

Amoebae strains on agar (6 week)

Note: The culturing conditions below are not necessarily the optimal growth conditions for each strain, as much variation is found between strains, and cultures are not always kept in optimal growth conditions at CCAP for practical reasons. There may be more info in the individual strain data on the website.

On receipt of culture: Check microscopically to ascertain if subculturing is needed imminently.

ACDP Hazard Gp: 1 - Non pathogenic / non hazardous. Unlikely to cause human disease.

Culture Medium: The amoebae are grown on Non-Nutrient agar plates (NN – freshwater strains) or MY75S agar plates (marine strains). Some strains feed on the bacteria already within the culture, some are provided with a food source, at CCAP we are using a non-pathogenic strain of *Escherichia coli*. Check the strain data for individual strains.

Freshwater strains can alternatively be grown in NCL liquid medium in tissue culture flasks (30ml medium) with two boiled wheat grains, marine strains in NCL75S liquid medium.

Media recipes can be found on our website: www.ccap.ac.uk/index.php/media-recipes/

Lighting: not required

Light Cycle: -

Temperature: 20 degrees C

Sub Interval: 6 weeks (at CCAP, may vary depending on environment)

Culture Vessel: petri dishes.

Culture Method:

Preparation of *E. coli* plates

Using a sterile swab, the bacteria from an established culture are spread over the entire surfaces of nutrient agar plates. The plates are incubated at 20 degrees C for a week, at this temperature *E. coli* will have grown up to an adequate lawn of bacteria (if the *E. coli* is needed in a shorter time-frame it can be grown at 37 degrees C).

Subculturing

An older culture is observed using a stereoscopic microscope, and high density areas are marked. These marked areas will be used as a guide to cut blocks for transfer to the new media. New agar plates are streaked with *E. coli* (if required) using a microbiology disposable loop, and the cut agar blocks placed, amoeba-side down, on top of the bacteria so the amoebae can feed on it. To minimise drying out of the agar and reduce the possibility of contaminants entering the dish, the lid is sealed with a narrow strip of parafilm.

Use strict aseptic techniques throughout and if possible carry out all subculturing within a laminar flow cabinet (particularly important for axenic strains).