Detection of a ubiquitinated protein from a yeast lysate:

1. Grow cells O/N at 26°C (shaking).
2. Next day, cut back cells to .2 ODU/ml in 20 ml medium and grow at 26°C until they reach .5-1.0 ODU/ml.
3. Spin cells out for 5 min at room temp. Aspirate medium (do not decant).
4. Resuspend cells in 1 ml 5mM N-Ethylmaleimide (NEM) and transfer to an eppi tube. Prepare NEM immediately before use (7 mg NEM/ 10 ml H_20).
5. Add 100 μl 100% TCA, vortex. Ice for 30 min.
6. Spin @ full speed for 10 min at 4°C, aspirate. Keep tubes on ice throughout the protocol.
7. Add 1 ml ice-cold acetone. Sonicate (using water bath sonicator) pellets into solution.
8. Repeat Steps 6 and 7.
9. Spin @ full speed for 10 min at 4°C, aspirate.
10. Dry pellets in speed vac.
11. Add 100 μl Urea Lysis Buffer + 5 mM NEM (fresh). Sonicate into solution.
12. Add 100 μl glass beads and vortex for 10 min.
13. Heat @ 75°C for 5 min, vortex 10 min.
14. Heat @ 75°C for 5 min.
15. Add 1 ml Tween IP buffer + 5 mM NEM (fresh).
16. Mix well by Inversion.
17. Spin full speed for 15 min at 4°C.
18. Transfer 950 μl of the sup to a new eppi tube. Careful not to disturb the pellet.
19. Add 5 μl of antibody against desired ubiquitinated protein.
20. Rotate O/N at 4°C.
21. Next day, add protein A sepharose beads to lysates.
22. Rotate for 2 hrs at 4°C.
23. Spin out beads full speed for 30 sec at 4°C. Carefully aspirate sup, leaving ~100μl sup so as not to disturb beads.
24. Wash beads 1x with Tween Urea buffer, 2x with Tween IP buffer and 1x with TBS.
25. Carefully remove the final 100μl of sup.
26. Dry beads in speed vac.
27. Add 50 μl sample buffer, tap beads into solution.
28. Boil samples at 75°C for 10 minutes.
29. Load 20 μl onto gel.
30. Run gel and transfer to nitrocellulose as usual.
31. See autoclaved blot protocol.

Urea Lysis Buffer
6 M Urea
1% SDS
50 mM Tris pH 7.5
1 mM EDTA

Tween IP Buffer
50 mM Tris pH 7.5
150 mM NaCl
0.5% Tween-20
0.1 mM EDTA

Tween Urea Buffer
100 mM Tris pH 7.5
200 mM NaCl
2 M Urea
0.5% Tween-20