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General Information

Qbiogene is a pioneer in developing kits for molecular biology research. We introduced the GENE CLEAN[®] Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

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FastDNA[®] Kit

Catalog # 6540-400

100 preps

- Purifies PCR-ready Genomic DNA from Plants, Animal Tissue, Cultured Cells, Bacteria, Yeast, and Insects, etc.
- For use with the FastPrep[®] Instrument

Shipping and Storage:

The **FastDNA[®] Kit** is shipped and stored at ambient temperature.

Revision # 6540-999-1D04

FastDNA[®] Protocol

Notes

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Kit Components 6540-400 (100 preps)

<u>Name</u>	<u>Volume</u>	<u>Cat #</u>
Lysing Matrix A	100	6910-100
1/4" Cylindrical Spheres	100	6540-424
Binding Matrix (DNA Binding Matrix Solution)*	66 ml	6540-408
SEWS-M (Salt/Ethanol Wash Solution)**	12 ml	6540-405
DES (DNA Elution Solution-Ultra Pure Water)	20 ml	6540-406
BBS gel loading dye	200 µl	6540-407
SELECT FROM THE FOLLOWING FOR SPECIFIC APPLICATION:		
<u>Plants</u>		
CLS-VF (Cell Lysis/DNA Solubilizing Solution for Vegetation - Requires PPS)	90 ml	6540-402
PPS (Protein Precipitation Solution)	25 ml	6540-403
<u>Animal Tissue</u>		
CLS-TC ‡ (Cell Lysis/DNA Solubilizing Solution for Animal Tissue, Bone Tissue, Culture Cells, Insects, and Bacteria)	110 ml	6540-409
<u>Yeast, Algae, and Fungi</u>		
CLS-Y (Cell Lysis/DNA Solubilizing Solution for Yeast, Algae, and Fungi)	110ml	6540-411
Optional:		
SPIN ™ Filters and Catch Tubes	100	2080-800
	60	2080-600
	40	2080-400

*Contains Guanidine Thiocyanate. Use with proper caution.

**Protocol for preparation of SEWS-M (salt/ethanol wash solution, DNase-free): Add 100 ml ethanol to the bottle labeled SEWS-M, which already contains 12 ml of DNase-free salt solution, to make a total of 112 ml. Shake and store tightly capped bottle at room temperature.

‡In case a precipitate is seen, heat bottle in 45-55°C water bath and allow to cool to room temperature.

Introduction

The FastDNA[®] Kit is for use with the FastPrep[®] Instrument and purifies PCR-ready Genomic DNA from plant and animal tissue, cultured cells, bacteria, yeast, whole insects, etc., in less than 30 minutes. The kit consists of three general classes of components:

A. Lysing Matrix

Lysing Matrix tubes include a 1/4" Ceramic Sphere and Garnet Matrix preloaded in 2.0 ml tubes. 1/4" Ceramic Spheres are included in a separate package. Depending on the difficulty of lysing the samples, the following matrix combinations can be made. See the Matrix Comparison Table on the next page as a guideline for choosing one of the four combinations for a particular sample type. Yield and size of the resulting DNA can be optimized by varying the Lysing Matrix Combination, the speed of the FastPrep[®], and the amount of time that samples are processed.

To make this

Lysing Matrix Combination:

- (1) 1/4" Sphere
- (2) 1/4" Sphere + 1/4" Sphere
- (3) 1/4" Sphere + Garnet Matrix
- (4) 1/4" Sphere + Garnet + 1/4" Sphere

Do This:

- Discard the garnet
- Discard the garnet; add sphere
- As shipped
- Add sphere

B. Sample-Specific Cell Lysis Solution (CLS)

- For plant tissues: **CLS - VF + PPS** (Protein Precipitation Solution)
- For bacteria and animal cells/tissues, from soft to hard: e.g. cultured cells, insects, bone: **CLS - TC**
- For yeast, algae, and fungi: **CLS - Y**

C. Purification and Elution Reagents

- **Binding Matrix Solution**
- **SEWS-M** (Salt Ethanol Wash)
- **DES** (DNA Elution Solution is ultra-pure water)
- **BBS** (Gel Loading Dye)

Outline of Protocol

1. Sample Processing:

Choose appropriate:

- a. Lysing Matrix.
- b. CLS.
- c. Sample size.

2. Homogenize in FastPrep[®] Instrument.

Note: When using the 1/4" sphere lysing matrix, do not set the FastPrep[®] Instrument to a speed greater than 5.0

3. Centrifuge to pellet debris; transfer supernatant to new tube.

4. Add **Binding Matrix**; wash.

5. Elute.

References:

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How the System Works

The FastPrep® Instrument shakes a tube up and down (and with a slight twisting motion) at very high speeds. The rotor holds 12 x 2 ml tubes enabling 12 samples to be processed simultaneously.

The FastDNA® Kits include 2 ml tubes with 1/4" Ceramic Spheres and Garnet Matrix. In addition, 1/4" Ceramic Spheres are supplied. The spheres can be added to the tubes after the addition of reagents and sample. For extremely difficult to homogenize samples, all three matrices (sphere, garnet, and sphere) can be used. For soft tissues (liver, brain, tissue culture cells, etc.) the sphere is sufficient to homogenize the sample. While all three matrices can homogenize any sample, there is a compromise between total yield and resulting fragment size. Use table (on page 5) as a reference for optimizing homogenization conditions.

The 2 ml tubes that contain the Lysing Matrix and sample also contain a chaotropic DNA stabilizing solution which is a proprietary mixture of detergents and salts. The detergents have been found to serve two functions. One, they contribute to inactivate nucleases. Two, they provide lubrication during the lysing step to control the degree of shearing of the DNA.

When processing samples in the FastPrep® Instrument complete lysis occurs in 10-40 seconds. The energy generated by friction during the lysis step is considerable causing a temperature increase in the tube. This rise in temperature facilitates the inactivation of nucleases and does not harm the DNA.

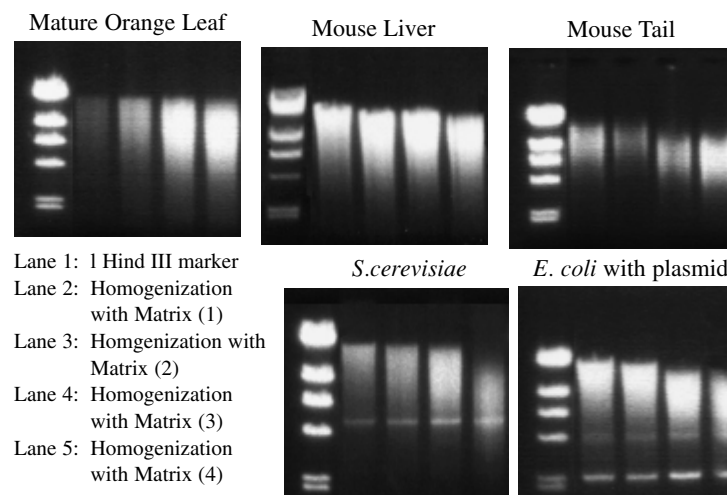
Summary of FastPrep® System

The FastPrep® System, which includes both the FastPrep® Instrument and FastDNA® and FastRNA® kits, has the ability to lyse cells with minimal shearing of the nucleic acids. The procedure eliminates the major concerns in isolation of nucleic acids from cells that are difficult to lyse without enzymes, manual grinding, or homogenizing. It is these laborious and time consuming lysing steps which allow nucleases to act and can make nucleic acid isolation a chore. The FastPrep® System, by use of highly energetic mechanical means and careful choice of reagents, disrupts whole tissues, lyses cells, and stabilizes nucleic acid from any source, thus eliminating the need for lysing enzymes or grinding and homogenizing equipment.

Lysing MATRIX Comparison Table

Sample	Lysing Matrix Comb.	Sample Weight	CLS	Yield	DNA Size	OD 260/280
Mature Orange Leaf	1	100 mg	VF	2.0 µg	20 kb	1.6
Leaf (<i>Citrus aurantium</i>)	2	100 mg	VF	2.5 µg	20 kb	1.9
	3	100 mg	VF	3.0 µg	20 kb	1.9
	4	100 mg	VF	4.5 µg	20 kb	1.8
Mouse (C57 Black6 SJ)	1	60 mg	TC	20 µg	22 kb	1.8
	2	60 mg	TC	24 µg	18 kb	1.7
	3	60 mg	TC	33 µg	18 kb	1.7
	4	60 mg	TC	34 µg	15 kb	1.6
Mouse (C57 Black6 SJ) Tail	1	20 mg	TC	2.5 µg	17 kb	1.9
	2	20 mg	TC	1.8 µg	16 kb	1.9
	3	20 mg	TC	2.0 µg	13 kb	2
	4	20 mg	TC	3.3 µg	11 kb	1.8
Yeast (<i>Saccharomyces Cerevisiae</i>)	1	28 mg	Y	10 µg	22 kb	1.7
	2	28 mg	Y	10 µg	22 kb	1.9
	3	28 mg	Y	10 µg	22 kb	2
	4	28 mg	Y	10 µg	18 kb	2
Bacteria (<i>E. coli</i>)	1	16 mg	TC	27 µg	23 kb	2
	2	16 mg	TC	23 µg	22 kb	1.9
	3	16 mg	TC	25 µg	19 kb	2
	4	16 mg	TC	28 µg	16 kb	2

Note: In each case the FastPrep® speed setting was 5 for 10 seconds.



Detailed Protocol

1. Sample Processing:

- a. Prepare appropriate **Lysing Matrix** for the sample to be processed. Depending on the level of difficulty in homogenizing a particular sample, the Lysing Matrix in the tubes may be altered (see Introduction, A. Lysing Matrix and Lysing Matrix Comparison Table).
- b. Choose appropriate **CLS** and add to tube with Lysing Matrix: Plant Samples: Add 800 µl **CLS-VF** and 200 µl **PPS** to a tube containing sample and Lysing Matrix. For bacteria and animal cells/tissues, from soft to hard: e.g. cultured cells, insects, bone: Add 1 ml **CLS-TC** to a tube containing samples and Lysing Matrix. Algae, Fungi, and Yeast: Add 1 ml **CLS-Y** to a tube containing samples and Lysing Matrix.
- c. Choose appropriate **sample size**: Samples consist of up to 200 mg of tissue or a 200 µl suspension of cells in water or isotonic saline solutions. [For single cells grown in suspension (bacteria, yeast, algae, tissue culture cell, e.g.): Centrifuge a sufficient volume of culture to provide a pellet size of 50-100 mg wet weight or up to 10⁹ bacteria, 10⁸ yeast/algae, 10⁷ mammalian cells. Resuspend pellets in 100 µl of water or isotonic saline to give a maximum suspension volume of 200 µl and add to tube]. Some tissues have high levels of nuclease activity and require additional measures to prepare DNA. Plants that contain high levels of phenolics may require the addition of 0.1-1% polyvinylpyrrolidone (PVP) or 25 mg of polyvinylpolypyrrolidone (PVPP, Sigma Chemical). The addition of DTT and sodium thiosulfate to 10 mM decreases the formation of brown discoloration; 1-3% beta-mercaptoethanol may be required in extreme cases.

Important: The volumes are calculated to leave a minimum air space of approximately 0.25 cc. If less air space is present, there is a likelihood of sample loss due to tube failure or deformation around the cap allowing sample to bubble out; this is caused by an increase in pressure with temperature increase during FastPrep[®] runs. The presence of 0.25 cc of air space in the tube is sufficient to prevent sample loss during routine FastPrep[®] runs.

2. Homogenize in FastPrep[®] Instrument.

For all applications, place tube in FastPrep[®] Instrument and process for 5-30 seconds at speeds from 4.0-5.0 for all applications. Speeds above 5.0 may damage FastPrep[®] tubes and result in loss of your sample.

Homogenization of hard or fibrous tissues can be facilitated by first placing the tissue between two weigh boats or pieces of parafilm and smashing with a hammer just prior to FastPrep[®] processing.

Additional processing time may be required for cartilage, some leaves, and other fibrous or dried samples. Ice-chill tubes for 1-2 minutes after each 30 second processing cycle.

Note: To improve chances of optimized recovery of DNA from extremely dry samples, like bone, an incubation period of 15 minutes to 2 hours is recommended after processing in the FastPrep[®] Instrument.

3. Centrifuge to pellet debris: Spin in microcentrifuge for 5 minutes at 14,000 x g to pellet protein and cell debris. Transfer 600 µl of the supernatant to a clean microcentrifuge tube.

Note: Extending spin to 15 minutes can enhance elimination of excessive debris from large samples or from cells with complex cell walls.

Warning: Please check that tubes are balanced by weight and that the bottom or side of the tubes will not scrape the wall of your microcentrifuge to avoid rapid loss of sample.

For improved convenience, the optional SPIN[™] Protocol on the next page uses steps 4a and 5a (instead of 4 and 5 below).

4. Add 600 µl of **Binding Matrix**; mix gently, and incubate for 5 minutes at room temperature. Spin for 1 minute; discard supernatant. Gently resuspend pellet with 500 µl **SEWS-M**. Spin for 1 minute and discard supernatant. Spin for 10 seconds and remove residual liquid with a small bore pipet tip.
5. Elute DNA from **Binding Matrix** by gently resuspending in 100 µl **DES** followed by a 2-3 minute incubation. Spin for 1 minute at 14,000 x g and transfer supernatant to a new tube. Be careful to avoid transferring particles of **Binding Matrix** pellet with your DNA sample. [Use of a large bore pipet will reduce the chance of mechanical shearing of the resulting DNA]. DNA is now ready for digestion, electrophoresis, PCR, and any other desired application.

SPIN[™] Protocol

Follow steps 1-3 of the Detailed Protocol and continue with steps 4a and 5a below.

Optional SPIN[™] Modules (See pg 3) are necessary for steps 4a and 5a.

- 4a. Add 600 µl **Binding Matrix**; mix gently, and incubate for 5 minutes at room temperature. Pulse spin for 5 seconds to pellet **Binding Matrix** and discard supernatant. Gently resuspend pellet with 500 µl **SEWS-M** and transfer suspension to a **SPIN[™] Filter**. Centrifuge for 1 minute and discard contents of Catch Tube. Centrifuge for 1 minute to "dry" **Binding Matrix**/DNA complex. Transfer **SPIN[™] Filter** to a new **Catch Tube**.
- 5a. Elute DNA by resuspending **Binding Matrix**/DNA complex in 100 µl **DES**. Wait for 2-3 minutes and centrifuge for 1 minute at 14,000 x g to transfer DNA-containing **DES** to the **Catch Tube**; discard **SPIN[™] Filter**. DNA is ready for use without further manipulation.