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Single cell isolation and monoclonal culture establishment of *Acanthamoeba castellanii* using migration on agar plates

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bc3eiyje

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

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ABSTRACT

Isolation of single *Acanthamoeba castellanii* cells from agar plates for establishment of monoclonal cultures. This protocol can be useful for isolating clones of a mixed wild-type population or of transformants. After isolation, growth can be re-established in typical Neff liquid medium.

MATERIALS TEXT

Materials needed:

- 10 cm Petri dishes
- 1% Page's Amoeba Saline agar (PAS: 1mM Na_2HPO_4, 1 mM KH_2PO_4, 0.016 mm $MgSO_4x7H_20$, 0.027 mM $CaCl_2x2H_2O$, 2 mM NaCl)
- *E. coli* K-12 in LB
- PYG medium with additives (0.75% yeast extract, 0.75% proteose peptone, 2 mM KH2PO4, 1 mM MgSO4, 1.5% glucose, 0.1 mM ferric citrate, 0.05 mM CaCl2, 1 µg/mL thiamine, 0.2 µg/mL D-biotin, and 1 ng/mL vitamin B12.)
- polymyxin B (optional)

E. coli preparation

1 Grow 24 hour culture of *E. coli* K-12 in LB

2 Pellet *E. coli* and resuspend in an equal volume of water or Page's Amoeba Saline in 15 mL Falcon tube

3 Heat-kill *E. coli* in 65 degree C water bath for 20 minutes.

Plate set-up

4 Pour PAS agar into several (3 to 5) Petri plates per 10 cells planned to isolate and let set.

5 Spread 1 mL aliquot of heat-killed *E. coli* onto each plate and continue spreading until dry.

6 Scrape the internal surface of a 7 to 14 day old *Acanthamoeba castellanii* liquid culture and gently shake the flask to suspend cells.

7 Pipette 1 microlitre of *Acanthamoeba* suspension into the centre of each plate.

Cell isolation

8 After 3 to 5 days, cells will have migrated far enough from the centre of the plate that the furthest ones may be 1 or more centimetres apart. Check this with an inverted microscope to determine when this is the case. There will be visible trails through the bacterial lawn to see where cells have migrated.

9 When cells have dispersed enough (1 cm or more apart), cut 1 cm squares out of the agar, containing only one cell each (not too difficult to see with an inverted microscope) and place one square into each well of a 12 well culture plate. You can cut squares from the middle of the plate into a few wells as positive controls.

10 Add enough PYG medium to fully cover the agar cubes (you can fully fill these wells) and allow growth at room temperature. Growth can be observed in these plates under an inverted microscope. When cells start to cover the entire area of the bottom of a well (at the same density you might see from 4-5 days growth in regular culture) you can scrape those wells and transfer the entire contents back into flasks for growth in typical culture conditions. 10 ug/ml polymyxin B can be added to control bacterial growth in case not all *E. coli* were heat killed. If you are isolating single transformed cells you can select immediately or wait a few days to allow your new monoclonal populations to establish in your flasks.