Transfected Naegleria Fluorescence Microscopy

Anastasios Tsaousis

Abstract

Citation: Anastasios Tsaousis Transfected Naegleria Fluorescence Microscopy. **protocols.io** dx.doi.org/10.17504/protocols.io.hpvb5n6
Published: 31 Jul 2017

Protocol

Step 1.

Transfected *Naegleria gruberi* was incubated in selection media for seven days at 28°C, in 2 or 4 well Permanox chamber slides (Sigma Aldrich C6682/6932).

Step 2.

For mitochondrial observation, Mitotracker Red CMXROS (ThermoFisher Scientific M7512) was added to wells containing *N. gruberi* to reach a final concentration of 200nM and incubated for a further full hour

Step 3.

Media was then aspirated from the wells and replaced with a room temperature PBS wash. The PBS wash was removed and replaced a further two times.

Step 4.

After the final PBS wash, freshly prepared paraformaldehyde at room temperature and a w/v concentration of 3% was added to the wells and incubated in fume cupboard for 15 minutes.

Step 5.

The parasformaldehyde was then removed and replaced with a further 3 rounds of PBS wash and one final wash with distilled water.

Step 6.

The chambers where then removed from the slide and dried by gently tapping against an absorbent material such as laboratory tissue paper.

Step 7.

Mounting media containing DAPI (Sigma Aldrich F6057) was then added to the slides, appropriate

cover slips mounted and sealed with nail varnish before visualisation on a fluorescence microscope.

Step 8.