Thiol Disulfide exchange protocol

Extracting yeast mitochondria from the culture (Meisinger, Pfanner, & Truscott, 2006)

1. Check that samples reach an OD600nm of ~0.8

2. Centrifuge 50mL of yeast culture at 3000g for 5 mins a

3. Discard the supernatant

4. Resuspend and wash pellet with ~300mL of pure water

5. Centrifuge at 3000g for 5 mins and discard the supernatant

6. Resuspend pellet with 5mL pre-warmed DTT buffer 100 mM Tris-H2SO4, pH 9.4, 10 mM dithiothreitol (DTT), prewarmed to 30°C. Add DTT prior to use. A 1 M Tris-H2SO4 stock can be stored at room temperature.

7. Shake yeast and DTT buffer mixture slowly at 30oC for 20 mins

8. Centrifuge at 3000g for 5 mins and discard supernatant

9. Resuspend pellet in 25mL zymolyase buffer

10. Centrifuge at 3000g for 5 mins and discard supernatant

11. Resuspend pellet in 25mL zymolyase buffer

12. Shake solution slowly at 30oC for 30-45 mins

13. Centrifuge at 3000g for 5 mins and discard supernatant

14. Resuspend pellet in 25mL zymolyase buffer

15. Centrifuge at 3000g for 5 mins and discard supernatant

16. Resuspend pellet in 30mL ice-cold homogenisation buffer (0.6 M sorbitol, 10 mM Tris-HCl, pH 7.4, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.2% (w/v) bovine serum albumin (BSA; essentially fatty acid-free, Sigma-Aldrich, Taufkirchen, Germany). Add PMSF from a freshly prepared 100 mM stock in ethanol just prior to use.)

17. Using a Dounce homogeniser, homogenise the spheroplasts with ~15 strokes at 4oC

18. Use 60mL of homogenisation buffer to wash out and collect as much of the sample as possible into a falcon tube

19. Centrifuge homogenate at 1500g for 5 mins at 4oC to pellet cell debris and nuclei. Collect the supernatant

20. Centrifuge the supernatant at 4000g for 5 mins at 4oC. Collect the supernatant and discard the pellet. Collect 50uL aliquots from each sample for 1D gel and quantitation.

21. Centrifuge the supernatant at 12 000g for 15 mins at 4oC. Keep the pellet

22. Resuspend the mitochondrial pellet in SEM (50 mM sucrose, 1 mM EDTA, 10 mM MOPS-KOH, pH 7.2)

23. To store the sample, use liquid nitrogen to flash freeze and store at -80oC