Diplonema Genomic DNA isolation

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Abstract

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Protocol

Step 1.
Spin down cells (10^6) for 5 mins at 1800g to get clear pellet and try to remove all the traces of sea salt

Step 2.
Resuspend cells in 0.5 ml (per 10^8 cells) lysis buffer (10mM Tris-HCl pH 8.0, 5mM EDTA, 200mM NaCl, 0.2% w/v SDS), add 5µl RNase A (20 mg/ml) (Fermentas) and mix by inverting the tube several times

Step 3.
Incubate for 10 min at 60°C and add 2.5µl Proteinase K (10 mg/ml) (Fermentas). Incubate 1 hour at 60°C.

Step 4.
Add 250 µl ice cold 5 M NaCl, and incubate on ice for 10 mins (protein precipitation step—salting out of protein).

Step 5.
Spin down at 16000-20000 RPM for 15 min and transfer supernatant to new tube

Step 6.
Add an equal volume (about 700-750 µl) of isopropanol and mix by inversion (genomic DNA precipitation). Pending on amount of gDNA, incubate 15 mins at room temperature (RT) or 1 hour at -20°C to improve the recovery

Step 7.
Spin down 10 min at RT to pellet gDNA precipitate

Step 8.
Wash pellet 2X times with 500 µl 70% (v/v) ethanol by centrifuge at 16000-20000 RPM for 15 min

Step 9.
Remove the supernatant completely by aspiration and air dry gDNA pellet for 10-25 min at room temperature

Step 10.

Resuspend gDNA in 10mM Tris-HCl (pH 8.0) leave overnight at RT for gDNA to resolve completely