Large Scale Immunoprecipitation

1. Harvest cells from 40 x 150 mm plates. Snap freeze and store in -80 ºC

2. Lyse cells in 30 mL of ice-cold lysis buffer (50 mM Tris, pH 7.5, 0.2 M NaCl, 0.5% NP-40, 1 mM DTT, 1X PIC, 10 mM NaF, 5 mM β-glycerophosphate, 10 µM cytochalasin B, 10% glycerol). Incubate on ice 15 min

3. Sonicate for 4 x 30 sec on ice and spin at 28,000 rpm with SW-28 for 45 min.

4. Filter lysate with 0.45 µm filter

5. Split lysate into two tubes

6. Incubate half supernatant with 100 µL anti-GST beads for 2 hr at 4 ºC

7. Combine anti-GST depleted supernatant with other half of supernatant. Split into half again and add 100 µL anti-MMS21 or 100 µL anti-NSE1. Incubate 2 hr at 4 ºC

8. Wash beads with 30 mL of lysis buffer once, then three times with 30 mL of wash buffer (50 mM Tris, pH 7.5, 0.4 M NaCl, 0.5% NP-40, 5 mM NaF, 2.5 mM β -glyercophosphate, 1 mM DTT), and finally once in 1 mL of lysis buffer

9. Transfer beads from last step in 1 mL of lysis buffer to eppendorf and elute with 150 µL of glycine buffer (0.1 M glycine, pH 2.5, 1 mM DTT, 1X PIC) three times

10. Spin the elute to remove the beads and neutralize with 50 µL of 1M Tris, pH 8.0.

11. Concentrate all 500 µL to ~20 µL. Remove supernatant.

12. Add hot SDS loading buffer to membrane and spin out

13. Run on one lane of 4-20% gradient SDS-PAGE gel

14. Wash gel three times with MiliQ water for 5 min each

15. Stain with Colloidal Blue overnight at 4 ºC in gel plastic tray