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SQUASH PCR: Rapid DNA Extraction from Filamentous Fungal Biomass for PCR Analysis

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SUMMARY

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Scope and applications

This Standard Operating Procedure outlines a protocol for the extraction of DNA from fungal biomass using the Squash PCR method, a rapid and streamlined approach suitable for downstream PCR-based applications. This technique has been previously employed for DNA preparation of filamentous fungi, but its application has been limited to species of industrial interest. In this protocol, the method has been adapted and validated for use with more than 150 filamentous fungal species belonging to Ascomycota, Basidiomycota and Mucoromycota, demonstrating its effectiveness in enabling rapid genotyping and molecular screening. Representative taxa tested belong to the genera *Acremonium*, *Agaricus*, *Alternaria*, *Aspergillus*, *Cordyceps*, *Fusarium*, *Ganoderma*, *Mortierella*, *Mucor*, *Mycena*, *Penicillium*, *Rhizopus*, *Trichoderma* etc. For yeast species, conventional colony PCR remains sufficient to achieve comparable outcomes. In contrast to earlier protocols relying on conidial suspensions, the present method has been performed on mycelium biomass, thereby extending its applicability to a broader range of fungal taxa (i.e. the one that do not sporulate). It is important to note that the procedure described here is intended for applications where speed and simplicity are prioritized over DNA purity, such as routine diagnostic PCR or strain verification. It is not recommended for applications requiring high-integrity or high-purity DNA, such as whole-genome sequencing.

Materials

- Distilled Sterile Water
- Sodium Phosphate Buffer 0.12M (Na₂HPO₄) or PBS
- Autoclaved Microscope Slides (washed slides can be used)
- Cover glass 24x32 (new ones, no sterilization needed)
- 200 µl autoclaved tips

Procedure

1. Grow the strain of interest on agar plates;
2. Under a laminar flow hood, scrape the mycelium to collect the biomass in Eppendorf tubes previously filled with 50 µl of distilled sterile water, avoiding agar and melanized conidia when possible. When working with more than one plate, take all the biomasses first before moving to step 3;
3. Using a sterile dissecting needle or an Eppendorf pestle, break the biomass in the Eppendorf tubes, then vortex at maximum speed for 5 seconds. In this way, DNA is released in the water;
4. Using 200 µl tips, take 10 µl of the sample and put it on a microscope slide, making sure to get a little amount of solid biomass together with the liquid;
5. Put the cover glass on top of the sample and apply pressure with your thumb;
6. Carefully lift the cover glass and add 10 µl of phosphate buffer to recover the biomass from the cover glass: all the material from the cover glass should be collected on the slide's surface.
7. At this point, in a new sterile Eppendorf tube, collect the liquid on the slide with a pipette, avoiding any pieces of solid biomass left;
8. Finally clean the slide by adding 10 µl of phosphate buffer and collect the material in the Eppendorf tube, avoiding solid biomass;
9. If the sample appears very turbid, consider diluting;
10. Assess nucleic acid concentration and quality with Nanodrop: the concentration should range from 100-400 ng/ul; amplification might be difficult to achieve with 260/230 ratios below 1.
11. Proceed directly with the PCR using 1 µl of extract. If not possible, samples can be stored for maximum 3 days at 4°.

Important considerations

- Wear nitrile gloves as much as possible when handling the Eppendorf tubes, the microscope slides and cover slips, but avoid wearing gloves when using a Bunsen burner, for safety reasons.
- Any traces of agar, melanized conidia and biomass will decrease the 260/230 ratio, possibly inhibiting amplification. It is advisable to collect the biomass before strong sporulation of the colony happens.
- Extracted samples can possibly be stored at -20° C, but no data are available on quantity/quality loss.

Reference

- YUAN, Guoliang, et al. Rapid and robust squashed spore/colony PCR of industrially important fungi. *Fungal Biology and Biotechnology*, 2023, 10.1: 15.
<https://doi.org/10.1186/s40694-023-00163-0>
- Woodman, Michael E. "Direct PCR of intact bacteria (colony PCR)." *Current protocols in microbiology* 9.1 (2008): A-3D.
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