.2 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

▪ **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 microorganism Sample**

Five kinds of different protocols in this handbook provide detailed instructions to use i-genomic BYF DNA Mini Kit for purifying genomic DNA from various bacteria, yeast, fungal tissue and fungi samples. These protocols are optimized for use.

▪ **Collection and Storage of Microorganism Samples**

In case of bacteria and yeast, progress newly culture before beginning experiment in order to user fresh cell. If gram positive bacteria and yeast will not be used freshly, divide cultured cell. After quickly froze in liquid nitrogen, and then store at -80°C. In case of fungal tissue such as mushroom, fungal tissue sample is big size. So cut off suitable size and grind with mortar and liquid nitrogen. If fungal tissue sample will not be used directly, cut off suitable size. After quickly froze in liquid nitrogen, and then store at -80°C.

When working with fungi such as mold, harvest mycelium directly from a cultured dish or liquid culture. For liquid culture, first pellet cells by centrifugation. Remove the supernatant completely. If fungi sample will not be used directly, pellet cells by centrifugation. After quickly freeze in liquid nitrogen, and then store at -80°C. Alternatively, store at room temperature after progress freeze-drying.

▪ **Disruption and Homogenization**

Most microorganism samples except fungal tissue can not be disrupted and homogenized. Fungal tissue only apply. Namely, disruption can be performed without lysis buffer by keeping the sample submerged in liquid nitrogen before and during disruption on a mortar. We do not recommend disrupting frozen material in lysis buffer as this can result in low yields and degraded DNA. 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.

ADDITIONAL REQUIRED EQUIPMENT

i-genomic BYF 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
- Vortex mixer
- Absolute ethanol (EtOH, 96~100%)
- 80% EtOH
- Liquid nitrogen
- Microcentrifuge with rotor for 2.0 ml tubes
- Microcentrifuge tubes (1.5 ml)
- Ice
- 15ml tube
- Lyticase or zymolase solution
- Other general lab equipments

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of i-genomic BYF 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 / Lysozyme : 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. Also in case of Lysozyme, the activity was determined 25 ~ 30 Kunit per mg of protein using *Pichia pastoris* lysis test.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below

Table 1. Quality control criteria of each components

Buffer	Conductivity	pH
Buffer MYP	10 ~ 12 mS/cm	7.9 ~ 8.3
Buffer MP	21 ~ 25 mS/cm	7.5 ~ 8.4
Buffer MG	13 ~ 16 mS/cm	7.9 ~ 8.9
Buffer MB	98 ~ 108 mS/cm	7.0 ~ 7.7
Buffer MW	10.5 ~ 13 mS/cm	7.0 ~ 7.7
Buffer ME	500 ~ 700 μS/cm	7.1 ~ 7.9

▪ Type C Protocol : Yeast

(A) DNA Yield and Purity

Lane	Samples	Amounts (ml)[1]	DNA Yield (μg)	$A_{260/280}$
1	*Candida albicans*	5	10 ~ 13	1.78
2	*Candida parapsilosis*	5	10 ~ 12	1.83
3	*Candida tropicalis*	5	10 ~ 13	1.79
4	*Saccharomyces type 1*	5	12 ~ 14	1.85
5	*Saccharomyces type 2*	5	12 ~ 15	1.84
6	*Saccharomyces type 3*	5	10 ~ 14	1.82
7	*YRG-2*	5	12 ~14	1.81

[1] The volume of cultured cell

(B) DNA Purification and Enzyme Digestion (RE)

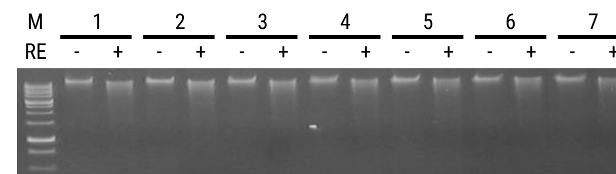


Figure 3. Results of DNA purification and enzyme digestion with *Eco*RI

After eluting genomic DNA with 50 μl Buffer ME, each 100 ng of DNAs 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.

◈ Determination of Yield and Purity Data of DNA

▪ **Type A & B Protocol : Gram Positive & Negative Bacteria**

(A) DNA Yield and Purity

Lane	Samples	Amounts (ml)[1]	DNA Yield (μg)	$A_{260/280}$
1	*Bcillus cereus*	2	6 ~ 13	1.78
2	*Bacillus subtilis*	2	5 ~ 12	1.84
3	*Lactobacillus sp.*	2	6 ~ 13	1.80
4	*Corynebacterium*	2	6 ~ 13	1.79
5	*Staphylococcus sp.*	2	5 ~ 11	1.88
6	*Streptococcus sp.*	2	6 ~ 12	1.84
7	*Escherichia coli*	2	7 ~ 14	1.85
8	*Pseudomonas aeruginosa*	2	7 ~ 14	1.86

[1] The volume of cultured cell

(B) DNA Purification and Enzyme Digestion (RE)

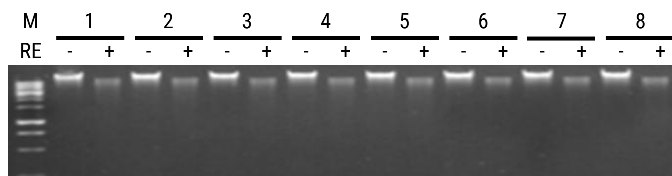


Figure 2. Results of DNA purification and enzyme digestion with *Eco*RI

After eluting genomic DNA with 50 μl Buffer ME, each 100 ng of DNAs 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.

APPLICATIONS

- Bacterial genomic research
- Pathogen detection study
- Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, microarray

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.

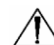
TECHNICAL ASSISTANCE

iNtRON customers are a major source of information regarding advanced or specialized uses 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 or new applications and techniques.

For technical assistance and more information please call iNtRON Technical Service Department or local distributors.

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 chaotropic 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.



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

PROTOCOL LIST

Table 2. Protocols according to the BYF Sample Groups (5 Protocols)

BYF Samples		Protocol Type
Bacteria	*Gram Positive Bacteria*	Type A Protocol
	Gram Negative Bacteria	Type B Protocol
Yeast		Type C Protocol
Fungi	*Fungal Tissue*	Type D Protocol
	Fungi	Type E Protocol

COLUMN INFORMATION

Table 3. Column information of i-genomic Series

Column Membrane [1]	Silica-based membrane
Spin Column [2]	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 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.

EXPERIMENTAL INFORMATION

◈ Electrophoresis Results from Various BYF Sample

i-genomic BYF DNA Mini Kits provide a reliable and practical method to purify efficiently genomic DNA from all kinds of gram-positive bacteria, yeast, fungal tissue and fungi. DNA purified by i-genomic BYF DNA 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 1 shows overall electrophoresis data from representative various BYF samples.

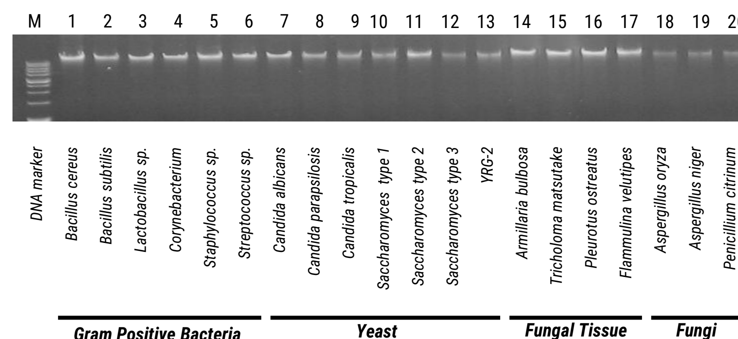


Figure 1. Agarose Gel Electrophoresis of Eluted Genomic DNA (1.0%)

After eluting genomic DNA with 50 µl Buffer ME, each 25 ~ 50 ng of DNAs were used in DNA Electrophoresis.
Electrophoresis condition: 1.0% Agarose Gel at 100 Volt for 30 min.

◈ Storage, quantification, and determination of quality and yield of gDNA

Storage of DNA

For long-term storage, DNA should be dissolved in TE buffer or Buffer ME, and stored at –20°C. DNA stored in water is subject to acid hydrolysis. Any contaminants in the DNA solution may lead to DNA degradation. Avoid repeated freeze-thawing as this will lead to precipitates. We recommend storing genomic DNA samples in aliquots.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer using a quartz cuvette. For the greatest accuracy, readings should be between 0.1 and 1.0.

An absorbance of 1 unit at 260 nm corresponds to 50 μg genomic DNA per ml (A260 =1 ⇒ 50 μg/ml). This relation is valid only for measurements made at neutral ~ slightly alkaline pH, therefore, samples should be diluted in a low-salt buffer with slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5~8.0)

Note : If you will use more than one cuvette to measure multiple samples, the cuvettes must be matched.

Note : Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration.

Note : Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher yields and higher purity, because of an upward shift in the A260 value.

Purity of DNA

The ratio of the readings at 260 nm and 280 nm (A260/ A280) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The A260/ A280 ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A260/ A280 ratio can vary greatly. Lower pH results in a lower A260/ A280 ratio and reduced sensitivity to protein contamination. For accurate A260/ A280 values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5~8.0). Make sure to zero the spectrophotometer with the appropriate buffer. Pure DNA has an A260/ A280 ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

RNA contamination

Depending on the DNA isolation method used, RNA will be copurified with genomic DNA. RNA may inhibit some downstream applications, but it will not inhibit PCR. Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration.

NOTES FOR SAMPLE SIZING

◈ Measuring the Amount of Sample Before Pre-Lysis

We recommend to measure the amount of starting material without disruption and homogenization except fungal tissue. In case of the gram positive bacteria and yeast samples, don't use high OD_{600} value sample. It makes to measure conveniently the amount of starting material. Followed table shows a recommended amount of starting material before pre-lysis. Please follow the manual instruction not to be over the required amounts.

◈ Sample Volume

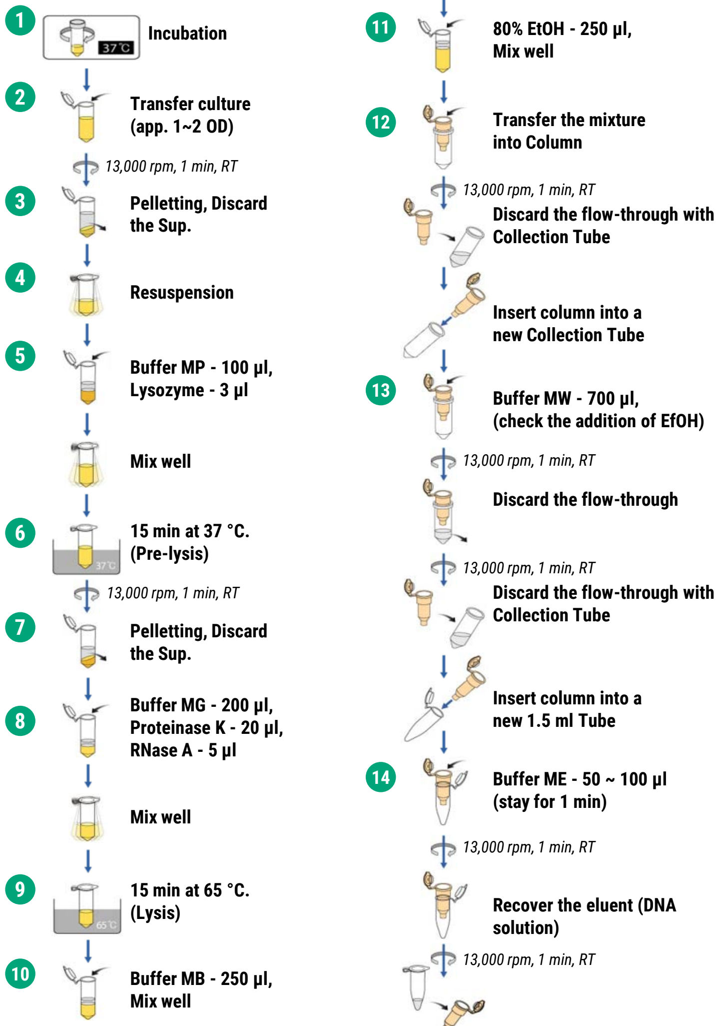
i-genomic BYF DNA Mini Kit procedures are optimized for 1 ~ 5 ml of bacteria culture and yeast culture, 50 ~ 100 mg of fungal tissue, and 2 ~ 3 piece of 0.5 x 1cm of fungi grown plate. Followed table provides guidelines according to microorganism. Exceeding the recommended amount of starting material will result in inefficient lysis, resulting in low yield and purity. In the large, DNA yields and purity vary depending on genome size, sample viscosity, and age of sample.

Table 4. Recommended Volume of Starting Material according to Microorganism

Sample	Amount
Bacteria	1~2 ml
Yeast	3~5 ml
Fungal Tissue	50~100 mg
Fungi	2~3 pieces of 0.5 x 1cm*

* The piece is cut of cultured plate

Quick Guide – G(+) Bacteria gDNA Extraction



TECHNICAL ADVICE

◈ General remarks on handling genomic DNA

DNA is a relatively stable molecule. However, introduction of nucleases to DNA solutions should be avoided as these enzymes will degrade DNA. Genomic DNA consists of very large DNA molecules, which are fragile and can break easily. To ensure the integrity of genomic DNA, excessive and rough pipetting and vortexing should be avoided. DNA is subject to acid hydrolysis when stored in water, and should therefore be stored in TE buffer or Buffer ME from iNtRON.

◈ Sample storage prior to isolation of genomic DNA

The quality of the starting material affects the quality and yield of the isolated DNA. The highest DNA yield and quality is achieved by purifying genomic DNA from freshly harvested tissues and cells. If samples cannot be processed immediately after harvesting, they should be stored under conditions that preserve DNA integrity. In general, genomic DNA yields will decrease if samples, particularly animal samples, are stored at either 2 ~ 8°C or −20°C without previous treatment. In addition, repeated freezing and thawing of frozen samples should be avoided as this will lead to genomic DNA of reduced size, and in clinical samples, to reduced yields of pathogen DNA.

Bacteria cell cultures and Yeast cell culture Centrifuge harvested cell cultures, remove the supernatant, and then store the cells at −20°C or −80°C.

Fungal material Mycelium of fungal sample should be harvested directly from a culture dish or liquid culture. For liquid cultures, the cells should be pelleted by centrifugation and the supernatant removed before DNA isolation or storage. Harvested samples can be either directly frozen or freeze dried, and stored at −80°C.

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.
	High viscosity of Lysate	Reduce the amounts of starting material.
		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.
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 MW.
		Store Buffer MW at room temperature (15 ~ 20°C).
	Amount of DNA used in experiments.	Optimize the amount of DNA used in your downstream experiments.

PROTOCOL A (Gram Positive Bacteria)

1. Prepare Gram positive bacteria sample.

Note : Innoculate the bacterial sample into 5ml of approprеate culture broth, then incubate the culture at adequate temperature until OD600 value of 0.8 ~ 1.0 on a spectrophotometer. OD600 values depend on the length of the light path and therefore differ between spectrophotometers.

2. Transfer 1 ~ 2 ml cultured bacteria cell into 2 ml tube.

Note : Ensure correct amount of start material. When exceed the recommended optimal amount of starting material, will result in inefficient lysis. As a result, obtain low yield and purity.

3. Pellet bacteria by centrifugation for 1 min at 13,000 rpm, then discard supernatant except 50 µl of supernatant.

Note : Remain about 50 µl supernatant for resuspension, and then resuspend by tapping or pipetting.

4. Resuspend completely the bacterial pellet into remnant supernatant by tapping or vigorously votexing.

Note : It is essential that the pellet and remnant suspernatant are mixed thoroughly yield a homogeneous solution.

5. Add 100 µl Buffer MP and 3 µl lysozyme solution into sample tube, and resuspend by vortex for 30 sec or pipetting vigorously.

Note : Cell wall of gram positive bacteria is composed of thick peptidoglycan. So normal lysis buffer doesn't break a cell wall. For efficient lysis of most gram positive bacteria, enzymes such as lysozyme may be necessary. For certain species, such as Staphylococcus spp., lysis is much more efficient with lysostaphin.

6. Incubate the lysate for 15 min at 37 °C.

Note : During incubation, the lysozyme enzymatically breaks down the bacterial cell wall, while the detergent in MP buffer ensure complete lysis of the bacteria. And for complete break of cell wall, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA.

7. Centrifuge the pre-lysate for 1 min at 13,000 rpm at room temperature. Discard supernatant, ensuring that all liquid is completely removed. And then resuspend by vortexing or tapping of cell pellet to pre-lysis cell perfectly.

Note : The cell wall is removed giving rise to the formation of spheroplasts. So, should be lysed bacteria cell more easily and rapidly. It is better that the cell pellet is resuspended by vortexing or tapping before adding Buffer MPG.

8. Add 200 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution into sample tube, and resuspend by vortex vigorously.

Note : Resuspend pellet by vortex or pipetting. After mix MG buffer, bacteria cell lysate change opaquely.

9. Incubate the lysate for 15 min at 65°C.

Note : 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.

10. After lysis completely, add 250 µl Buffer MB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : This step is an equilibration step for binding genomic DNA to column membrane.

11. Add 250 µl 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. It is possible to apply various method of sample mixing (pipetting, inverting or gently vortexing) until not showing 2-phase which is not mixed. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA.

12. Pipette 750 µl of the mixture from step 11 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 and collection tube altogether.

Note : The maximum volume of the spin column reservoirs is 800 µl. In case of the larger volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture. If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

13. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 µl Buffer MW 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 (reuse the collection tube).

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. Ensure that 40 ml of ethanol (EtOH) has been added to Buffer MW.

14. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ~ 100 µl Buffer ME 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 µl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 µl increases generally overall DNA yield. 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

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.
	High viscosity of Lysate	Reduce the amounts of starting material.
		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.
		Increase lysozyme or lyticase volume in Pre-Lysis step.
	Error in DNA binding	After adding Buffer MB in DNA Binding step, please mix well by gently inverting.
		Check that the amount of Buffer MB is added correctly to the supernatant.
		Check that the amount of 80% EtOH is added correctly to binding mixture.
	Incorrect Washing step	Check again that the amount of ethanol (EtOH) is added correctly to Washing buffer.
		When storing Washing Buffer, always keep a lid shut tightly without evaporation.
	Insufficient DNA elution	Increase the volume of Buffer ME or water to 100 ml.
		Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.
	Excess addition of elution buffer	Reduce the amount of Buffer ME.
		Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.
		Reload the elute into used spin column, and then repeat elution step.

11.After lysis completely, add 250 µl Buffer MB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : This step is an equilibration step for binding genomic DNA to column membrane.

12.Add 250 µl 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. It is possible to apply various method of sample mixing (pipetting, inverting or gently vortexing) until not showing 2-phase which is not mixed. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA, Do not use alcohols other than ethanol since this may result in reduced yields.

13.Pipette 750 µl of the mixture from step 12 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 and collection tube altogether.

Note : The maximum volume of the spin column reservoirs is 800 µl. In case of the larger volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture. If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

14.Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 µl Buffer MW 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 (reuse the collection tube).

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. Ensure that 40 ml of ethanol (EtOH) has been added to Buffer MW.

15. Place the spin column into a new 1.5 ml tube (not supplied), and 30 µl ~ 50 µl Buffer ME 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 30 µl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 µl increases generally overall DNA yield. 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 (Gram Negative Bacteria)

1. Prepare Gram negative bacteria sample.

Note : Innoculate the bacterial sample into 5ml of appropreate culture broth, then incubate the culture at adequate temperature until OD600 value of 0.8 ~ 1.0 on a spectrophotometer. OD600 values depend on the length of the light path and therefore differ between spectrophotometers.

2. Transfer 1 ~ 2 ml cultured bacteria cell into 2 ml tube.

Note : Ensure correct amount of start material. When exceed the recommended optimal amount of starting material, will result in inefficient lysis. As a result, obtain low yield and purity.

3. Pellet bacteria by centrifugation for 1 min at 13,000 rpm, then discard supernatant except 50 µl of supernatant.

Note : Remain about 50 µl supernatant for resuspension, and then resuspend by tapping or pipetting.

4. Resuspend completely the bacterial pellet into remnant supernatant by tapping or vigorously votexing.

Note : It is essential that the pellet and remnant suspernatant are mixed thoroughly yield a homogeneous solution.

5. Add 300 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution into sample tube, and resuspend by vortex vigorously.

Note : Resuspend pellet by vortex or pipetting. After mix MG buffer, bacteria cell lysate change opaquely.

6. Incubate the lysate for 15 min at 65°C.

Note : 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.

7. After lysis completely, add 250 µl Buffer MB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : This step is an equilibration step for binding genomic DNA to column membrane.

8. Add 250 µl 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. It is possible to apply various method of sample mixing (pipetting, inverting or gently vortexing) until not showing 2-phase which is not mixed. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA.

9. Pipette 750 µl of the mixture from step 8 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 and collection tube altogether.

Note : The maximum volume of the spin column reservoirs is 800 µl. In case of the larger volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture. If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

10. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 µl Buffer MW 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 (reuse the collection tube).

Note : It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Ensure that 40 ml of ethanol (EtOH) has been added to Buffer MW.

11. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ~ 100 µl Buffer ME 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 µl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 µl increases generally overall DNA yield. A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate.

PROTOCOL E (Fungi)

1. Prepare Fungi sample.

Note : Fungi reproduce by a spore, and well grow in humid weather. So seed on CSZ (Czapek agar), MEA (Malt Extract agar), or PDA (Potato Dextrose agar) plate, and then incubate for 4 ~ 6 day in adequate temperature condition(15 ~ 37°C).

2. Cut off 2 ~ 3 pieces of 0.5 x 1 cm by scalpel.

Note : Because difficult pick up fungi sample directly, cut off a state that be grown fungi on plate.

3. Transfer sliced piece to 15 ml tube.

Note : Progress for pre-treating step.

4. Add 5 ml of 1x PBS buffer into sample tube and vortex vigorously.

Note : When progress vortex, spores and hypha separate from fungi on plate.

5. Transfer suspended fluid into 1.5 ml tube.

Note : In order to collect spore and hypha, transfer suspended fluid except plate piece.

6. Centrifuge suspended fluid for 5 min at 13,000 rpm at room temperature. Discard supernatant, remaining 50 µl of suspernantant without disturbed pellet. And then resuspend by vortexing or tapping of cell pellet to pre-lysis cell perfectly.

Note : It is better that the cell pellet is resuspended by vortexing or tapping before adding Buffer MP.

7. Add 100 µl Buffer MP and 3 µl lyticase or zymolase solution (not supplied, 20mg/ml) into sample tube, and resuspend by vortex for 30 sec or pipetting vigorously.

8. Incubate the lysate for 15 min at 37°C.

Note : For complete break of cell wall, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA.

9. Add 200 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution into sample tube, and mix by vortexing vigorously.

Note : The spore and hypha of fungi sample is low amount. Also, when MG buffer add rapidly, should be prevent degradation of genomic DNA by Dnase.

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

Note : 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.

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

Note : 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.

10. After lysis completely, add 250 µl Buffer MB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : This step is an equilibration step for binding genomic DNA to column membrane.

11. Add 250 µl 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. It is possible to apply various method of sample mixing (pipetting, inverting or gently vortexing) until not showing 2-phase which is not mixed. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA, Do not use alcohols other than ethanol since this may result in reduced yields.

12. Pipette 750 µl of the mixture from step 8 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 and collection tube altogether .

Note : The maximum volume of the spin column reservoirs is 800 µl. In case of the larger volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture. If a small amount will not pass through, please centrifuge again for 1 min at 13,000 rpm.

13. Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 µl Buffer MW 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 (reuse the collection tube).

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. Ensure that 40 ml of ethanol (EtOH) has been added to Buffer MW.

14. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ~ 100 µl Buffer ME 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 µl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 µl increases generally overall DNA yield. 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 C (Yeast)

1. Prepare Yeast sample.

Note : Pick up the single colony from agar plate. Inoculate picked single colony to 5 ml liquid culture media (ex. YPD, 2xYT etc), then incubate for overnight in adequate temperature condition until OD600 value of 0.8 ~ 1.0 on a spectrophotometer. OD600 values depend on the length of the light path and therefore differ between spectrophotometers.

2. Transfer 3 ~ 5 ml cultured yeast cell into 15 ml tube.

3. Pellet yeast by centrifugation for 1 min at 13,000 rpm, then discard supernatant except 50 µl of supernatant.

Note : Remain about 50 µl supernatant for resuspension, and then resuspend by tapping or pipetting.

4. Resuspend completely the yeast pellet into remnant supernatant by tapping or vigorously votexing.

Note : It is essential that the pellet and remnant suspernatant are mixed thoroughly yield a homogeneous solution.

5. Add 200 µl Buffer MYP and 2 µl β-mercaptoethanol into sample tube, and mix well by vortex for 30 sec or pipetting vigorously.

Note : Major structural constituents of the cell wall of yeast are polysaccharides (80 ~ 90%), mainly glucans and mannans, with a minor percentage of chitin. Glucans are very sturdy and weave together tightly to form a tough ball protecting the inside of the yeast cell ,and chitin is only 2 ~ 4% of the cell wall. Also, cell wall is composed with glycoprotein. Glycoprotein is most mannoprotein. So, normal lysis buffer doesn't break cell wall

6. Incubate the lysate for 15 min at 37°C.

7. Centrifuge the pre-lysate for 1 min at 13,000 rpm at room temperature. Discard supernatant, ensuring that all liquid is completely removed. And then resuspend by vortexing or tapping of cell pellet to lysis cell perfectly.

Note : It is better that the cell pellet is resuspended by vortexing or tapping before adding Buffer MG.

8. Add 100 µl Buffer MP and 3 µl lyticase or zymolase solution (not supplied, 20mg/ml) into spheroplast sample tube, and mix well by vortex for 30 sec or pipetting vigorously.

Note : Lyticase or zymolase enzyme break cell wall components such as glycoprotein and polysaccharide. For efficient lysis of some yeast species, zymolase rather than lyticase is recommended. Please use the appropriate enzyme for the particular species.

9. Incubate the lysate for 15 min at 37°C.

Note : During incubation, the lyticase or zymolase enzymatically breaks down the yeast cell wall. And for complete lysis, mix 5 ~ 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA.

10.Centrifuge the pre-lysate for 1 min at 13,000 rpm at room temperature. Discard supernatant, ensuring that all liquid is completely removed. And then resuspend by vortexing or tapping of cell pellet to lysis cell perfectly.

Note : Because pre-lysate pellet is removed cell wall, should be lysed yeast cell more easily and fast. It is better that the cell pellet is resuspended by vortexing or tapping before adding Buffer MG.

11.Add 200 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution into sample tube, and vortex vigorously.

Note : Resuspend pellet by vortex or pipetting. After mix MG buffer, bacteria cell lysate change opaquely.

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

Note : 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.

13.After lysis completely, add 250 µl Buffer MB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : This step is an equilibration step for binding genomic DNA to column membrane.

14.Add 250 µl 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.

Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution.

15.Pipette 750 µl of the mixture from step 14 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 and collection tube altogether.

16.Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 µl Buffer MW 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 (reuse the collection tube).

Note : It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions.

17. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ~ 100 µl Buffer ME 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 µl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 µl increases generally overall DNA yield.

PROTOCOL D (Fungal Tissue)

1. Prepare Fungal tissue sample.

Note : We recommend to collect the fresh fungal tissue such as mushroom sample. The more fungal tissue sample is like to plant leaf or stem tissue sample. Also, fungal tissue sample is big size, therefore it is disrupt and homogenize. To later use, should slice off fungal tissue sample to suitable size and then stored at -80 ℃ after frozen in liquid nitrogen.

2. Slice prepared sample to suitable size by pre-chilled scalpel or scissor.

Note : When the handling of liquid nitrogen, be careful and wear a glove and protective suit. When homogenize large size of sample, bound sample's pieces out of mortar. Also, sample of small size is homogenized easily and speedy.

3. Place the sliced sample into prepared a grinding jar (mortar). Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and grind carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to next step.

Note : We recommend to be homogenized completely until tissue clumps are not visible. If fungal tissue clump remain homogenized sample, do not lyse completely in lysis step. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step. The keeping frozen sample inhibit low DNA yields and degraded DNA.

4. Measure 50 ~ 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 pre-chilling the spatula and 1.5ml tube in liquid nitrogen. The freeze-thaw 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. Add 100 µl Buffer MP and 3 µl lyticase or zymolase solution (not supplied, 20mg/ml) into sample tube, and resuspend by vortex for 30 sec or pipetting vigorously.

6. Incubate the lysate for 15 min at 37°C.

Note : For complete break of cell wall, mix 5 or 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA.

7. Centrifuge the pre-lysate at 13,000 rpm for 1 min (RT). Then discard supernatant, ensuring That all liquid is completely removed.

8. Add 200 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution into sample tube, and vortex vigorously.

Note : With fungal tissue sample absorbs lysis buffer, and becomes swollen. When apply exceeding the recommended amount of starting material, it may be difficult to handle fungal tissue due to it's sticky. Therefore, always keep the recommended amount of starting material. Also, when MG buffer and rapidly, should be prevent degradation of DNA by DNase.