


Apr 24, 2019

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

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DOI

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

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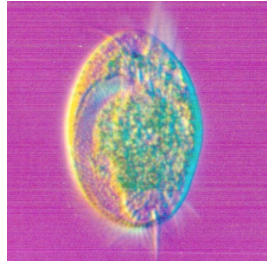
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Protocol Citation: Lawrence A. Klobutcher, Larry Klobutcher 2019. Culturing *Euplotes crassus* to high densities using a combination of algae and bacteria as the food source.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.2aggabw>

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Protocol status: Working

We use this protocol and it's working

Created: April 24, 2019

Last Modified: April 24, 2019

Protocol Integer ID: 22568

Keywords: dense cultures of algae, growing euplotes crassus, culturing euplotes crassus, euplotes cell density, bacteria as the food source, euplotes crassus to high density, using algae, euplotes crassus to higher density, using bacteria, euplotes culture, combination of algae, euplotes crassus, bacterial feeding, bacteria, species of euplote, algae, euplote, utility of rnai, possible use of rnai, cell, cell density, essential nutrient, dense culture, rnai, particular gene, small culture

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 quantities

Troubleshooting

- 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.