EasyPrep™ Fungus Genomic

DNA Miniprep Manual

Catalog#: GD06-01, GD06-02

For research use only

(March 2017)

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Introduction

EasyPrep™ Fungal gDNA Miniprep Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of Fungal species and tissues. Up to 100mg of wet tissue (or up to 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Bioland's matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the EasyPrep™ Fungal gDNA Miniprep Kit for the first time, please read this booklet to become familiar with the procedures before beginning. Dry or fresh fungal tissue is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated. Binding conditions are then adjusted and the sample is applied to a DNA spin-column. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the EasyPrep™ Fungal DNA Miniprep Kit are stable for at least 12 months from date of purchase when stored at 22°C-25°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer FG 3. It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Content

Catalog#	GD06-01	GD06-02
DNA Mini Columns	50	250
Colletion Tubes	100	500 mL
Buffer FG1	40 mL	180 mL
Buffer FG2	12mL	50 mL
Buffer FG3	15mL	200 mL
Buffer CFG1	30 mL	130 mL
DNA Wash Buffer	12 mL	54 mL
Elution Buffer	15 mL	50 mL
RNase A	100 µL	500 µL
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Caution: Buffer CFG1 and FG3 contain chaotropic salts. Wear gloves and protective eyeware when handling. Don't mix waste with bleach.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps. Important:

- Pre-heat Elution Buffer to 65°C
- Dilute Wash Buffer Concentrate with ethanol as follows and store at room temperature.

GD06-01	Add 48mL absolute (96%-100%) ethanol
GD06-02	Add 216 mL absolute (96%-1 00%) ethanol/bottle

- Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens.

A. Dry Specimens

For processing ≤50 mg powdered tissue. DNA yields range from 50µg to 100µg per 100mg sample.

B. Fresh or Frozen

For processing ≤200 mg fresh or frozen tissue. DNA yields is about the same as in A.

Fungal Genomic DNA Miniprep Protocols

A. Dry Specimens

Materials supplied by users:

- Centrifuge capable of at least 13,000rpm
- Nuclease-free 1.5 mL or 2.0 mL microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile ddH2O water at 65°C
- Absolute (96%-100%) ethanol
- ß-mercaptoethanol

This is the most robust method for isolation of total cellular (mitochondrial. chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping.

Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples place ~50 mg of dried tissue into a microfuge (2 mL tubes are recommended for processing of >50 mg tissue) tube and grind using a pellet pestle. Disposable Kontes pestles work well. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process in sets of four to six tubes until Step 2 before starting another set.

1. To **10-50 mg powdered dry tissue** add **600µl Buffer FG1 in a microcentrifuge tube.** *Optional: Add 10 µL ß-mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps.*

***Tip**: Process in sets of four to six tubes: grind, add Buffer FG1 and ß-mercaptoethanol, and proceed to Step 2 before starting another set. Do not exceed 50 mg dried tissue.*

2. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube. *Optional: If necessary, add 2 µL of RNase into the lysate before incubation to remove the RNA.*

3. Add **200µl Buffer FG2** and mix well by vortexing for 10s. Incubate on ice for 5 min. Centrifuge at 13,000rpm for 5 min.

4. Carefully transfer the supernatant to a new microfuge tube. Add 0.7 volume isopropanol. Mix well by vortexing for 5s and centrifuge at 13,000rpm for 2 min to precipitate the DNA. *This step removes polysaccharides and improves DNA binding ability to the spin column.*

5. Carefully decant the supernatant and discard, avoid dislodging the DNA pellet. Inverting the tube on absorbance paper towel for 1 min to drain any residual ethanol. *The pellet doesn't have to be dry.*

6. Add **300µl** preheated (65°C) **ddH_2O** and vortex for 10s to mix the DNA well. *A brief incubation at 65°C may help dissolve the DNA.*

7. Add **150µl Buffer FG3** and **300µl 100% ethanol**, mix well by vortexing for 5s. *A precipitation may form but does not interfere DNA binding to the column.*

8. Transfer the sample to a column and centrifuge at 13,000rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

9. Add **500 µl DNA Wash Buffer** and centrifuge at 13,000rpm for 1 min. Discard the flow-through and put the column back to the collection tube. Repeat once and put the column, with the lid open, back to the collection tube.

10. Centrifuge the column 13,000rpm for 1 min . *This step is **critical** for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.*

11. Transfer column to a clean 1.5 mL tube. Add **100 µl Elution Buffer** (or sterile deionized water) pre-warmed to 65°C and centrifuge at 13,000 rpm for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200 µL of buffer for elution is not recommended.

12. Optional: Add the eluted DNA back to the column for another elution. The first elution normally yields 60-70% of the DNA while a second elution yields 20-30% of the DNA.

B. Fresh or Frozen Specimens

Materials supplied by users:

- Centrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 mL or 2.0 mL microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile ddH2O water at 65°C
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples
- ß-mercaptoethanol

Note: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples, allowing efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of various fungi, sample size should be limited to □200 mg. Best results are obtained with young leaves or needles. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples, collect tissue in a 1.5 mL or 2 mL microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable Kontes pellet pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70□ for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

1. Collect grounded **fungal tissue** (start with **100mg**) in a microfuge tube and immediately add **600µl Buffer FG1.** Optional: Add 10 µl ß-mercaptoethanol and vortex vigorously. *Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.* ***TIP****: Process in sets of four to six tubes: fill all tubes with liquid nitrogen, grind, and add Buffer FG1 and β-mercaptoethanol; proceed to Step 2 before starting*

another set. As a starting point, use 100 mg tissue per tube and if yield and purity are satisfactory increase to 200 mg.

2. Incubate at 65°C for 10 min. Mix sample twice during incubation by inverting tube. **Optional:** If necessary, add 2 µl of RNase into the lysate before incubation to remove the RNA.

3. Add **140µl** Buffer FG2 and mix well by vortexing for 10s. Centrifuge at 13,000 rpm for 10 min.

4. Carefully transfer the supernatant to a clean 2.0 mL tube. Add **0.7 volume isopropanol**. Mix well by vortexing for 5s and centrifuge at 12,000 x g for 2 min to precipitate the DNA. *This step removes polysaccharides and improves DNA binding ability to the spin column.*

5. Carefully decant the supernatant and discard, avoid dislodging the DNA pellet. Inverting the tube on absorbance paper towel for 1 min to drain any residual ethanol. *The pellet doesn't have to be dry.*

6. Add **300µl** preheated (65°C) **Elution Buffer** and vortex for 10s to mix the DNA well. *A brief incubation at 65°C may help dissolve the DNA.*

7. Add **150µl Buffer FG3** and **300µl 100% ethanol**, mix well by vortexing for 5s. *A precipitation may form but does not interfere DNA binding to the column.*

8. Transfer the sample to a DNA column and centrifuge at 13,000rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

9. Add **500 µl DNA Wash Buffer** and centrifuge at 13,000 rpm for 30s. Discard the flow-through and put the column back to the collection tube. Repeat once. Put the column, with the lid open, back to the collection tube.

10. Centrifuge the column at 13,000 rpm for 1 min. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

11. Transfer the column to a clean 1.5 mL tube. Add **100µl Elution Buffer** pre-warmed to 65°C and immediately centrifuge at 13,000 rpm for 1 min to elute DNA. *Smaller volumes will significantly increase DNA concentration but give lower yields. The first elution normally yields 70% of the DNA bound to the column.*

12. Optional: Add the eluted DNA back to the column for a 2nd elution yields another 20-30% of the DNA bound. *To increase DNA concentration, add Elution Buffer and incubate the column at 60-70°C for 5 min before elution.*

Protocol for DNA isolation from Fungus rich in polyphenols and/or polysaccharides.

1. Collect ground Fungal tissue (start with 100 mg) in a 2.0 mL microfuge tube and immediately add **500µl Buffer CFG1**.
2. Incubate at 65℃ for 15 min. Mix sample twice during incubation by inverting tube. *Optional: If necessary, add 2 µL RNase into the lysate before incubation to remove the RNA.*
3. Add **800µl chloroform /Isoamyl alcohol** (24:1) and vortex to mix. Centrifuge at ≧10,000 x g for 5 min. Carefully transfer 300 µl of supernatant to a new tube making sure not to disturb the pellet or any precipitates.
4. Add **150µl Buffer FG3** and **300µl absolute ethanol,** vortex to obtain a homogenous mixture. *A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.*

5. Apply the entire sample to a DNA column with the collection tube. Centrifuge at 13,000 rpm for 1 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.

6. Add **500µl DNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through liquid and reuse the collection tube. Repeat once.

7. Centrifuge the column at 13,000 rpm (or top speed) for 1 min.

8. Transfer the column to a clean 1.5 mL tube and add **100µl Elution Buffer** (Pre-warmed to 65°C) and incubate at room temperature for 1 min.

9. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Optional: Add the eluted DNA back to the column for another elution. The first elution normally yields 60-70% of the DNA while a second elution yields 20-30% of the DNA.

Trouble Shooting

Problem	Possible reason	Suggestions
Clogged well	Carry-over of debris	Following precipitation with Buffer FG2, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column	In protocols A and B, ensure that DNA is dissolved in water before adding Buffer FG3 and ethanol. This may need repeated incubation at 65□and vortexing.
	Sample too viscous	In protocol C, do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers FG1 and FG 2 and use two or more columns per sample.
	Incomplete precipitation following addition of FG 2.	Increase RCF or time of centrifugation after addition of Buffer FG2.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer FG1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers FG1 and FG 2.
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over	DNA Wash Buffer must be at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human. This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. For technical support or learn more product information, please visit our website at www.bioland-sci.com or contact us at (562)602-8882.

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