BECKMAN COULTER Life Sciences

DNA Extraction from Filamentous Fungi using GenFind V3

Researchers working on filamentous fungi e.g. *Aspergillus* and *Penicillium* may use this protocol.
Please reference the current GenFind V3 protocol for product information. (Part Number C34880, C34881)

Purpose

Filamentous fungi have tough cell walls that can make lysis and DNA extraction difficult. The most common method for lysing filamentous fungi is bead beating, which is labor intensive and not amenable to automation. This protocol provides two other methods for lysis that are more automation friendly and we show that they produce results that are very similar to the industry standard of bead beating.

Materials Used:

Material	Part Number	Supplier
7 Bar magnet for 96-well plate	771MWZM-1ALT	V&P Scientific
2 mL 96-well plate	609681	Beckman Coulter
Lysozyme from chicken egg white	L6876	Sigma-Aldrich
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
2xYT	AB15063-01000	AmericanBio
PBS, pH 7.4	10010023	ThermoFisher Scientific
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
0.5 mm glass beads	Z250465	Sigma-Aldrich

Protocol

This GenFind V3 supplemental protocol provides sample DNA isolation with three different protocols. Protocol choice is dependent on workflow needs and the particular strain(s) used.

Fungal growth should be fresh

- Fungal growth can be done either on a plate or in an overnight culture of a rich media such as 2xYT
- If using fungal growth from a plate, combine fungi with 200 µL of 1 x PBS and vortex to mix

Isolation from up to 200 µL of fresh culture using a freeze/thaw lysing method

1. **Lysis**
   a. Transfer **200 µL** of **fungal growth** to a 2 mL 96-well plate
   b. Add **500 µL** of **Lysis (LBB)** to the sample
   c. Add **30 µL** of **Proteinase K** to the sample
   d. Add **4 µL** of **lysozyme (10mg/mL)** the sample
   e. **Incubate** the plate for **10 minutes** at **-80°C**
   f. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   g. **Incubate** the at **room temperature** for **5 minutes** or until samples are completely thawed
   h. **Incubate** the samples for **30 minutes** at **37°C**

2. Bind

a. Vortex the bottle of **Bind (BBB)** to fully resuspend the beads
b. Add **300 µL** of **Bind (BBB)** to the sample
c. Add **100 µL** of **80% ethanol** to the sample
d. **Mix** by pipetting up and down 10 times, or until thoroughly mixed

   Note: If this step is skipped not all of the beads will settle.
e. **Incubate** for **5 minutes** at **room temperature**
f. Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
g. Remove and discard the supernatant without disrupting the beads
h. Remove the plate from the magnet

3. Wash

a. Add **800 µL** of **Wash (WBB)** to the sample
b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
d. Remove and discard the supernatant without disrupting the beads
e. Remove the plate from the magnet
f. Repeat steps 3.a-3.e for a total of **2 washes**
g. Add **1600 µL** of **Wash (WBC)** to the sample
h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
i. Place the plate on a **magnet** for **6 minutes** (or until supernatant is clear)
j. Remove and discard the supernatant without disrupting the beads
k. Remove the plate from the magnet
l. Repeat steps 3.g-3.k for a total of **2 washes**

4. Elute

a. Add **40 µL** of **Nuclease-free water** to the sample
b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
c. **Incubate** for **2 minutes** at **room temperature**
d. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
e. Remove and **Save** the supernatant without disrupting the beads

Isolation from up to 200 µL of fresh culture by lysing at 65°C for 1 hour

1. **Lysis**
   a. Transfer **200 µL** of **fungal growth** to a 2 mL 96-well plate
   b. Add **500 µL** of **Lysis (LBB)** to the sample
   c. Add **30 µL** of **Proteinase K** to the sample
   d. Add **4 µL** of **lysozyme (10mg/mL)** the sample
   e. **Incubate** the plate for **1 hour** at **65°C**
   f. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   g. **Incubate** the plate for **1 hour** at **65°C**
2. **Bind**
   a. Vortex the bottle of **Bind (BBB)** to fully resuspend the beads
   b. Add **300 µL** of **Bind (BBB)** to the sample
   c. Add **100 µL** of **80% ethanol** to the sample
   d. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
      Note: If this step is skipped not all of the beads will settle.
   e. **Incubate** for **5 minutes** at **room temperature**
   f. Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
   g. Remove and discard the supernatant without disrupting the beads
   h. Remove the plate from the magnet
3. **Wash**
   a. Add **800 µL** of **Wash (WBB)** to the sample
   b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
   d. Remove and discard the supernatant without disrupting the beads
   e. Remove the plate from the magnet
   f. Repeat steps 3.a-3.e for a total of **2 washes**
   g. Add **1600 µL** of **Wash (WBC)** to the sample
   h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   i. Place the plate on a **magnet** for **6 minutes** (or until supernatant is clear)
   j. Remove and discard the supernatant without disrupting the beads
   k. Remove the plate from the magnet
   l. Repeat steps 3.g-3.k for a total of **2 washes**
4. **Elute**
   a. Add **40 µL** of **Nuclease-free water** to the sample
   b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   c. **Incubate** for **2 minutes** at **room temperature**
   d. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
   e. Remove and **Save** the supernatant without disrupting the beads

Isolation from up to 200 µL of fresh culture using Bead Beating

1. **Lysis**
   a. Add 0.5 g of 0.5 mm glass beads to a 2 mL 96-well plate
   b. Transfer **200 µL** of **fungal growth** to the plate
   c. Add **100 µL** of **Lysis (LBB)** to the sample
   d. Add **100 µL** of **Nuclease-free water** to the sample
   e. Bead beat at **max speed** (≥1200rpm) for **3 minutes**
   f. Let the beads settle and transfer the supernatant to a new 2 mL 96-well plate
   g. Add **30 µL** of **Proteinase K** to the sample
   h. Add **4 µL** of **lysozyme (10mg/mL)** to the sample
   i. Add **400 µL** of **Lysis (LBB)** to the sample
   j. Remove the plate from the magnet
   k. **Incubate** the samples for **30 minutes** at **37°C**
2. **Bind**
   a. Vortex the bottle of **Bind (BBB)** to fully resuspend the sample
   b. Add **300 µL** of **Bind (BBB)** to the sample
   c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   d. **Incubate** for **5 minutes** at **room temperature**
   e. Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
   f. Remove and discard the supernatant without disrupting the beads
3. **Wash**
   a. Add **800 µL** of **Wash (WBB)** to the plate
   b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
   d. Remove and discard the supernatant without disrupting the beads
   e. Remove the plate from the magnet
   f. Repeat steps 3.a-3.e for a total of **2 washes**
   g. Add **1600 µL** of **Wash (WBC)** to the sample
   h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   i. Place the plate on a **magnet** for **6 minutes** (or until supernatant is clear)
   j. Remove and discard the supernatant without disrupting the beads
   k. Remove the plate from magnet
   l. Repeat steps 3.g-3.k for a total of **2 washes**
4. **Elute**
   a. Add **40 µL** of **Nuclease-free water** to the plate
   b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   c. **Incubate** for **2 minutes** at **room temperature**
   d. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
   e. Remove and **Save** the supernatant without disrupting the beads

Example Data

Genomic DNA extraction from *A. niger*

Data shown below resulted from the use of Aspergillus niger streaked onto a YT plate that was grown for 2 weeks and kept at 4°C for 2 months. The fungal growth and spores were scraped off the plate into 1.5 mL of 1xPBS and thoroughly vortexed. The fungal PBS mixture was distributed evenly among the three treatment groups.

Bead beating resulted in the highest yield of DNA; this is most likely due to the large amounts of spores that were used in this study. The other two treatment groups while resulting in about half the total yield are more permissible to high throughput applications. Other than one outlier the 65°C for 1 hour treatment group had the best $A_{260/A280}$ ratios of the other treatment groups.

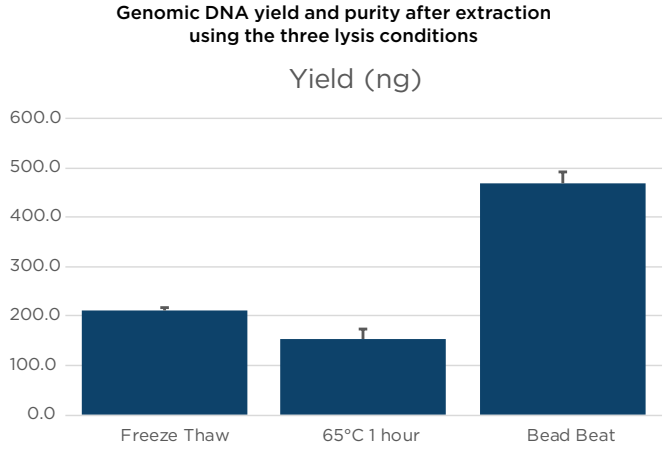

Figure 1. The average yield of DNA from A. niger using one of the three lysis conditions. Error bars represent the standard deveiation of three technical replicates.

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AAG-4717SP01.19