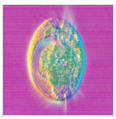

Apr 24, 2019

Working

Culturing Euplotes crassus to high densities using a combination of algae and bacteria as the food source.

Forked from Culturing Euplotes crassus to high densities using a combination of algae and bacteria as the food source.

Lawrence A. Klobutcher[1], Larry Klobutcher[2]

[1]University of Connecticut Health Center, [2]University of Connecticut, School of medicine

dx.doi.org/10.17504/protocols.io.2aggabw

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

Angela Piersanti
University of Camerino

ABSTRACT

- This procedure was developed to investigate the possible use of RNAi through bacterial feeding as a means of knocking down expression of particular genes.
- While the utility of RNAi is still being investigated, the method is effective in growing *Euplotes crassus* to higher densities than is possible using algae alone. Using moderately dense cultures of algae, we typically achieve cell densities of 500-1,000 cells/ml, while with the E. coli supplemented cultures we describe here, *Euplotes* cell densities exceed 3,000 cells/ml. In fact, preliminary studies mixing a volume of a *Euplotes* culture with an equal volume of resuspended E. coli have produced a density of ~8,000 cells/ml.
- While the described procedure involves small cultures, it can likely be scaled up as required.

For some species of *Euplotes*, long-term culture using bacteria as a food source has not been successful (C. Miceli, personal communication), suggesting that some essential nutrient is not being provided in sufficient quantitities

1 Grow a 10 ml culture of *E. coli* overnight using L-broth. (Notes: we have used strain HT115, but any strain of *E. coli* will likely do. Do not use antibiotics.)

2 Distribute the bacterial culture to 2 ml microcentrifuge tubes and pellet by centrifugation in a microcentrifuge for 1.5 min.

3 Pour off the supernatant and resuspend the pellet in each tube in 2 ml of a moderately dense culture of *Dunaliella salina* grown in artificial seawater.

4 In a 15 ml disposable plastic tube, combine 1 ml of a lightly starved *Euplotes crassus* culture, 7 ml of a moderately dense culture of the algae *Dunaliella salina* grown on artificial seawater, and 2 ml of the resuspended *E. coli* culture.

5 Incubate at room temperature with the tubes at an angle.

6 Gently invert tubes daily to disperse settled material.

7 The *Euplotes* will likely consume all bacteria and algae after about 5 days and should achieve a density of ~3,000 cells/ml.
Notes:
- This procedure was developed to investigate the possible use of RNAi through bacterial feeding as a means of knocking down expression of particular genes.
- While the utility of RNAi is still being investigated, the method is effective in growing *Euplotes crassus* to higher densities than is possible using algae alone. Using moderately dense cultures of algae, we typically achieve cell densities of 500-1,000 cells/ml, while with the *E. coli* supplemented cultures we describe here, *Euplotes* cell densities exceed 3,000 cells/ml. In fact, preliminary studies mixing

a volume of a *Euplotes* culture with an equal volume of resuspended *E. coli* have produced a density of ~8,000 cells/ml.

- While the described procedure involves small cultures, it can likely be scaled up as required.
- For some species of *Euplotes*, **long-term culture** using bacteria as a food source has not been successful (C. Miceli, personal communication), suggesting that some essential nutrient is not being provided in sufficient quantities.