Using cycloheximide (CHX) to inhibit protein synthesis in S pombe

- 1. CHX stock solution (x1). 10 mg/ml in EMM (highly toxic, gloves, weigh out in fume cupboard). Make sure solution does not contain any crystals. It may be necessary to warm the solution to 60°C to dissolve completely. Solution should be freshly prepared.
- 2. Grow pombe cells in EMM to log phase. Pellet 2.5 x 10⁷ cells (=1.5 OD equivalent) and wash in EMM. Resuspend in 0.5 ml EMM containing 10 mg/ml CHX. 15 min incubation is required for CHX to penetrate cells and inhibit protein synthesis.

Note that S. pombe requires CHX concentrations x50 those used in S. cerevisiae.

Reference:

 $\frac{http://books.google.com/books?id=3KSzVtiT0jEC\&lpg=PA122\&dq=S.\%20pombe\%20requires\%20cycloheximide\%20concentrations\%2050\%20times\%20higher\%20than\%20do\%20S\&pg=PA113#v=onepage&q=S.\%20pombe\%20requires\%20cycloheximide%20concentrations\%2050\%20times\%20higher%20than\%20do\%20S\&f=false$