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## Embedding of Yeast in Spurr resin

### FIXATION

1. Filter 10 OD units of cells (~1 x 10<sup>8</sup> cells) on a 0.45 mm Millipore filter unit without letting the cells dry.
2. Wash on the filter with 10ml of 0.1M sodium cacodylate buffer (pH 6.8).
3. Add 10ml of 3% glutaraldehyde in 0.1M sodium cacodylate buffer pH 6.8 and immediately disconnect filter unit from vacuum line. Transfer to a 15ml centrifuge tube. Continue fixation for 1 hour @ RT, then overnight @ 4°C.

### ZYMOLASE DIGESTION

1. Wash twice in 10ml of 50mM KPO<sub>4</sub> pH 7.5, each time centrifuging the cells @ 2,000 RPM @ 4°C.
2. Make a 0.25mg/ml solution of zymolase in 50mM KPO<sub>4</sub> pH 7.5. Incubate mixture @ 37°C for 15 min while shaking. Remove insoluble particles by centrifuging @ 2,000rpm for 5 min @ RT (20°C). Resuspend cells in 2ml of zymolase supernatant. Incubate tubes for 25 minutes @37°C while shaking.
3. Stop zymolase digestion by centrifuging cells @ 2,000 RPM for 5 min @ 4°C.

### POSTFIXATION

1. Wash 2 x in 5ml of ice-cold 0.1M sodium cacodylate pH 6.8, each time centrifuging them @ 2,000 RPM @ 4°C. Resuspend them in 1 ml of buffer and transfer to Eppendorf tube.
2. Pellet the cells @ 14,000 RPM for 2 min @ RT.
3. Add 0.5ml of cold 2% osmium tetroxide in 0.1M sodium cacodylate pH 6.8. Dislodge the pellet from the bottom of the tube using a needle to ensure good penetration of the osmium. Incubate for 1 hour on ice in the hood.
4. Wash 3 x 5 minutes with water.

### EN BLOC STAINING

1. Add 1ml of 2% aqueous uranyl acetate and incubate for 1 hour @ RT in the dark.
2. Wash 2 x 5 min with water.

### DEHYDRATION

1. Dehydrate in the following order: 50% ethanol 2 x 5 minutes  
70% ethanol 2 x 5 minutes  
90% ethanol 2 x 5 minutes  
100% ethanol 3 x 5 minutes
2. Wash 4 more times with 100% ethanol from a fresh bottle, 5 min each.
3. Wash with 100% acetone for 5 min.

### INFILTRATION

- 1 Add 50% acetone / 50% Spurr and incubate for 2 hours on wheel. Change to 100% Spurr and incubate overnight.
- 2 Change Spurr 2 x over the next day. Bake @ 80°C for at least 24 hours.