Jul 29, 2017

Preparation of Genomic DNA of Microsporidia

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.igacbse

ShiNan Dong

Chen Gong

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.igacbse

External link: https://doi.org/10.1371/journal.pone.0181703

Protocol Citation: ShiNan Dong 2017. Preparation of Genomic DNA of Microsporidia. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.igacbse**

Manuscript citation:
Chen G, Wang W, Chen H, Dai W, Peng X, Li X, Tang X, Xu L, Shen Z (2017) Functional characterization of an aquaporin from a microsporidium, *Nosema bombycis*. PLoS ONE 12(7): e0181703. doi: **10.1371/journal.pone.0181703**

Protocol status: Working

Created: June 14, 2017

Last Modified: March 14, 2018

Protocol Integer ID: 6370

Keywords: genomic dna of microsporidia, purified spore, spore, microsporidia, precipitated dna, phenol extraction, extracted dna, genomic dna, ml eppendorf tube

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.

Attachments

PDF

Sequence and Phyloge...

274KB

Troubleshooting