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Working

Long staining procedure of nuclei in Euplotes crassus using DAPI

Forked from Long staining procedure of nuclei in Euplotes crassus using DAPI

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

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1 Pellet Euplotes crassus cells at 400 rcf for 3 minutes, and remove as much supernatant as possible by pipetting.

Both algae and bacteria are autofluorescent. Better to have a completely starved Euplotes crassus culture.

2 Add 1 ml of 2% PFA in 1X PHEM or 4% PFA in 1X PBS to the cells, and incubate them for 10 minutes at room temperature.

3 Pellet Euplotes crassus cells by centrifugation at 400 rcf for 3 minutes, and remove as much supernatant as possible by pipetting.

4 Wash cells twice with 1X PBS (400 rcf for 3 minutes each time).

5 Add 1 ml of TBSTEM - 3% BSA and 0.5 µl of DAPI (0.1 mg/ml) to the cells, and stain for 10 minutes at room temperature.

6 Pellet Euplotes crassus cells by centrifugation at 400 rcf for 3 minutes.

7 Add 50 µl of Prolong medium.

8 Place an approx. 10 µl droplet of Euplotes crassus cells on a slide for observation by fluorescence microscopy.