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Euplotes crassus micronuclear enrichment by PFGE V.1

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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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 esuspended 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).