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Working

Euplotes crassus micronuclear enrichment by PFGE

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Protist Research to Optimize Tools in Genetics (PROT-G)



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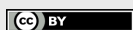


PROTOCOL STATUS

Working

We use this protocol in our group and it is working

- 1 Starved *Euplotes crassus* cells (treated overnight with ampicillin 100 mg/ml) were filtered and harvested by centrifugation at 400 rcf for 3 min.
- 2 As much as possible of the sea water was removed and cells were resuspended in 1V of 1XTE buffer and mixed with 1V of 2% Certified Low-Melt Agarose (Bio-Rad) in 1XTE buffer, then quickly transferred into the wells of the plug mold to produce the plugs (around 150,000 cells in 80 ul of volume in each well).
- 3 Plugs were set at 4°C for at least 30 min and then placed in 5 ml cells lysis buffer (1% N-Lauroylsarcosine sodium salt, 1% SDS, 1 mg/ml Proteinase K, 0.5 M EDTA pH 8.0) at 60°C overnight.
- 4 The plugs were washed three times in 0.5 M EDTA pH 8.0 for 10 min each, then inserted into the wells of the 1% Certified Low-Melt Agarose gel (Bio-Rad).
- 5 The instrument used for the PFGE was CHEF Mapper XA Pulsed Field Electrophoresis System (Bio-Rad). 2.2 L of 0.5X TBE buffer were poured into the electrophoresis chamber for a pre-run of 30 min at 14°C, with a pump speed setting of 70 (flow rate of ~ 1 liter/minute).
- 6 The run was set with a gradient of 6 V/cm, a time of 30 h, an included angle of 120°, an initial switch time of 70 sec, a final switch time of 70 sec and a linear ramping factor.
- 7 When the run was completed the gel was stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) for 60 min and visualized with blue light.
- 8 The bands of interest were cut and melted at 65°C for around 30 min. The gel volume was determined and digested with Agarase from *Pseudomonas atlantica* (Sigma) at 40°C overnight and then with RNase A (Thermo Fisher Scientific) 10 µg/ml for 2 h at 37°C.
- 9 The DNA was extracted by Phenol/Chloroform, precipitated by 3 M sodium acetate pH 5.2/absolute EtOH, resuspended in 30 ml of ddH₂O, and quantified by NanoDropTM 1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific).



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