OmniPrep™ for Fungus

G-Biosciences

Abstract

High Quality Genomic DNA Extraction from Fungal Samples

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Guidelines

INTRODUCTION

The OmniPrep™ for Fungus kit isolates high quality genomic DNA from fungal samples. The kit isolates high purity (A260/A280 ratios of 1.7 to 2) DNA between 100-200kbp and the yield is 0.2-1µg/5mg fungal samples. If used according to the protocols this kit purifies DNA from 1-2gm fungal tissues.

ITEM(S) SUPPLIED (Cat. # 786-399)

Description	Size
Genomic Lysis Buffer	100ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
Longlife™ RNase (5mg/ml; >60U/mg)	0.5ml
LongLife™ Proteinase K (5mg/ml)	2 x 0.5 ml
Molecular Grinding Resin™	1ml
TE Buffer	20ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label.

ADDITIONAL ITEMS REQUIRED

Chloroform, Isopropanol and 70% Ethanol

Materials

OmniPrep™ for Fungi 786-399 by G-Biosciences

Protocol

PREPARATION BEFORE USE

Step 1.

Proteinase K Solution: To avoid repeated freezing-thaw, dispense the Proteinase K solution into aliquots of 30µl/tube and freeze at -20°C.

PREPARATION BEFORE USE

Step 2.

Genomic Lysis Buffer & DNA Stripping Solution: If a precipitate forms due to cold storage allow to warm to room temperature until precipitate dissolves.

PREPARATION BEFORE USE

Step 3.

Molecular Grinding Resin: Centrifuge the Molecular Grinding Resin tube for 2 minutes at 2,500x g and remove the water. Add 0.5ml Genomic Lysis Buffer.

DURATION
00:02:00

Step 4.

Collect fungal tissue from liquid culture and wash 2-3 times in sterile water.

Step 5.

Fungal mycelia are best prepared by grinding samples using Molecular GrindingResin™ in Genomic Lysis Buffer.

NOTES
Colin Heath 12 Aug 2016

For fungal teliospores, grinding samples in liquid nitrogen to a fine powder and quickly adding to an appropriate volume of GenomicLysis Buffer is recommended.

Step 6.

Add 10-20mg fungal mycelia to a microcentrifuge tube containing 500µl Genomic Lysis Buffer. Resuspend Molecular Grinding Resin by vigorous mixing or vortexing.

Step 7.

Add 30µl Molecular Grinding Resin™ using a wide bore pipette tips and grind with a microcentrifuge pestle.

NOTES
Colin Heath 12 Aug 2016

For teliospoes, add ground powder to 500µl Genomic Lysis Buffer and vortex to wet sample.

Step 8.

Add 1µl Proteinase K solution for every 100µl Lysis Buffer and incubate at 60°C for 1-2 hours. Invert the tube periodically each hour.

DURATION
01:00:00
Step 9.

Allow the sample to cool to room temperature.

Step 10.

Add 200µl chloroform and mix by inverting the tube several times.

Step 11.

Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.

DURATION
00:10:00
Step 12.

Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.

DURATION
00:05:00
Step 13.

Add 100µl Precipitation Solution and mix by inverting the tube several times. A white precipitate

should be produced, if not add 50µl aliquots of Precipitation Solution until a white precipitate forms.

Step 14.

Centrifuge the sample at 14,000xg for 5 minutes.

DURATION
00:05:00

Step 15.

Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol. Invert the tubes 10 times to precipitate the DNA.

Step 16.

Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.

DURATION
00:05:00

Step 17.

Add 700µl 70% ethanol to the tube and invert several times to wash the DNA pellet.

Step 18.

Centrifuge for 1 minute at 14,000xg.

DURATION
00:01:00

NOTES
Colin Heath 07 Jun 2016

In some samples, the pellet may behard to see at this point and will be loosely attached to the tube.

Step 19.

Decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not let the pellet dry completely or it will be difficult to rehydrate.

Step 20.

Add 50 to 100µl TE Buffer to the pellet. Incubate at room temperature for at least 15 minutes to rehydrate. Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60 minutes.

OPTIONAL: Add 1µl LongLife™ RNase for every 100µl TE Buffer at this stage.

Step 21.
Store DNA at 4°C, for long-term storage store at -20°C or -80°C.