

Immunofluorescence Analysis of Yeast Proteins

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[Abstract] Many important regulatory proteins such as transcription factors are regulated through subcellular localization. Protein localization can be examined by fusing a GFP tag. However, GFP is relatively big in size, and potentially may affect correct protein localization. Several small tags have been developed, such as myc, HA or Flag. By using immunostain and fluorescence microscopy as described in this protocol, one can easily probe the regulation of a selected yeast protein with the application of the aforementioned small tags.

Materials and Reagents

1. Yeast cells
2. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P8416)
3. Potassium phosphate dibasic ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$) (Sigma-Aldrich, catalog number: P9666)
4. Sorbitol ($\text{C}_6\text{H}_{14}\text{O}_6$) (Sigma-Aldrich, catalog number: S1876)
5. BSA (Albumin from bovine serum) (Sigma-Aldrich, catalog number: A4503)
6. Potassium chloride (KCl) (Thermo Fisher Scientific, catalog number: BP366-1)
7. 37% formaldehyde solution (Thermo Fisher Scientific, catalog number: F75P1GAL)
8. Zymolyase (USB, catalog number: Z1001)
9. Vectorshield
10. Poly-L-lysine
11. DAPI
12. Cytoseal 60
13. Phosphate buffer (see Recipes)
14. Sorbitol buffer (see Recipes)
15. Blocking buffer (see Recipes)

Equipment

1. Centrifuges
2. Shaker

3. Conical tube
4. Fluorescence microscope
5. 15 ml conical tube
6. Light microscope
7. Heat blot

Procedure

1. Inoculate yeast cells overnight in a 30 °C shaker.
2. Subculture cells at $OD_{600}=0.1$ in 5 ml YPD or defined media.
3. Continue to shake at 30 °C for 4-6 h to a concentration of $1-5 \times 10^7$.
4. Add 0.6 ml of 37% formaldehyde solution directly to the cells and continue to incubate with shaking for 90 min (to be exact) at the same temperature as growth.
5. Transfer cells to a 15 ml conical tube and pellet by centrifugation at $660 \times g$ for 3 min at 4 °C.
6. Aspirate supernatant and wash cells with ice-cold 5 ml phosphate buffer. Be gentle at this step because yeast cells are very fragile after fixation by formaldehyde.
7. Repellet cells and wash with ice-cold 5 ml sorbitol buffer.
8. Pellet cells and aspirate supernatant. Resuspend in ~1 ml sorbitol buffer.
9. Pre-warm the cells at 30 °C for 5 min.
10. Add 25 μ l of zymolyase, mix gently and incubate on 30 °C heat blot for 15-30 min.
11. Check the digestion under a light microscope (fully digested cells are gray while undigested cells are bright).
12. When 80% of cells are digested, Pellet cells $660 \times g$ for 3 min at 4 °C. Re-suspend cells gently in 0.5 ml ice-cold sorbitol buffer.
13. Repeat step 12.
14. Prepare slides by coating them with Poly-L-lysine for 10 min at room temperature (RT).
15. Wash slides 5 times with water and let them dry at RT.
16. Place 20 μ l of cell suspension into each well on the slide. Incubate in a wet chamber for at least 10 min.
17. Immediately immerse slide in ice-cold methanol for 6-7 min.
18. Remove and immerse immediately in ice-cold acetone for 30 sec.
19. Dry the slides on 30 °C heat blot.
20. From this step on, don't let the wells dry. Wash wells several times with blocking buffer and incubate a moist chamber for at least 10 min.

Note: Longer incubation time may give better results, for example, incubate in blocking buffer at 4 °C overnight.

21. Remove supernatant and add 20 μ l of primary antibody (diluted in blocking buffer). Incubate in a moist chamber for at least 2 h.
Note: Can put the slides at 4 °C overnight.
22. Aspirate excess solution and wash 5x with blocking buffer.
23. Add 20 μ l of fluorescence conjugated-secondary antibody (diluted in blocking buffer).
24. Place in a dark/moist chamber for ~1 h. Remember to keep slides in the dark as much as possible to prevent bleaching of fluorescence.
25. Aspirate excess solution and wash 5x with blocking buffer.
26. Then, wash once with 1x PBS.
27. Dilute DAPI (1:1,000) in 1x PBS.
28. Add DAPI solution to wells for 2 min.
29. Aspirate excess solution and wash once with 1x PBS. Do not allow the slide to dry and add 2 μ l Vectorshield to the well and immediately cover with cover slide.
30. Seal the edge of the slides with Cytoseal 60 and examine under a fluorescence microscope.

Recipes

1. Phosphate buffer
Make 0.1 M KH_2PO_4 in H_2O
Make 0.1 M K_2HPO_4 in H_2O
Add K_2HPO_4 solution to KH_2PO_4 solution to bring the pH to 6.5.
2. Sorbitol buffer
0.1 M potassium phosphate buffer (pH 6.5)
1.2 M sorbitol
3. Blocking buffer
5 % BSA in 1x PBS (pH 8.0)

Acknowledgments

This protocol was adapted from and used in Wei and Zheng (2009) and Wei *et al.* (2009).

References

1. Wei, Y. and Zheng, X. F. (2009). [*Sch9* partially mediates TORC1 signaling to control ribosomal RNA synthesis.](#) *Cell Cycle* 8(24): 4085-4090.

2. Wei, Y., Tsang, C. K. and Zheng, X. F. (2009). [Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1](#). *EMBO J* 28(15): 2220-2230.