

User-Developed Protocol

DNA Extraction Protocol for Paraffin Embedded-Tissue Sections using the MycXtra DNA extraction kit

A. Deparaffinization of Tissue Sections

The DNA extraction was performed as previously described by Bialek *et al.* with minor modifications. A first step of deparaffinization was performed by adding of two 10 µm sections of paraffin wax embedded tissue to 1,500µl xylene to an Eppendorf tube. This was vortexed, then incubated at room temperature for 5 minutes, centrifuged at 16,000g for 5 minutes and the supernatant discarded. This step was repeated once. Then 1,500 µl methanol (100%) was added, vortexed and incubated for 5 minutes at room temperature. It was centrifuged at 16,000 g for 5 minutes and supernatant was removed. This step was also repeated once. Then tubes were incubated with opened lids in a heating block at 37°C until there was complete evaporation of the methanol was observed.

B. Basic DNA extraction

750 µL of sterile dH₂O was added to deparaffinised tissue and centrifuged for 2 min at 10,000 x g. Then 700 µL of the supernatant was removed. The pellet was re-suspended in approximately 50 µL and the entire amount was transferred to the 2 mL Bead Solution tube provided with the MycXtra DNA extraction kit. Subsequent steps were performed in accordance with the manufacturer's instructions and DNA was eluted in 40 µl buffer S5.

[This step differs from Bialek's modified QIAamp tissue kit method, which requires 3 steps of freezing in liquid nitrogen and boiling for 5 minutes].

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C. Further DNA purification

To further purify the DNA (only ~50% of samples will amplify without this next step), 100 µl 10 mmol Tris pH 8.0 was added to the extracted DNA from the previous extraction. Then 50 µl phenol-chloroform-isoamyl alcohol (pH 6.7-8 in the ratio of 25:24:1) was added, vortexed and centrifuged at 10,000 rpm for 5 min. Approximately 120 µl of the aqueous phase was transferred to a new tube and 0.1 vol of 3 M sodium acetate pH 4.1 was added. Then 2.5 vol of 96% ethanol was added and the tubes were centrifuged at 12,000 rpm for 15 min. The pellet was washed in 200 µl 70% ethanol and the tubes were centrifuged at 8,000 rpm for 2 min. The supernatant was carefully discarded. The pellet was dried by vacuum centrifugation and the DNA was resuspended in 30 µl of distilled H₂O.

Reference

This protocol was kindly supplied by Professor David Denning (personal communication).

Bialek R, Konrad F, Kern J, Aepinus C, Cecenas L, Gonzalez GM, *et al.* PCR based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. *J Clin Pathol* 2005; 58: 1180-4.

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