Preparation of Genomic DNA of Microsporidia

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Abstract

To prepare the genomic DNA of microsporidia, 400 ll suspension of purified spores (1010 spores/ml) was mixed with 40 ll KOH (2 mol/l) in a 1.5 ml Eppendorf tube and incubated at 27C for 1 h, TEK buffer (1 mmol/l Tris–HCl, 10 mmol/l EDTA, 0.17 mol/l KCl, pH 8.0) was added and continued incubating at 27C for 1 h. Adjusted pH to 8.0 with 1 mol/l HCl, added 10% SDS in order to attain 0.5% in the mixture and kept it in ice-bath for 15 min. 20 mg/ml proteinase K (TaKaRa Biotechnology Co. LTD) was added in order to attain 200 lg/ml in the mixture and incubated at 50C for 4 h. Subsequently, isovolumetric tris–phenol extraction was performed twice, and washed with chloroform: isoamyl alcohol (24:1 v/v). 10% NaOAC was added to the recovered aqueous phase after centrifuging (10,000 r/min, 5 min), and then the DNA was precipitated by 2.5 times volume of cold ethanol at -20C for 40 min before centrifuging (12,000 r/min, 10 min). In addition, the precipitated DNA was rinsed twice with 500 ll cold 70% ethanol before drying at 37C for 5–10 min. Then, the extracted DNA was stored at -20C after dissolution in 50 ll TE buffer at 65C for 10 min.

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