Arcis Fungal Nucleic Acid Extraction Protocol

As classic culture-based detection methods for fungal pathogens may be time consuming, and show low sensitivity and poor specificity; PCR based assays have been developed to amplify the DNA of fungal pathogens. Protocols for the extraction of DNA from fungal cells may be laborious, and require lysis steps such as mechanical disruption with beads, sonication, freeze-thaw or heating with alkali as the cell walls are very resistant. The method presented here was designed to take advantage of the Arcis extraction technology to produce a rapid, inexpensive and reliable procedure for the extraction of total nucleic acids from fungal cells. This simple method provides nucleic acids suitable for a range of downstream processes including qPCR and sequencing. The method was tested for seven common fungi, covering a range of cell wall strengths and the integrity of the DNA extracted was tested using Sanger sequencing and PCR.

The following protocol describes the extraction of DNA from fungal samples, and varies from the standard Arcis DNA Sample Prep Kit method to maximize cell disruption.

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 90 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Samples

A primary solution was obtained through preparation of a +2 Mc Farland solution of fungal cells in sterile water and vortex mixed thoroughly.

Method

- The suspended fungal samples were added (30µl) to 150µl Arcis reagent 1 and incubated for 10 minutes at room temperature and vortex-mixed every 3 minutes.
- The samples were then centrifuged for 10 minutes at 12,00rpm and the supernatant containing the released nucleic acids retained.
- The lysed mixture was then added to reagent 2 in a 1:4 ratio.
- 10µl was then added to the PCR Mastermix.
- Detection and identification of fungal DNA target through PCR based on the PCR primers targetting a 500-bp region of the 18 S rRNA gene, was performed using a Perkin Elmer GeneAmp end-point PCR System (Applied Biosystems). All PCR products were subjected to electrophoresis and standard Sanger sequencing.

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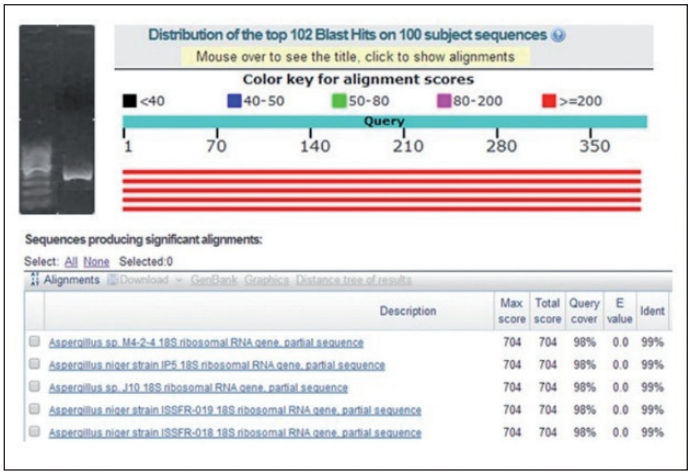
Results

The results obtained using Arcis extraction protocol in combination with PCR and subsequent sequence analysis are shown in Table1 and Figure1. The results show that this new extraction Arcis technology generates amplification products which are 100% compliant with standard PCR-based sequencing methods. All fungi identified from plates were subjected to a redundant cycle of extraction-amplification-sequencing so as to prove reproducibility for its identification through Sanger sequencing.

Figure 1



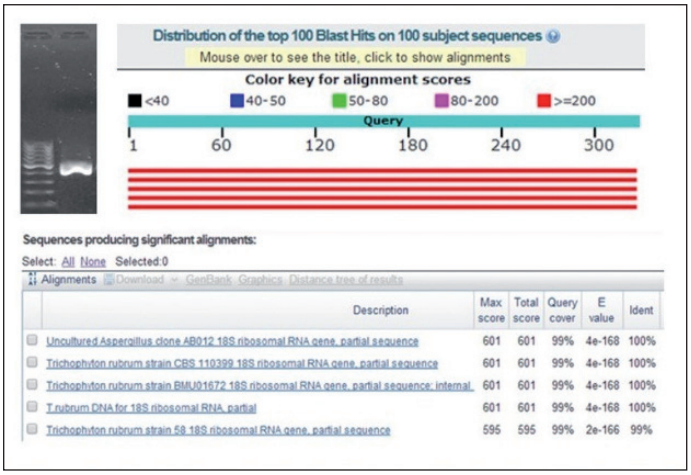


Table 1 shows the comparison between the detection of the fungal species by culture based methods and PCR. Complete concordance was observed between culture based and nucleic-acid based identification methods.

Micro and macro identification (Microbiology)	PCR	Sequencing results from ARCIS extraction and identification fungi kit (MK1)
C.albicans	+	*C.albicans, C.dublinensis, C.parapsilosis*
Aspergillus sp	+	*A.sojae, A.tamarii, A.flavus*
A.flavus	+	*A.sojae, A.tamarii, A.flavus, A.fumigatus, Penicillium sp*
A.fumigatus	+	*A.sojae, A.tamarii, A.flavus, A.fumigatus*
A.niger	+	*A.niger, A.terreus, A.turingiensis*
Penicillium sp	+	*A.sydowii, A.versicolor, Ascomycota, A.terreus*
Alternaria	+	*Alternaria alternata*
Microsporum canis	+	*M.canis, M.audouinii*
Trichophyton rubrum	+	*T.rubrum, T.soudanense, T.schoenleinii, T.mentagrophytes*

Conclusion

This low-tech and rapid process releases DNA from fungal samples which is stable at ambient temperatures, thus facilitating a quicker and easier diagnosis in low-resource settings. There was 100% concordance between the culture-based identification and the PCR results. Furthermore, 100% condordance was observed between the PCR results and the Sanger sequencing results, indicating that the released nucleic-acids are of good integrity.