Affinity Purification of Yeast Protein-interacting Metabolites for ESI-MS Analysis

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[Abstract] The method described here can be used to discover *in vivo* protein-metabolite interactions. Metabolite-protein complexes are purified from yeast cell lysates by an affinity tag that recognizes the protein of interest. The protein-bound metabolites are extracted for identification by mass spectrometry, while the protein is concurrently analyzed by gel electrophoresis. A parallel experiment using cell lysate without target protein should be used as a negative control. The metabolite extract should be analyzed within 1-2 days to avoid undesired chemical reaction.

Materials and Reagents

1. Cells
2. 1x phosphate buffered saline (PBS)
3. Methanol (mass spec grade)
4. Water (mass spec grade)
5. 2x laemmli buffer (for SDS-page)
6. NH_4Ac
7. EGTA
8. DTT
9. PMSF
10. Roche protease inhibitor tablets (Roche Diagnostics)
11. Lysis buffer (see Recipes)
12. Wash buffer 1 (see Recipes)
13. Wash buffer 2 (see Recipes)

Equipment

1. Zirconia silica beads (Bio Spec Products)
2. Rabbit IgG-conjugated dynabeads
3. Eppendorf protein Lobind tubes
4. FastPrep cell lyser with an adapter for 2 ml tubes

5. Hula mixer (Life Technologies, Invitrogen™) or similar product
6. Magnetic stand for 1.5/2.0 ml tubes
7. Heat block

Procedure

1. Add equal volume of 0.5 mm Zirconia silica beads (stored at -20 °C) to cells from 150 ml culture, add in 950 µl lysis buffer, homogenate on FastPrep 24, 3 x 40 sec min at 6.5 m/sec with 2 min interval on ice.
   Note: Wash the IgG Dynabeads 2x in 1x PBS, 3x in lysis buffer. Re-suspend in lysis buffer. Use 50 µl per sample.
2. Spin down lysate at 14,000 rpm, 10 min, and transfer supernatant (lysate) to 2.0 ml Lobind tubes. Store at 4 °C.
3. Add 950 µl lysis to the cell pellet and lyse again as step 1.
4. Repeat step 2 and combine the lysate. Add 50 µl IgG beads.
5. Incubate 30 min at 4 °C with end-over-end invertion on Hula mixer.
6. Use magnetic stand to separate beads from lysate.
7. Wash the beads in 0.8 ml wash buffer 1, and 0.8 ml in wash buffer 2. Transfer beads with wash buffer 2 to a new tube. Each time buffer is added to the beads, invert at 4 °C until homogenate, briefly spin down the beads (10 sec), put on magnetic stand for at least 30 sec, and pipet off the buffer.
8. Add 50 µl methanol (MS grade) to the beads, pipette mix, 15 min at room temperature, and separate on magnetic beads, repeat and combine the methanol extracts in MS vials.
9. Add 30 µl 2x SDS sample buffer to the beads, boil 15 min, load 15 µl on SDS-page for protein yield evaluation.

Recipes

1. Lysis buffer
   200 mM NH_4Ac (stock: 5 M)
   1 mM EGTA (stock: 500 mM EGTA)
   1 mM DTT (stock: 1 M)
   1 mM PMSF (stock: 100 mM in ethanol, 4 °C)
   Roche Protease inhibitor tablets (1x), if no EDTA, add to final 1 mM
2. Wash buffer 1
   500 mM NH_4Ac (stock: 5 M) ligation reaction
3. Wash buffer 2

50 mM NH_4Ac (stock: 5 M)

References

1. Li, X., Gianoulis, T. A., Yip, K. Y., Gerstein, M. and Snyder, M. (2010). Extensive *in vivo* metabolite-protein interactions revealed by large-scale systematic analyses. *Cell* 143(4): 639-650.