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

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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

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Protocol

Step 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.)

Step 2.

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

Step 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.

Step 4.

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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.

Step 5.

Incubate at room temperature with the tubes at an angle.

Step 6.

Gently invert tubes daily to disperse settled material.

Step 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.

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