Transmission electron microscopy protocol for anaerobic ciliates

Johana Rotterova[1], Ivan Čepička[1]

[1]Charles University

Nov 09, 2019

1 Works for me

dx.doi.org/10.17504/protocols.io.85uhy6w

Johana Rotterova
Charles University

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rotterová, J., Bourland, W. and Čepička, I., 2018. Tropidoatractidae fam. nov., a deep branching lineage of metopida (Armophorea, Ciliophora) found in diverse habitats and possessing prokaryotic symbionts. Protist, 169(3), pp.362-405. Beinart, R.A., Rotterová, J., Čepička, I., Gast, R.J. and Edgcomb, V.P., 2018. The genome of an endosymbiotic methanogen is very similar to those of its free-living relatives. Environmental microbiology, 20(7), pp.2538-2551.

MATERIALS

NAME	CATALOG #	VENDOR
Osmium tetroxide solution 2%	75633	Sigma Aldrich
25% Glutaraldehyde		
Acetone		
Sodium cacodylate trihydrate	View	ProSciTech
Ethanol		
Poly/Bed 812	08791-500	Polysciences

SAFETY WARNINGS

Please see Osmium Tetroxide safety data sheet here - PrintMSDSAction.pdf

Please see Sodium Cacodylate buffer safety data sheet here - https://archive.proscitech.com/?navaction=pages&page=sds&catno=c0205

Please see Glutaraldehyde safety data sheet here - 354400_SDS_ZA_EN.PDF

BEFORE STARTING

Get a well growing culture of anaerobic ciliates following protocol listed here - dx.doi.org/10.17504/protocols.io.85why7e or in Rotterová et al. 2018.

Rotterová J, Bourland W, Čepička I (2018). Tropidoatractidae fam. nov., a Deep Branching Lineage of Metopida (Armophorea, Ciliophora) Found in Diverse Habitats and Possessing Prokaryotic Symbionts.. Protist.
https://doi.org/10.1016/j.protis.2018.04.003

Fixation

1 Isolate 1 ml of thriving culture of ciliate cells, fix with 2.5% (v/v) glutaraldehyde (Polysciences) and subsequently centrifuge at 800×g for 5 min at 4 °C. 10m

2 Carefully replace supernatant with 1 ml of 2.5% (v/v) glutaraldehyde in 0.2M SCB (Sodium Cacodylate buffer, pH 7.2) and incubate 1 hour on ice. 1h 10m

3 Then, rinse the pellet carefully with SCB three times per 15 minutes. 50m

4 Postfix the cells with 1% OsO4 in distilled water for 1 hour on ice. 3h 10m

Rinsing and embedding

5 Rinse the cells with distilled water (5 minutes), dehydrate them in a graded ethanol series from 30% to absolute ethanol: through 30%, 50%, 70%, 80%, 90%, 95%, and finally three times in absolute ethanol, each per eight minutes. 1h

6 Incubate cells two times in 1:1 ethanol-acetone mixture per 15 minutes.

7 Incubate cells once in 2 ml of 100% acetone per 15 minutes, and finally substitute aceton with acetone-resin mixture (EPON resin Poly/Bed 812, Polysciences) and incubate per 6-12 hours.

8 Substitute absolute EPON resin (Poly/Bed 812, Polysciences) three times and finally embed cells in in the resin into capsules.

9 Let the embedded cells get polymerized at 70 °C for 48 hours. 48h 30m

Cutting ultrathin sections

10 Cut serial ultrathin sections on an Ultracut E ultramicrotome (Reichert) using a diamond knife and stain with lead citrate and uranyl acetate (2–3%). 3h

Protocol is adapted from Rotterova et al. (2018) and Beinart et al. (2018).

Rotterová J, Bourland W, Čepička I (2018). Tropidoatractidae fam. nov., a Deep Branching Lineage of Metopida (Armophorea, Ciliophora) Found in Diverse Habitats and Possessing Prokaryotic Symbionts.. Protist. https://doi.org/10.1016/j.protis.2018.04.003

Beinart RA, Rotterová J, Čepička I, Gast RJ, Edgcomb VP (2018). The genome of an endosymbiotic methanogen is very similar to those of its free-living relatives.. Environmental microbiology.
https://doi.org/10.1111/1462-2920.14279