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HPF-FS of *Solarion arianae* for transmission electron microscopy V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is an optimized version of the protocol for standard High Pressure Freezing followed by Freeze Substitution (HPF-FS) for transmission electron microscopy that was used for the fixation of the culture of *Solarion arianae*.



Cell harvest

- 1 Centrifuge a well-grown culture at 1500 g at 4 °C.

5m



- 1.1 Discard supernatant and collect pellet.

Cryo-immobilization by high-pressure freezing

- 2 The obtained pellet was cryo-immobilized by high-pressure freezing (Leica EM ICE) in gold-plated, 3 mm wide copper carriers with a cavity of 0.1 mm (Wohlwend GmbH).

- 3 After high-pressure freezing, the samples were transferred to precooled (-90 °C) fixative medium (2% OsO₄ in 100% acetone) and processed by automatic freeze substitution (Leica EM AFS), as follows:



- 3.1 incubation at -90 °C for 96 hours

4d



- 3.2 warm up to -20 °C (5°C/hour for 14 hours)

14h

- 3.3 incubation at -20 °C for 24 hours

1d

- 3.4 warm up to 4°C (3°C/hour for 8 hours)

8h

- 3.5 Incubation at 4°C for 18 hours

18h

- 4 After AFS, the samples were transferred to room temperature and washed with 100% acetone (3x).

- 5 After washing, infiltrate sequentially for 1h each with:



- 5.1 resin-acetone mixture 1:2



1h



5.2 resin-acetone mixture 1:1

1h



5.3 resin-acetone mixture 2:1

1h



6 Finally, the samples were infiltrated with pure resin overnight (EMbed 812)

7 Lastly, polymerize for 48 hours at 60 °C

2d



Section preparation

8 Cut sections.
For *Solarion* HPF-FS, 90nm thick sections were cut with a diamond knife on an an EM UC6 (Leica) ultramicrotome.

9 post-contrast with uranyl acetate and lead citrate.

10 Lastly, coat with a carbon layer.