

***Rosculus* spp.**

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: These amoebae are cystformers and may have encysted whilst in transit. To encyst the culture, subculture onto fresh medium as detailed below.

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) with a food source, at CCAP we are using a non-pathogenic strain of *Escherichia coli*. For liquid cultures we use WMY 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: 4 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

Rosculus is grown in petri dishes. 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 spread with *E. coli* 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 bottle, the lid is sealed with a narrow strip of parafilm.

Watch the video: [Subculturing cyst-forming amoebae on Vimeo](#)

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