***The Best Way to GENOMIC DNA***

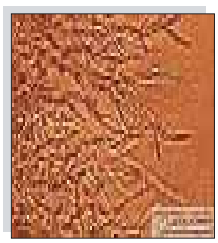
***Never Before ! 4-28-260 !***
***4 kinds of Products divided !***
***28 Protocols provided !***
***260 Samples adjusted !***
***i-genomic series***

# PART III

## *i-genomic BYF*
## DNA Extraction Mini Kit Handbook

Bacteria (Gram+)

Type A Protocol



Yeast

Type B Protocol

Fungi

Type C Protocol
Type D Protocol

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# Contents

# i-genomic BYF DNA Mini Kit Contents

**i-genomic BYF DNA Extraction Mini Kit**
**(Cat. No. 17361, 50 columns)**

## Table 1. Kit Contents

| Label | Description | Contain |
|---|---|---|
| **Buffer MYP** | Yeast Pre-Lysis Buffer | 12 ml |
| **Buffer MP**[1] | Pre-Lysis Buffer | 7 ml |
| **Buffer MG** | Lysis Buffer | 15 ml |
| **Buffer MB** | Binding Buffer | 15 ml |
| **Buffer MW(concentrate)**[2] | Washing Buffer | 10 ml (add 40 ml of EtOH) |
| **Buffer ME**[3] | Elution Buffer | 20 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) | 0.6 ml |
| **Lysozyme solution** [5] | 100 mg/ml (store at -20°C) | 0.3 ml |

[1] Contains a chaotropic salt. Carefully handle. See page 7 for safety information.
[2] Buffer MW is supplied as concentrate. Add 40 ml of ethanol (96 ~ 100%) according to the bottle label before use.
[3] Buffer ME is finally 10mM 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 and Lysozyme solution. Use carefully according to the instruction manual (page 19).

Figure 1. i-genomic BYF DNA Extraction Mini Kit

## Storage

We recommend that all components of i-genomic BYF DNA Extraction Mini Kit is stored dry at room temperature (15~25°C). However, three components, including RNase A, Proteinase K and Lysozyme stock solution should be stored at -20°C, 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 BYF DNA Mini Kit is tested to ensure the absence of DNase contamination. All buffers are each incubated with 1 mg pUC18 DNA for 6 hours at 37°C. 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 MG 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 Kit or other products in general, please do not hesitate to contact us.
Your informations 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 product 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 performace and Southern blotting less than 1~2 hours. Purification requires no phenol or chloroform extraction or alchol precipitation. Pure DNA extracted by i-genomic series Kits 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**<br>**i-genomic CTB**<br>**DNA Extraction Mini Kit** | **C**ells<br>**T**issues<br>Gram(-) **B**acteria | 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**<br>**i-genomic Blood**<br>**DNA Extraction Mini Kit** | Blood | Whole blood / Buffy Coat / Dried Spot / Blood Swab / Plasma / Serum / Others |
| **CAT. NO. 17361**<br>**i-genomic BYF**<br>**DNA Extraction Mini Kit** | Gram(+) **B**acteria<br>**Y**east<br>**F**ungi | *Azotobacter sp.*<br>*Staphylococcus sp.*<br>*Saccharomyces sp.*<br>*Aspergillus sp.*<br>Others |
| **CAT. NO. 17371**<br>**i-genomic Plant**<br>**DNA Extraction Mini Kit** | Plant | Leaf / Root / Stem / Fruit / Seed /Others |

***Whole blood*** ***Buffy Coat*** ***Dried Spot*** ***Blood Swab*** ***Plasma*** ***Serum***

# 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 | | | | | | | | | | | Blood | | | | | | BYF | | | | Plant | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protocol Type | | A | B | C | D | E | F | G | H | I | J | K | A | B | C | D | E | F | A | B | C | D | 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 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |

■ Perform
□ Do Not Perform

Total : 11 Types — Cultured Cell, Animal Tissue, Rodent Tail, Formalin Fixed Tissue, Paraffin Embedded Tissue, Animal Hairs, Insect / Worm, Stool, Bone, Buccal Swab, Gram(-) Bacteria

Total : 6 Types — Whole Blood, Buffy Coat, Dried Spot, Blood Swab, Plasma, Serum

Total : 4 Types — Gram(+) Bacteria, Yeast, Fungal Tissue, Fungi

Total : 7 Types — Lyophilized Leaf, Leaf, Stem, Root, Fruit, Seed (Others), Seed (Gramineae)

### 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

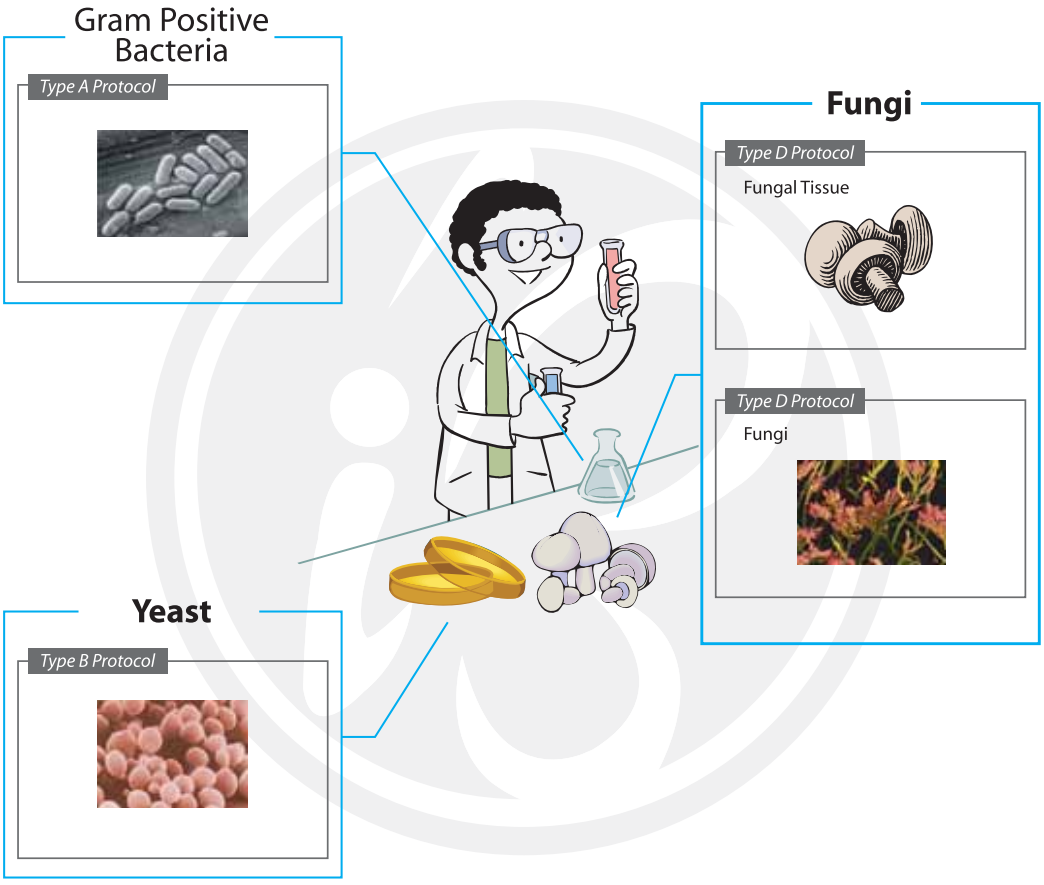
# i-genomic BYF DNA Extraction Mini Kit

i-genomic BYF DNA Extraction Mini Kit provides a fast and easy way to purify DNA from BYF 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 23 BYF samples. You can see vast sample photos, vast samples, and vast practical data.

i-genomic BYF DNA Mini Kit provides 4 kinds of protocols, Type A, Type B, Type C and Type D. You can also extract genomic DNA 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 List (see Figure 2). If you need some more information in selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

**Table 4. Four Kinds of Protocols according to the BYF Samples**

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


Gram Positive Bacteria
Type A Protocol
Fungi
Type D Protocol
Fungal Tissue
Type D Protocol
Fungi
Yeast
Type B Protocol

# BYF Samples Grouping according to Protocols

i-genomic BYF DNA Mini Kit provides four kinds of protocols according to BYF samples. We recommend to select an appropriate protocol for your samples. The samples show 23 samples we have tested with i-genomic BYF DNA Mini Kit (see Figure 2). You can extract efficiently genomic DNA from various BYF 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 new techniques.

*See next pages.*

Figure 2. BYF Samples Grouping (Sample tested by iNtRON)

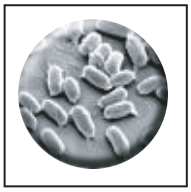
**Gram Positive Bacteria**

# *Bacteria*

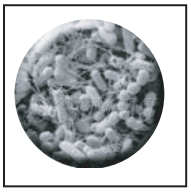
*For Gram Positive Bacteria*

***Type A Protocol***

*Bacillus cereus*



*Bacillus subtilis*



*Lactobacillus sp.*

*Corynebacterium*

*Straphylococcus aureus*

*Streptococcus salivarrius*

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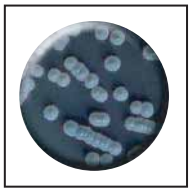
Yeast

# *Yeast*

*For Yeast*

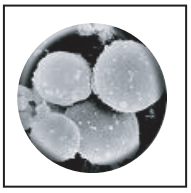
***Type B Protocol***

*Candida tropicalis*

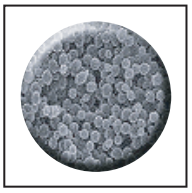


*Candida parasilosis*

*Candida albicans*

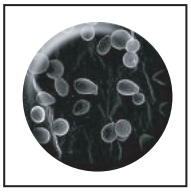


*Saccharomyces Cerevisiae* type 1

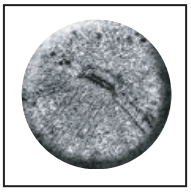


*Saccharomyces Cerevisiae* type 2

*Saccharomyces Cerevisiae* type 3



YRG-2



iNtRON Waits For Your Data.

**Fungal Tissue**

# *Fungi*

*For Fungal Tissue*

***Type C Protocol***

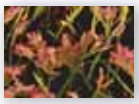
*Armillaria bulbosa*

*Tricholoma Matsutake*

*Pleurotus ostreatus*

*Flammulina veluipes*

Fungi

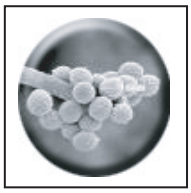


# *Fungi*

*For Fungi*

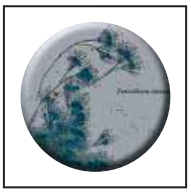
***Type D Protocol***

*Aspergillus oryza*



*Aspergillus niger*

*Penicillium citrinum*



iNtRON Waits For Your Data.

# Equipments and Reagents to Be Supplied by User

i-genomic BYF DNA Mini Kit provides almost all reagents for extracting DNA, including RNase A, Proteinase K and Lysozyme 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.

- **Common equipments and reagents**

✓ Pipettes and pipette tips
✓ Water bath or heating block
✓ Vortex mixer
✓ Microcentrifuge with rotor for 2.0 ml tubes
✓ Microcentrifuge tubes (1.5 ml)
✓ Absolute ethanol (EtOH, 96 ~ 100%)
✓ 80% EtOH
✓ Liquid nitrogen
✓ Ice
✓ Other general lab equipments

- **For Type B Protocol**

✓ Lyticase or zymolase solution
✓ Beta-Mercaptoethanol

- **For Type C Protocol**

✓ Equipment for disruption and homogenization, including grinding jar set (mortar)
✓ Liquid nitrogen

- **For Type D Protocol**

✓ 15 ml tube
✓ 1x PBS buffer

# Notice Before Use

## Important Points Before Starting

- **Buffer MW (Washing Buffer)**
  : Buffer MW 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.

- **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.

- **Preheat a water bath or a heating block to 65℃.**

- **Centrifugation**
  : All centrifugation steps are carried out at RT (15 ~ 25℃ ) 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 ㎕ |
| **DNA Binding Capacity** | Maximum 45 ㎍ |
| **Recovery** | 85 ~ 95% depending on the elution volume |
| **Elution Volume** | Generally, eluted with 30 ~ 200 ㎕ 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 year 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 BYF Sample

Four kinds of different protocol in this handbook provide detailed instructions to use i-genomic BYF DNA Mini Kit for purifying genomic DNA from various gram positive bacteria, yeast, fungal tissue and fungi samples (see Figure 2). These protocols are optimized for use.
Especially, with iNtRON's 23 samples in more than three categories, we have verified practically by several experiments to ensure the quality and the application of i-genomic BYF DNA Mini Kit. We recommend to choose the right protocol according to your gram positive bacteria, yeast, fungal tissue and fungi samples. For more information, please contact iNtRON Technical Assistance Team.

### Collection and Storage of BYF Samples

In case of gram positive 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℃.
In case of fungal tissue such as mushroom, sample size is big. 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℃.
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 froze in liquid nitrogen, and then store at -80℃. Alternatively, store at room temperature after progress lyophilizing.

### Disruption and Homogenization

Most BYF 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.

# Notes for Sample Sizing

### Measuring the Sample Amount 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.

Table 5 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 Kits procedures are optimized for 1 ~ 5 ml of gram positive bacteria and yeast, 50 ~ 100 mg of fungal tissue, and 2 ~ 3 piece of 0.5 x 1 cm of fungi grown plate. Table 5 provides guidelines according to BYF sample. 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 5. Recommended Volume of Starting Material according to BYF sample**

- **Type A Protocol : Bacteria (Gram Positive)**

| Bacteria | Amount |
|---|---|
| *Bacillus cereus* | 1 ~ 2 ml |
| *Bacillus subtilis* | 1 ~ 2 ml |
| *Lactobacillus sp.* | 1 ~ 2 ml |
| *Corynebacterium* | 1 ~ 2 ml |
| *Staphylococcus sp.* | 1 ~ 2 ml |
| *Streptococcus sp.* | 1 ~ 2 ml |

▪ **Type B Protocol : Yeast**

| Yeast | Amount |
|---|---|
| *Candida albicans* | 3 ~ 5 ml |
| *Candida parapsilosis* | 3 ~ 5 ml |
| *Candida tropicalis* | 3 ~ 5 ml |
| *Saccharomyces cerevisiae type 1* | 3 ~ 5 ml |
| *Saccharomyces cerevisiae type 2* | 3 ~ 5 ml |
| *Saccharomyces cerevisiae type 3* | 3 ~ 5 ml |
| *YRG-2 (Saccharomyces)* | 3 ~ 5 ml |

▪ **Type C Protocol : Fungal Tissue**

| Fungal Tissue | Amount |
|---|---|
| *Armillaria bulbosa* | 50 ~ 100 mg |
| *Tricholoma matsutake* | 50 ~ 100 mg |
| *Pleurotus ostreatus* | 50 ~ 100 mg |
| *Flammulina velutipes* | 50 ~ 100 mg |

▪ **Type D Protocol : Fungi**

| Fungi | Amount |
|---|---|
| *Aspergillus oryza* | 2 ~ 3 pieces* of 0.5 x 1 cm |
| *Aspergillus niger* | 2 ~ 3 pieces* of 0.5 x 1 cm |
| *Penicillium citrinum* | 2 ~ 3 pieces* of 0.5 x 1 cm |

* The piece is cut of cultured plate

# Standard Protocols


# BYF Type A Bench Protocol

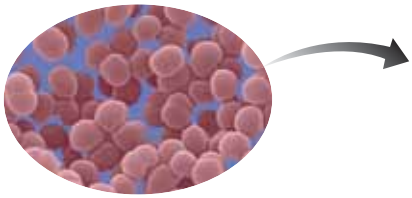
## Type A Protocol
## For Bacteria (Gram Positive)

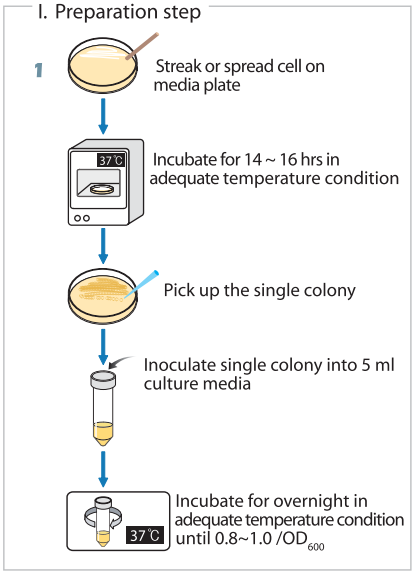
A-1. Sample Treatment Step

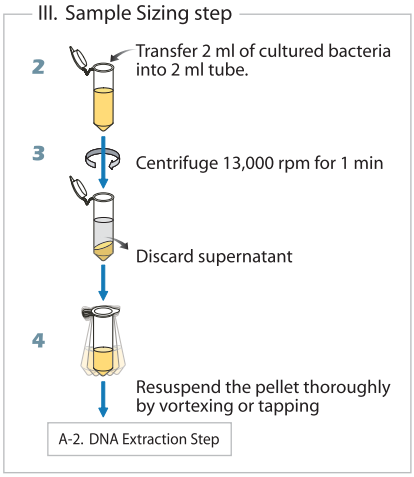


▪ ***Bacteria (Gram Positive)***

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





A-2. DNA Extraction Step

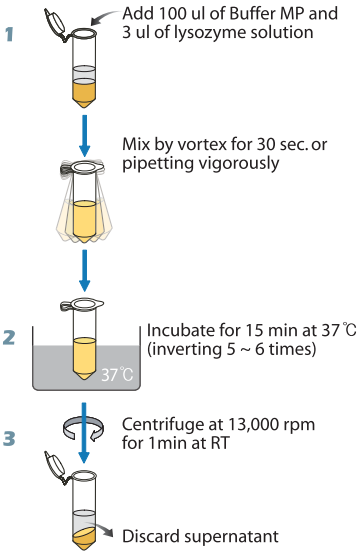
▪ ***Bacteria (Gram Positive)***

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

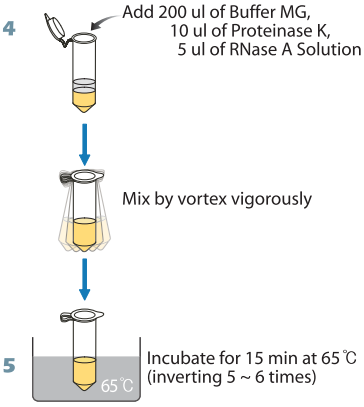
□ V. Washing step A ■ VI. Washing step B ■ VII. Elution step



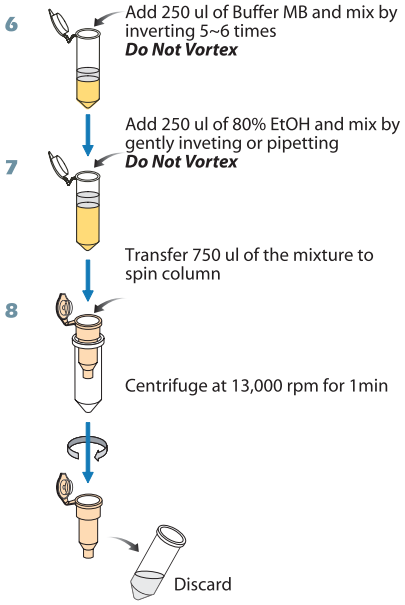
I. Pre-Lysis step




II. Lysis step

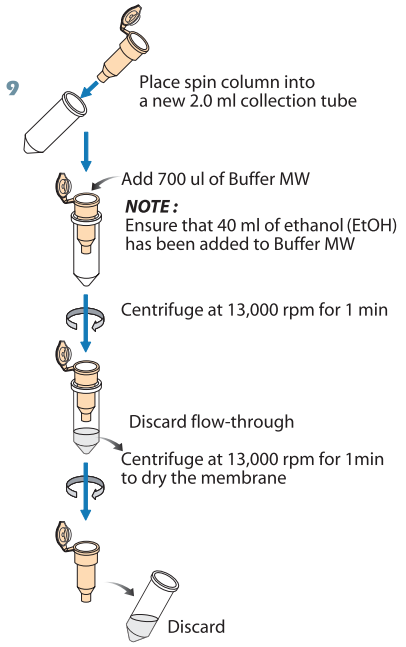



IV. DNA Binding step

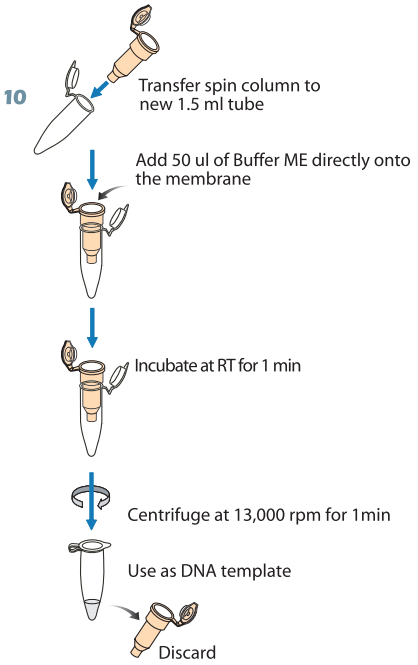


Type A Protocol
Bacteria

VI. Washing step B




VII. Elution step



A-1. Sample Treatment Step

- ***Bacteria (Gram Positive)***

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

**I. Preparation step**

**1. Prepare gram positive bacteria sample.**

Streak or spread cell on agar plate (ex. LB, SOB etc.). Incubate for 14 ~ 16 hrs in adequate temperature condition. Pick up the single colony from agar plate. And inoculate picked single colony to 5 ml liquid culture media (ex. LB, SOB etc), then incubate for overnight in shaking incubator of adequate temperature until $OD_{600}$ value of 0.8 ~ 1.0 on a spectrophotometer. $OD_{600}$ values depend on the length of the light path and therefore differ between spectrophotometers.

**III. Sample Sizing step**

**2. Transfer 2 ml cultured bacteria cell into 2 ml tube.**

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 at 13,000 rpm for 1 min, then discard supernatant.**

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

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

A-2. DNA Extraction Step

- ***Bacteria (Gram Positive)***

■ 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 37°C for use in step 2 and to 65°C for use in step 5.
- All centrifugation steps should be carried out at room temperature.

**I. Pre-Lysis step**

**1. Add 100 ㎕ Buffer MP and 3 ㎕ lysozyme solution into sample tube, and mix well by vortex for 30 sec or pipetting vigorously.**

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.

**2. Incubate the lysate for 15 min at 37°C.**

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.

**3. Centrifuge the pre-lysate at 13,000 rpm (RT) for 1 min. Discard supernatant, and then resuspend by vortexing or tapping of cell pellet to pre-lysis cell perfectly.**

The cell wall is removed giving rise to the formation of spheroblasts. 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.

**II. Lysis step**

**4. Add 200 ㎕ Buffer MG, 10 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and mix by vortex vigorously.**

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

**5. Incubate the lysate for 15 min at 65°C.**

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.

**IV. DNA Binding step**

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

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

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

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, or inverting) 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.

**8. Pipette 750 ㎕ of the mixture from step 7 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 and collection tube altogether.**

The maximum volume of the spin column reservoirs is 800 ㎕. 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 at 13,000 rpm for 1 min.

## VI. Washing step B

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

## VII. Elution Step

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

# BYF Type B Bench Protocol

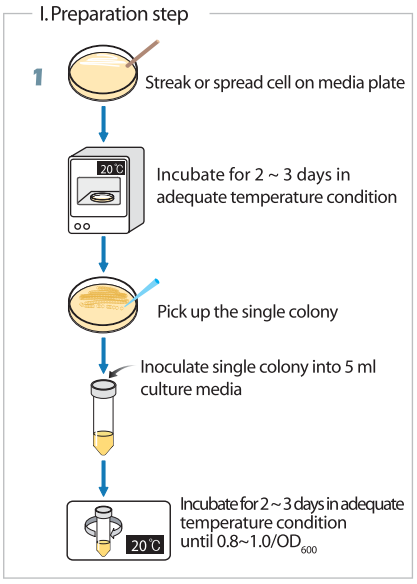
## Type B Protocol For Yeast

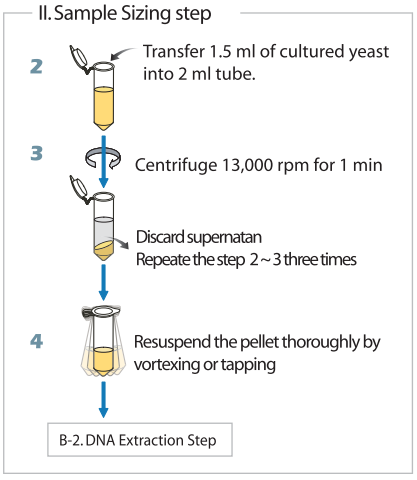
B-1. Sample Treatment Step

▪ ***Yeast***

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





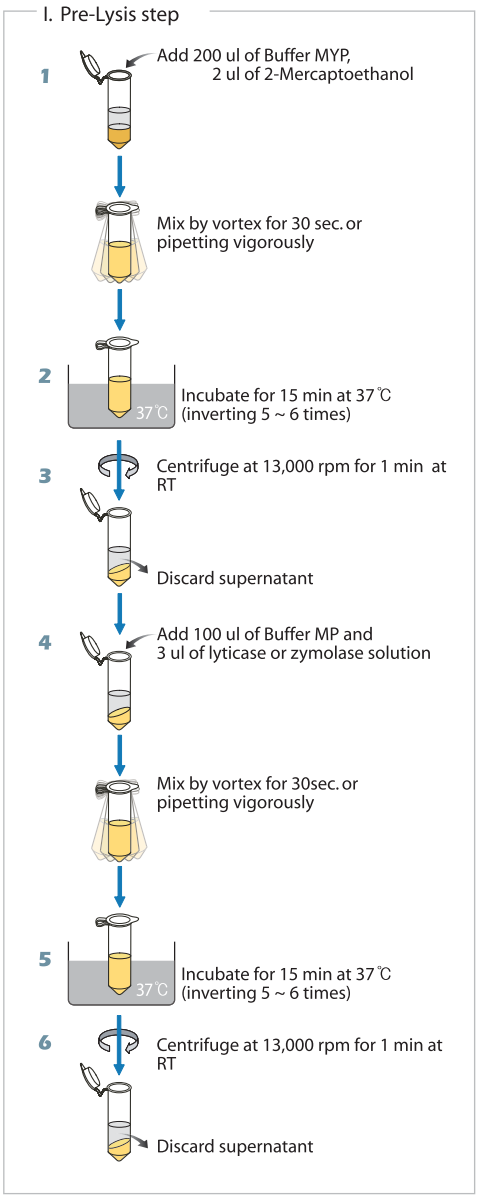



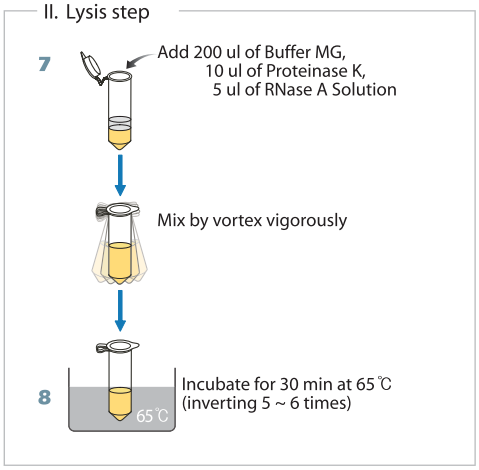
## B-2. DNA Extraction Step

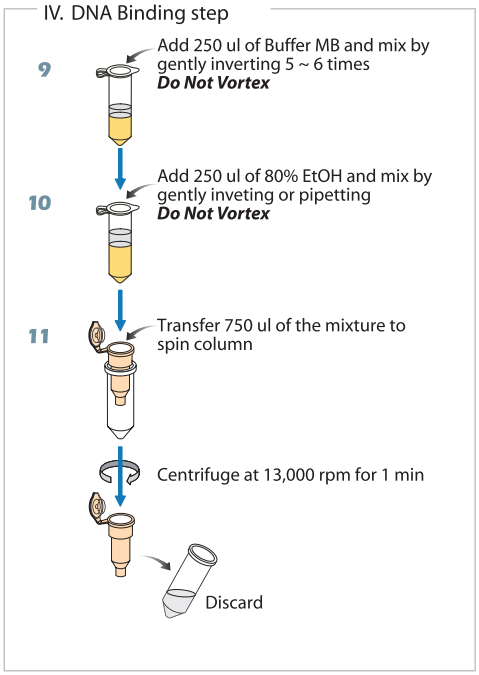
- ***Yeast***

■ 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

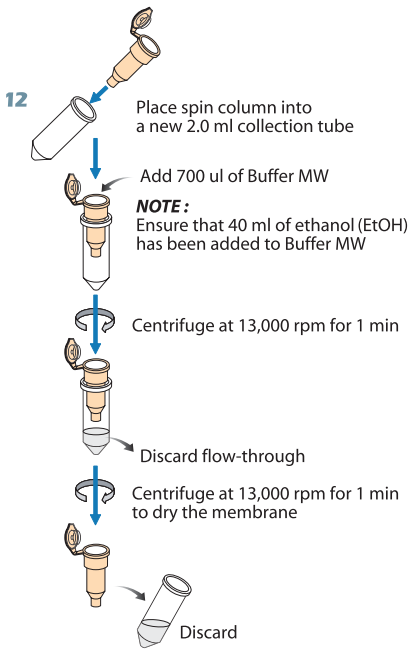


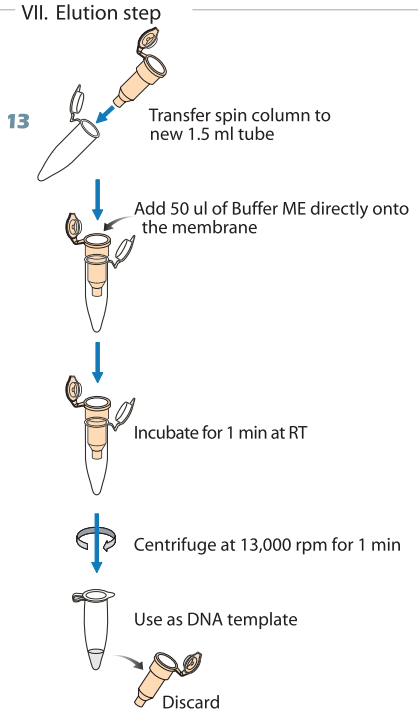



Type B Protocol
Yeast

VI. Washing step B




VII. Elution step



B-1. Sample Treatment Step

▪ ***Yeast***

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

**I. Preparation step**

**1. Prepare Yeast sample.**

Streak or spread cell on agar plate (ex. YPD, 2xYT etc.) . Incubate for 2 ~ 3 days in adequate temperature condition. 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 2 ~ 3 days in adequate temperature condition until $OD_{600}$ value of 0.8 ~ 1.0 on a spectrophotometer. $OD_{600}$ values depend on the length of the light path and therefore differ between spectrophotometers.

**III. Sample Sizing step**

**2. Transfer 1.5 ml cultured yeast cell into 2 ml tube.**

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 yeast by centrifugation at 13,000 rpm for 1 min, then discard supernatant. And the repeat the step 2 ~ 3 three times.**

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

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

B-2. DNA Extraction Step

▪ ***Yeast***

■ 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 37°C for use in step 2, 5 and to 65°C for use in step 8.
- All centrifugation steps should be carried out at room temperature.

## I. Pre-Lysis step

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

2. **Incubate the lysate for 15 min at 37℃.**

3. **Centrifuge the pre-lysate at 13,000 rpm for 1 min at room temperature. Discard supernatant, and then resuspend by vortexing or tapping of cell pellet to lysis cell perfectly.**
   It is better that the cell pellet is resuspended by vortexing or tapping before adding Buffer MP.

4. **Add 100 ㎕ Buffer MP and 3 ㎕ lyticase or zymolase solution into spheroblast sample tube, and mix well by vortex for 30 sec or pipetting vigorously.**
   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.

5. **Incubate the lysate for 15 min at 37℃.**
   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.

6. **Centrifuge the pre-lysate at 13,000 rpm for 1 min(RT). Discard supernatant, and then resuspend by vortexing or tapping of cell pellet to lysis cell perfectly.**
   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.

## II. Lysis step

7. **Add 200 ㎕ Buffer MG, 10 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and vortex vigorously.**
   Resuspend pellet by vortex or pipetting. After mix MG buffer, bacteria cell lysate change opaquely.

8. **Incubate the lysate for 30 min at 65℃.**
   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.

## IV. DNA Binding step

9. **After lysis completely, add 250 ㎕ Buffer MB to the lysate, and mix by pipetting or gently inverting 5 ~ 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**
   This step is an equilibration step for binding genomic DNA to column membrane.

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

11. **Pipette 750 ㎕ of the mixture from step 10 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 and collection tube altogether.**
    The maximum volume of the spin column reservoirs is 800 ㎕. 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.



## VI. Washing step B

12. **Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ㎕ Buffer MW to the spin column, and centrifuge at 13,000 rpm for 1 min.**
    **Discard the flow-through, and again centrifuge for additionally 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 MW.

## VII. Elution Step

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

# BYF Type C Bench Protocol

## Type C Protocol
## For Fungal tissue

C-1. Sample Treatment Step

- ***Fungal Tissue***

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

I. Preparation step

1 Prepare fresh fungal tissue sample

II. Disruption & Homogenization step

2 Slice off the prepared sample to small pieces

3 Place the sliced sample into mortar

Add liquid nitrogen for sample freezing

Pouring liquid nitrogen slowly. Disrupt and homogenize completely

III. Sample Sizing step

4 Measure 50 ~ 100mg of sample powder

Transfer to 1.5 ml tube

C-2. DNA Extraction Step

Type C Protocol
Fungal Tissue

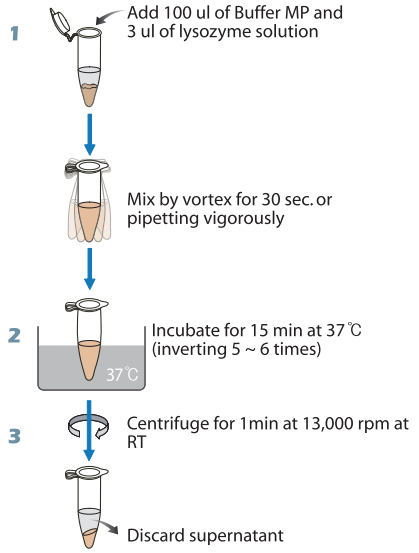
## C-2. DNA Extraction Step

- ***Fungal Tissue***

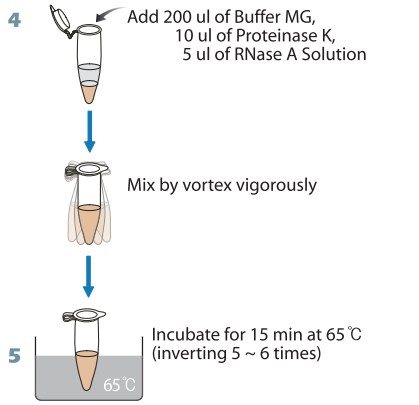
■ I. Pre-Lysis step ■ II. Lysis step □ III. Precipitation step ■ IV. DNA Binding step

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

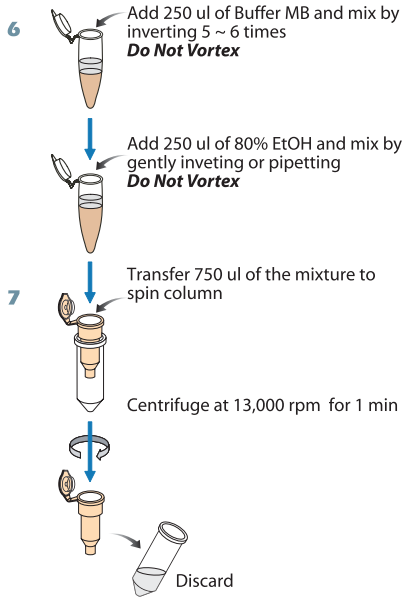
### I. Pre-Lysis step




### II. Lysis step

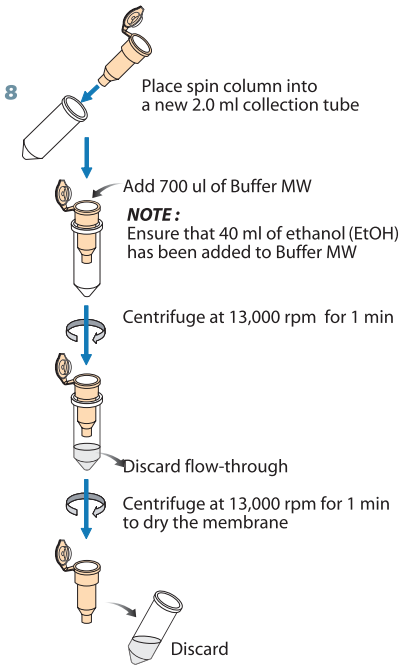



### IV. DNA Binding step

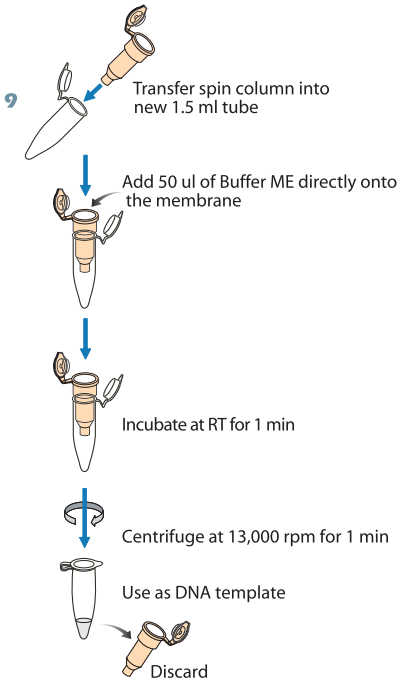



Type C Protocol
Fungal Tissue

VI. Washing step B




VII. Elution step



C-1. Sample Treatment Step

- ***Fungal Tissue***

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

## I. Preparation step

**1. Prepare Fungal tissue sample.**

We recommend to collect the fresh fungal tissue such as mushroom sample. The more fungal tissue samples are like to plant root or stem tissue sample. Also, fungal tissue sample's size is big, therefore it is disrupt and homogenize. Because fungal tissue is not cultivated when dry, it is important that keep temperature and humidity when cultivate. To later use, should slice off fungal tissue sample to suitable size and then stored at -80°C after frozen in liquid nitrogen.

## II. Disruption & Homogenization Step

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

When the handling of liquid nitrogen, be careful and wear a glove and protective suit. When homo -genize large size of sample, bound sample's pieces out of mortar. Also, sample of small size is homogenized easily and rapidly.

**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 step III.**

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. Observe lest sample or liquid nitrogen should bound. When the handling liquid nitrogen, be careful and wear a glove and protective suit.

## III. Sample Sizing Step

**4. Measure 50 ~ 100 mg of sample powder, and then transfer into 1.5 ml tube using a spatula.**

To prevent thaw 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.

C-2. DNA Extraction Step

▪ ***Fungal Tissue***

■ 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 37°C for use in step 2 and to 65°C for use in step 5.
- All centrifugation steps should be carried out at room temperature.

**I. Pre-Lysis step**

1. **Add 100 ㎕ Buffer MP and 3 ㎕ lysozyme solution into sample tube, and mix well by vortex for 30 sec or pipetting vigorously.**
2. **Incubate the lysate for 15 min at 37°C.**
   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.
3. **Centrifuge the pre-lysate at 13,000 rpm for 1 min(RT).**
   **Discard supernatant, ensuring that all liquid is completely removed.**

**II. Lysis step**

4. **Add 200 ㎕ Buffer MG, 10 ㎕ Proteinase K, and 5 ㎕ RNase A Solution into sample tube, and mix by vortex vigorously.**
   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 add rapidly, should be prevent degradation of DNA by Dnase.
5. **Incubate the lysate for 30 min at 65°C.**
   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.

**IV. DNA Binding step**

6. **After lysis completely, add 250 ㎕ Buffer MB to the lysate, and mix by pipetting or gently inverting 5 ~ 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**
   This step is an equilibration step for binding genomic DNA to column membrane.

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

**8. Pipette 750 ㎕ of the mixture from step 7 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 and collection tube altogether .**
The maximum volume of the spin column reservoirs is 800 ㎕. 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 at 13,000 rpm for 1 min .

## VI. Washing step B

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

## VII. Elution Step

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

# BYF Type D Bench Protocol

## Type D Protocol For fungi

D-1. Sample Treatment Step

- ***Fungi***

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

I. Preparation step

1 Prepare fungi by incubate 4 ~ 6 days in adequate temperature condition (15 ~ 17°C)

III. Sample Sizing step

2 Cut off 2 ~ 3 pieces size as 0.5 x 1 cm

3 Transfer sliced piece to 15 ml tube

IV. Pre-Treating step

4 Add 5 ml of 1x PBS buffer and vortex viogorously

5 Transfer 1 ml of suspended fluid into 1.5 ml tube

6 Centrifuge at 13,000 rpm for 5 min at RT

Discard supernatant, and then repeat the step 5 ~ 6 three times

7 Resuspend by vortexing or tapping

D-2. DNA Extraction Step

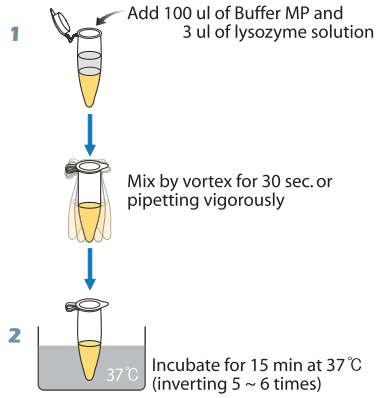
## D-2. DNA Extraction Step

- ***Fungi***

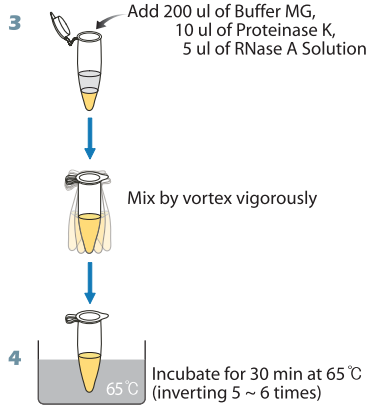
■ I. Pre-Lysis step ■ II. Lysis step □ III. Precipitation step ■ IV. DNA Binding step

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

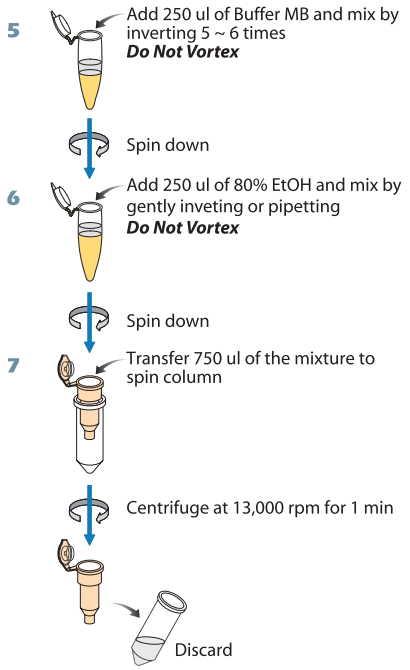
### I. Pre-Lysis step



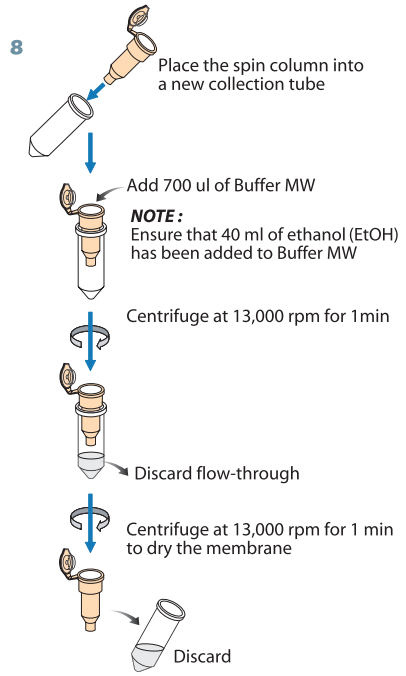

### II. Lysis step



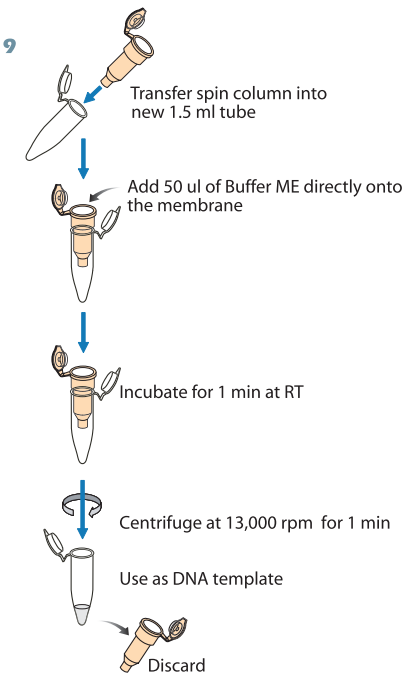

### IV. DNA Binding step



VI. Washing step B




VII. Elution step




Type D Protocol
Fungi

D-1. Sample Treatment Step

▪ ***Fungi***

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

**I. Preparation step**

**1. Prepare Fungi sample.**

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).

**III. Sample Sizing step**

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

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

**3. Transfer sliced piece into 15 ml tube.**

Progress for pre-treating step.

**IV. Pre-Treating step**

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

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

**5. Transfer 1 ml of suspended fluid into 1.5 ml tube.**

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

**6. Centrifuge suspended fluid at 13,000 rpm for 5 min at room temperature.**
**Discard supernatant, repeat step 5~ 6 three times.**

**7. Resuspend by vortexing or tapping of fungi pellet to pre-lysis cell perfectly.**

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

D-2. DNA Extraction Step

▪ ***Fungi***

| | | | |
|---|---|---|---|
| ■ 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 37°C for use in step 2 and to 65°C for use in step 4.
- All centrifugation steps should be carried out at room temperature.

**I. Pre-Lysis step**

**1. Add 100 μl Buffer MP and 3 μl lysozyme solution into sample tube, and mix well by vortex for 30 sec or pipetting vigorously.**

**2. Incubate the lysate for 15 min at 37°C.**

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.

**II. Lysis step**

**4. Add 200 μl Buffer MG, 10 μl Proteinase K, and 5 μl RNase A Solution into sample tube, and mix by vortexing vigorously.**

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.

**5. Incubate the lysate for 30 min at 65°C.**

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.

**IV. DNA Binding step**

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

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

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

**8. Pipette 750 ㎕ of the mixture from step 7 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 and collection tube altogether.**
The maximum volume of the spin column reservoirs is 800 ㎕. 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 at 13,000 rpm for 1 min.

## VI. Washing step B

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

## VII. Elution Step

**10. Place the spin column into a new 1.5 ml tube (not supplied), and 50 ㎕ Buffer ME directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge at 13,000rpm for 1 min to elute.**
Elution with 30 ㎕ increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 ㎕ 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 BYF 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 Assist Team. Our Technical Assist Teams are 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 (in Type C Protocol).
(2) Increase the incubation time at 65°C in Lysis step.

✓ High viscosity of Lysate
(1) Reduce the amounts of starting material.
(2) Increase the incubation time at 37°C in Pre-Lysis step.
(3) 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***

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

✓ Error in DNA binding
(1) After adding Buffer MB in DNA Binding step, please mix well by gently inverting.
(2) Check that the amount of Buffer MB is added correctly to the supernatant.
(3) Check that the amount of 80% EtOH is added correctly to binding mixture.

✓ 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.

✓ Insufficient DNA elution
(1) Increase the volume of Buffer ME or water to 100 $\mu\ell$.
(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 ME.

(2) Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.

(3) Reload the elute into used spin column, and then repeat elution step.

***DNA sheared***

✓ Incorrect storage of microorganism sample

(1) When store frozen tissues, always keep the samples frozen below -80°C.

(2) If possible, it is preferable to use fresh microorganism sample.

✓ Vigorously vortex

(1) Do not vortex the mixture after adding Buffer MB as described in protocol.

✓ Debris of cell 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, 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 MW.

(2) Store Buffer MW at room temperature (15 ~ 20°C).

✓ Amount of DNA used in experiments.

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

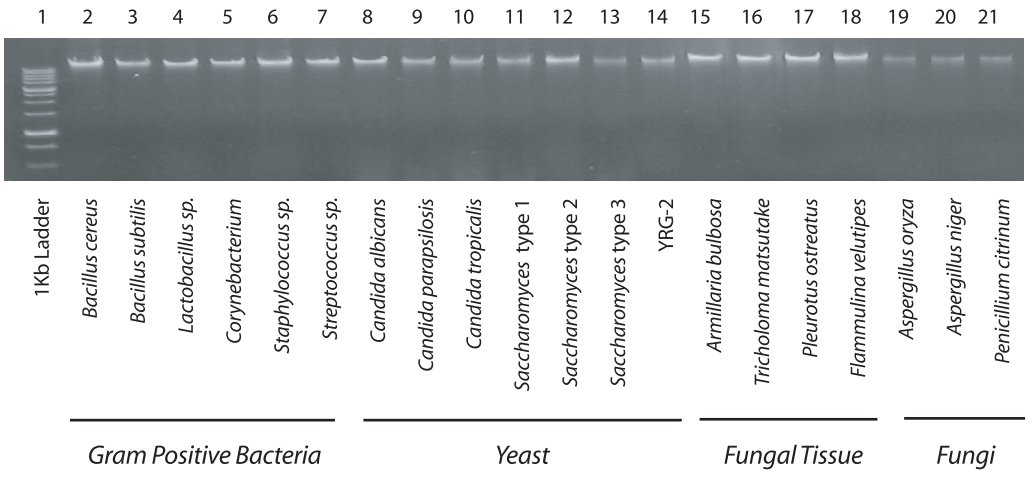
# Data Information
# Determination of Yield and Purity


# *Appendix A*

## *Electrophoresis Results from Various BYF Sample*

i-genomic BYF DNA Mini Kit provides 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 3 shows overall electrophoresis data from representative various BYF samples.

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




After elutin genomic DNA with 50 $\mu\ell$ Buffer ME, each 25 ~ 50 ng of DNA were used in DNA Electro-phoresis.

- Electrophoresis condition: 1.0% Agarose Gel at 100 volt for 30 min.
- DNA yields : Refer to Appendix B

# Appendix B

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

- **Type A Protocol**
  **: *Bacteria (Gram Positive )***

**(A) DNA Yield and Purity**

| Lane | Samples | Amounts (ml)[1] | DNA Yield (㎍) | $A_{260/280}$ |
|---|---|---|---|---|
| 1 | *Bacillus cereus* | 2 | 6 ~ 13 | 1.92 |
| 2 | *Bacillus subtilis* | 2 | 5 ~ 12 | 1.97 |
| 3 | *Lactobacillus sp.* | 2 | 6 ~ 13 | 1.90 |
| 4 | *Corynebacterium* | 2 | 6 ~ 13 | 1.94 |
| 5 | *Staphylococcus sp.* | 2 | 5 ~ 11 | 1.92 |
| 6 | *Streptococcus sp.* | 2 | 6 ~ 12 | 1.96 |

[1] The volume of cultured cell

**(B) DNA Purification and Enzyme Digestion (RE)**

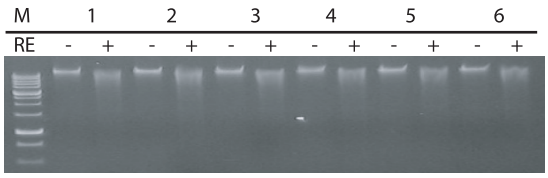



**Fig 4. Results of DNA purification and enzyme digestion with *Eco*RI**
After eluting genomic DNA with 50 ㎕ Buffer ME, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37℃ for 1 hour. (Lane M : 1 Kb Ladder, Cat. 24022)

- **Type B Protocol**
  **: *Yeast***

**(A) DNA Yield and Purity**

| Lane | Samples | Amounts (ml)[1] | DNA Yield (㎍) | $A_{260/280}$ |
|---|---|---|---|---|
| 1 | *Candida albicans* | 5 | 10 ~ 13 | 1.92 |
| 2 | *Candida parapsilosis* | 5 | 10 ~ 12 | 1.93 |
| 3 | *Candida tropicalis* | 5 | 10 ~ 13 | 1.91 |
| 4 | *Saccharomyces type 1* | 5 | 12 ~ 14 | 1.95 |
| 5 | *Saccharomyces type 2* | 5 | 12 ~ 15 | 1.91 |
| 6 | *Saccharomyces type 3* | 5 | 10 ~ 14 | 1.95 |
| 7 | *YRG-2* | 5 | 12 ~14 | 1.97 |

[1] The volume of cultured cell

**(B) DNA Purification and Enzyme Digestion (RE)**

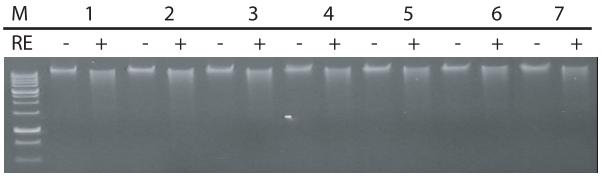



**Fig 5. Results of DNA purification and enzyme digestion with *Eco*RI**
After eluting genomic DNA with 50 ㎕ Buffer ME, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37℃ for 1 hour. (Lane M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

▪ **Type C Protocol**
**: *Fungal tissue***

**(A) DNA Yield and Purity**

| Lane | Samples | Amounts (mg)[1] | DNA Yield (㎍) | $A_{260/280}$ |
|---|---|---|---|---|
| 1 | *Armillaria bulbosa* | 50 | 9 ~ 13 | 1.91 |
| 2 | *Tricholoma matsutake* | 50 | 9 ~ 12 | 1.92 |
| 3 | *Pleurotus ostreatus* | 50 | 9 ~ 13 | 1.95 |
| 4 | *Flammulina velutipes* | 50 | 10 ~ 13 | 1.90 |

[1] The amount of ground fungal tissue powder

**(B) DNA Purification and Enzyme Digestion (RE)**

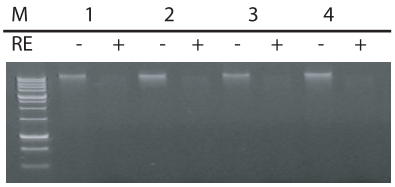



**Fig 6. Results of DNA purification and enzyme digestion with *Eco*RI**
After eluting genomic DNA with 50 ㎕ Buffer ME, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37℃ for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

▪ **Type D Protocol**
**: *Fungi***

**(A) DNA Yield and Purity**

| Lane | Samples | Amounts (ea)[1] | DNA Yield (㎍) | $A_{260/280}$ |
|---|---|---|---|---|
| 1 | *Aspergillus oryza* | 2 | 5 ~ 7 | 1.91 |
| 2 | *Aspergillus niger* | 2 | 5 ~ 8 | 1.90 |
| 3 | *Penicillium citrinum* | 2 | 5 ~ 6 | 1.89 |

[1] The piece of cut off cultured plate

**(B) DNA Purification and Enzyme Digestion (RE)**

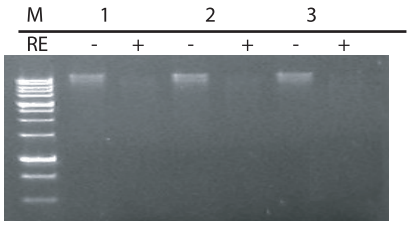
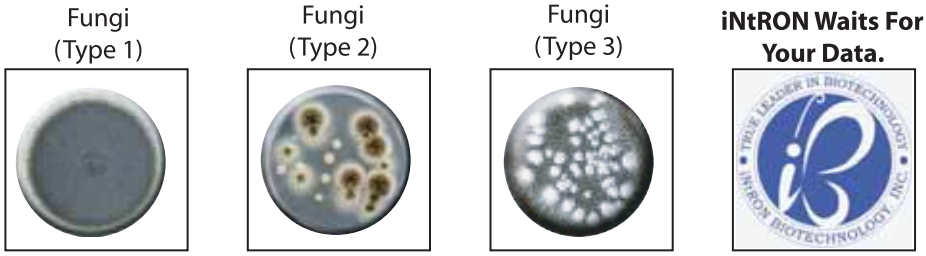



**Fig 7-1. Results of DNA purification and enzyme digestion with *Eco*RI**
After eluting genomic DNA with 50 ㎕ Buffer ME, each 100 ng of DNA were used in DNA electrophoresis and enzyme digestion, respectively. Restriction enzyme digestion was performed with 5 units of *Eco*RI at 37℃ for 1 hour. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

***Additional Data***
***(Type D protocol-based modification)***

When we have been developing i-genomic BYF 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 fungi sample supplied by customers with Type D protocol (occasionally, we slightly have modified Type D protocol). You can show good results from various fungi sample.

**Fig 7-2. Additional Data (sample morphology)**




**(A) DNA Yield and Purity**

| Lane | Samples | Amounts (ea)[1] | DNA Yield ($\mu$g) | $A_{260/280}$ |
|---|---|---|---|---|
| 1 | *Fungi (Type 1)* | 2 | 3 ~ 4 | 1.84 |
| 2 | *Fungi (Type 2)* | 2 | 2 ~ 5 | 1.88 |
| 3 | *Fungi (Type 3)* | 2 | 2 ~ 4 | 1.82 |

[1] The piece of cut off cultured plate

**(B) DNA Purification**

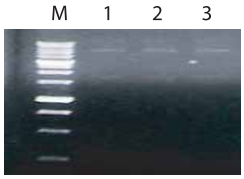



**Fig 7-3. Results of DNA purification**
After eluting genomic DNA with 50 $\mu\ell$ Buffer ME, each 1 $\mu\ell$ of DNA were used in DNA electrophoresis. (Lane M : 1 Kb DNA Ladder, Cat. 24022)

## *Note*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