

TEM: Preparation of samples for thin sectioning (ref. 1)

Samples of *E. coli* strains containing the artificial constructs were prepared for thin sectioning to visualize the interior of the cells.

The *E. coli* strain BL21(DE3) was transformed with the constructs of interest and 20 ml cell cultures were grown at 37°C to an OD₆₀₀ of 1.5.

The cultures were split in half and protein production was induced with 1 mM IPTG at 18 ° C in one culture whereas the other culture was incubated at 37°C overnight.

The cells were harvested by centrifugation and fixed for 12~24 hours in 1.5 ml 2.5 % Glutardialdehyde in PBS. Subsequently, the cells were pelleted and washed twice with PBS to remove traces of the fixing solution.

The cells were stained for 1 hour in 1% osmium tetroxide and then washed twice with PBS before dehydration. This was accomplished by subjecting the samples to a solvent gradient: 70 % Ethanol for 20 minutes, 100 % Ethanol for 20 minutes, 100 % Ethanol for 30 minutes, twice 100 % Propylene oxide for 30 minutes.

The cells were embedded by first washing them in 50 : 50 Agar Low Viscosity Resin : propylene oxide for 60 minutes and then embedding them in 100 % Agar Low Viscosity Resin for 180 minutes constituted for a block with medium hardness.

The Samples were placed in 0.5 ml embedding tubes, centrifuged for 5 minutes at 4,000 x g to concentrate the cells to the tip and incubated at 60°C overnight to polymerise.

[1] Martin J. Warren, et al. 2010. Synthesis of empty bacterial microcompartments, directed organelle protein incorporation, and evidence of filament-associated organelle movement. *Molecular Cell*. 2010 Apr **38** (2), 305–315