User-Developed Protocol:

Isolation of genomic DNA from fungi (culture and blood) using the QIAamp® DNA Mini Kit

This procedure has been adapted by customers from the QIAamp® Tissue Protocols, and is for use with the QIAamp DNA Mini Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

These procedures have been used successfully for isolation of genomic DNA from *Aspergillus* and *Candida* species, from both fungal cultures and blood.

Please be sure to read the QIAGEN® *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook* and the detailed Tissue Protocol carefully before beginning this procedure.

Procedure

From ***Candida* spp. cultures**

1. **Grow isolates on Sabouraud agar plates at 30°C for 48 h.**
   See below for medium composition.
2. **Harvest colonies using an inoculation loop and resuspend in 1 ml fungal saline (0.9% w/v NaCl) to obtain a suspension containing 1–5 x 10^6 cells (measured photometrically at A_{530}, or McFarland 0.5).**
   Note: 1–5 x 10^6 cells yield about 20–30 µg fungal genomic DNA.
3. **Centrifuge and collect the cell pellet. Continue with step 7 on the following page.**

From *Aspergillus* spp. cultures

1. **Grow isolates on Sabouraud agar plates at 30°C for 72 h.**
2. **Flood the plate surface with 10 ml fungal saline (0.9% w/v NaCl) or preferred reagent, to harvest the conidia. Prepare a 1 ml saline suspension containing 1–5 x 10^6 conidia (measured either photometrically at A_{530} or with a hemocytometer).**
   Note: 1–5 x 10^6 cells yield about 20–30 µg fungal genomic DNA.
3. **Centrifuge and collect the cell pellet. Continue with step 7 on the following page.**

From EDTA-anticoagulated blood

1. **Mix 3 ml EDTA-anticoagulated blood with 15 ml red-cell lysis buffer (RCLB) and incubate on ice for 10–15 min.**

   RCLB:
   10 mM Tris pH 7.6
   5 mM $MgCl_2$
   10 mM NaCl.

   Note: 3 ml EDTA-anticoagulated blood yields about 70–100 µg total DNA.

2. **Centrifuge at 3000 rpm for 10 min. Discard the supernatant.**
3. **Repeat steps 1 and 2, then continue with step 4.**
4. **Resuspend the cell pellet in 1 ml white-cell lysis buffer (WCLB: RCLB containing 200 µg/ml Proteinase K) and incubate at 65°C for 45 min.**
5. **Centrifuge at 5000 rpm for 10 min. Discard the supernatant.**
6. **Optional: Add 200 µl NaOH (50 mM). Cover with mineral oil and incubate at 95°C for 10 min. Centrifuge at 5000 rpm for 10 min. Discard the supernatant.**

   This optional treatment with NaOH is required only for fungi that are difficult to digest with lyticase alone (e.g., *Aspergillus niger*).

7. **Add 500 µl lyticase solution and incubate at 37°C for 30 min to produce spheroplasts.**

   Lyticase solution:
   10 U/ml lyticase
   50 mM Tris, pH 7.5
   10 mM EDTA
   28 mM ß-mercaptoethanol.

   Note: If a very low fungus titer is expected, the use of carrier DNA is recommended. See the *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook* General Comments.

8. **Centrifuge at full speed for 10 min. Discard the supernatant.**
9. **Resuspend the pellet in 180 µl Buffer ATL and 20 µl Proteinase K stock solution (provided in the QIAamp DNA Mini Kit). Incubate at 55°C for 15 min.**
10. **Continue with step 3 of the Tissue Protocol in the *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook.***
11. **Elute the DNA once with 50 µl Buffer AE or distilled water.**

Suppliers

Sabouraud agar plates (ready to use, without gentamycin and chloramphenicol) are available from Becton Dickenson (cat. no. 297739).

Lyticase (recombinant — to minimize the risk of introducing foreign DNA into the preparation), is available from Sigma (product no. L4276).

Alternative recipes for Sabouraud agar media

(for cultivation and maintenance of fungi)

1. Sabouraud Maltose Agar (available from Difco, BBL, and Oxoid)

40.0 g maltose
15.0 g agar
5.0 g pancreatic digest of casein
5.0 g peptic digest of animal tissue

made up in 1 liter distilled water, pH 5.6±0.2 at 25°C.

2. Sabouraud Dextrose Agar Extra (personal recommendation)

32.5 g Sabouraud Dextrose Agar
(Difco; 5.0 g neopeptone, 20.0 g dextrose, 7.5 g agar)
30.0 g dried malt extract
0.3 g yeast nutrient

made up in 500 ml distilled water. Boil to dissolve before autoclaving.

Reference

Loeffler, J. et al*. (1996) Extraction of fungal DNA from cultures and blood using the QIAamp Tissue Kit*. QIAGEN News 4, 16–17.*

* *The QIAamp Tissue Kit is now available as the QIAamp DNA Mini Kit.*