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# HPF-FS of *Solarion arianae* for transmission electron microscopy

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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.