Routine EM Embedding for *Pichia pastoris*:
(revised 01/10/2005)

1. Fix pelleted cells in 3% glutaraldehyde, 0.1 M cacodylate buffer, 5 mM CaCl₂, and 5 mM MgCl₂ for 1 hour rotating at room temperature.

2. Wash with 0.1 M cacodylate buffer 2 times, then once with ddH₂O. Disperse/ embed the cells in 2% low temperature agarose (~1:1), cool, then cut into small blocks.

3. Post-fix blocks in 4% potassium permanganate in ddH₂O for 1 hour at room temperature. Then wash 4 times with ddH₂O.

4. Treat with 0.5% sodium meta-periodate for 15 min. at room temperature. Wash 2 times with ddH₂O.

5. Place the blocks into filtered 2% UA overnight at room temperature and in the dark.

6. Dehydrate in graded series of ethanol (50%, 70%, 95%), followed by three washes in 100% ethanol for 15 min each.

7. Quickly place block in 1:1 100%ethanol/ propylene oxide, then wash 2 times with propylene oxide.

8. Place the block in 1:1 mixture of propylene oxide/ Embed 812 resin overnight at room temperature.

9. Place the samples in 100% Embed 812 resin for ~6 hours, changing the resin 2-3 times in that time.

10. Place the samples in a flat embedding mold with fresh resin and polymerize for two days in an 80°C oven.