Co-immunoprecipitation in Yeast

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[Abstract] This protocol describes investigation of protein-protein interactions in baker yeast by co-immunoprecipitation (CoIP). CoIP is a technique to identify physiologically relevant protein-protein interactions in the cell. The interesting protein can be isolated out of solution using antibody that specifically binds to that particular protein (antigene protein). The partner proteins that are bound to a specific target protein can be co-immunoprecipitated together with an antigen. These protein complexes can then be analyzed to identify new binding partners, binding affinities, the kinetics of binding and the function of the target protein. Here I describe the protocols that allow to immunoprecipitate different protein complexes, for example NAC complex (Panasenko *et al*., 2009), Ccr4-Not complex (Panasenko and Collort, 2011), ribosomes (Panasenko and Collort, 2012) and investigate their partners. For each CoIP I used the different lysis buffer, as indicated below in recipes.

Materials and Reagents

1. Glass beads for cells breaking 0.5 mm (Bio Spec Products, catalog number: 110/9105)
2. Cycloheximide (CHX) (Sigma-Aldrich, catalog number: C7698) solution 100 mg/ml prepared on ethanol.
3. Bradford reactive (Bio-Rad Laboratories, catalog number: 500-0006).
4. Different types of the beads can be used depending on the particular antigene protein and antibodies.
   a. Protein G magnetic Dynabeads (Life Technologies, Invitrogen™, catalog number: 100.04D) or Protein A magnetic Dynabeads (Life Technologies, Invitrogen™, catalog number: 100.02D)
   b. Protein G Sepharose (Amersham biosciences, catalog number:17-0618-01) or Protein A Sepharose (Amersham biosciences, catalog number: 17-0780-01)
5. Magnet, in case of using magnetic beads (Life Technologies, Invitrogen™, catalog number: 123.21D)
6. Antibodies
   For example Peroxidise-anti-peroxidase soluble complex (PAP-antibodies) were bought

from Sigma-Aldrich (catalog number: P1291). Antibodies against HA (HA.11) (Clone 16B12, catalog number: MMS-101R) and Myc [c-myc (9E10), catalog number: MMS-150R] were bought from Covance.

7. Protease inhibitor cocktail (F. Hoffmann-La Roche, catalog number: 13560400)
8. Phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich, catalog number: P7625) 100 mM solution prepared on isopropanol
9. Laemmli sample buffer
10. Lysis buffer for protein complexes IP (see Recipes)
11. Lysis buffer for ribosome IP (see Recipes)

Equipment

1. Table Centrifuges
2. Glass bead beater Genie Disruptor (Scientific Industries, catalog number：SI-DD38)

Procedure

A. Nascent associated complex (NAC) and Ccr4-Not complex IP

1. Yeast cultures were grown on YPD or selective media. In the morning dilute night culture till OD_{600} = 0.15. Grow 100 ml of culture at 30 °C until OD_{600} = 0.6-0.8.
2. Centrifuge the cells for 5 min at 4，000 *x g*. Wash the cells with 1 ml of sterile H_2O and transfer to the Eppendorf tube. Cells can be stored at -20 °C.
3. Work on ice! Resuspend the pellet in 0.5 ml of lysis buffer, add 0.5 ml of glass beads and disrupt 15 min on the glass bead beater at 4 °C.
4. Transfer the liquid phase into the new tube. Wash the beads with 0.5 ml of lysis buffer. Combine all liquid phases.
5. To avoid the contamination with cells derby preclean the total extracts. For this transfer the liquid phase into the new tube. Centrifuge 1 min 16,000 *x g* at 4 °C.
6. Transfer the supernatant into the new Eppendorf tube and centrifuge 20 min 16,000 *x g* at 4 °C.
7. Measure the total protein concentrations in the supernatants [= TE (Total Extracts)]. For this dilute the total extracts 1: 20 and load 5 and 10 µl to the Bradford sample (0.8 ml of water and 0.2 ml of Bradford reactive).
8. Use Protein-A or Protein-G beads 20 µl of 100% beads per reaction. Wash the beads for 20 sec 2 times with 1 ml of water and 1 time with 1 ml of lysis buffer, after each washing spin the beads at 800 *x g* for 1 min. Resuspend till 20% in lysis buffer. Take 100 µl of this suspension per reaction.

9. Mix 0.4 ml of the total extracts containing 2 mg of total protein (5 mg/ml) with 100 µl of beads suspension and 1-2 µl of antibodies.
10. Incubate from 4 h till over night at 4 °C with mild rotation.
11. Wash the beads for 20 sec with 1 ml of lysis buffer 3 times, after each washing spin the beads at 800 *x g* for 1 min.
12. Add 50 µl of Laemmli sample buffer 2x to the beads and incubate 10 min at 65 °C.
13. Analyze the CoIP by SDS-PAGE and western blot. Load the total extracts (30 µg) and immunoprecipitated fraction (20 µl) on the gel.

    Notes: For the control there are 2 possibilities.

    a. *Use the total extracts from the strains lacking the immunoprecipitated antigen.*

    b. *Use the same total extract that you use for IP but do not add the antibodies.*

Ribosome IP

B. During the cyclic process of translation, a small (40S) and large (60S) ribosomal subunit associate with mRNA to form an 80S complex (monosome). This ribosome moves along the mRNA during translation. Further ribosomes can initiate translation on the same mRNA to form polysomes. It is possible to immunoprecipitate separate ribosome particles (40S or 60S) or polysomes. To disrupt the polysomes, 25 mM of EDTA is added to the buffers. To maintain the polysomes, 0.1 mg/ml of cycloheximide (CHX) is added to the culture before collection and to all the solution.

1. Yeast cultures were grown on YPD or selective media. In the morning dilute night culture till OD_{600} = 0.15. Grow 100 ml of culture at 30 °C until OD_{600} = 0.6-0.8.
2. To keep the polysomes add cycloheximide (CHX) till final concentration 0.1 mg/ml and incubate 10 min on ice. To disrupt the polysomes avoid this step.
3. To keep the polysomes spin the cells for 5 min at 4,000 *x g* and wash with 50 ml of cold water containing 0.1 mg/ml of CHX. To disrupt the polysomes wash with 50 ml of cold water without CHX.
4. Resuspend the pellets in 1 ml of lysis buffer containing 0.1 mg/ml of CHX (in case of polysomes), spin. Cells can be frozen and stored at -20 °C.
5. Transfer the liquid phase into the new tube. Wash the beads with 0.5 ml of lysis buffer. Combine all liquid phases.
6. To avoid the contamination with cells derby preclean the total extracts. For this transfer the liquid phase into the new tube. Centrifuge 1 min 16,000 *x g* at 4 °C.
7. Transfer the supernatant into the new Eppendorf tube and centrifuge 20 min 16,000 *x g* at 4 °C.

8. Measure the total protein concentrations in the supernatants [= TE (total extracts)]. For this dilute the total extracts 1: 20 and load 5 and 10 µl to the Bradford sample (0.8 ml of water and 0.2 ml of Bradford reactive).
9. Use Protein-A or Protein-G beads 20 µl of 100% beads per reaction. Wash the beads for 20 sec 2 times with 1 ml of water and 1 time with 1 ml of lysis buffer, after each washing spin the beads at 800 *x g* for 1 min. Take 100 µl of this suspension per reaction.
10. Mix 0.4 ml of the total extracts containing 2 mg of total protein (5 mg/ml) with 100 µl of beads suspension and 1-2 µl of antibodies.
11. Incubate from 2-4 h at 4 °C with mild rotation.
12. Wash the beads for 20 sec with 1 ml of lysis buffer, containing 0.5 % of Triton X-100, 3 times. After each washing spin the beads at 800 *x g* for 1 min.
13. Add 50 µl of Laemmli sample buffer 2x to the beads and incubate 10 min at 65 °C.
14. Analyze the CoIP by SDS-PAGE and western blot. Load the total extracts (30 µg) and immunoprecipitated fraction (20 µl) on the gel.
    Notes: For the control there are 2 possibilities.
    a. *Use the total extracts from the strains lacking the immunoprecipitated antigen.*
    b. *Use the same total extract that you use for IP but do not add the antibodies.*

Recipes

1. Lysis buffer for protein complexes (for example NAC or Ccr4-Not complex) IP (Panasenko *et al.*, 2009; Panasenko and Collort, 2011)
   Usually we use 2 types of the buffers. In both of them protein complexes can be efficiently immunoprecipitated.
   Lysis buffer 1 (Panasenko *et al.*, 2009)
   40 mM HEPES-KOH (pH 7.5)
   100 mM KCl
   150 mM K-acetate
   1 mM EDTA (pH 8.0)
   20% glycerol
   Lysis buffer 2 (Panasenko and Collort, 2011)
   50 mM Tris-HCl (pH 8.0)
   100 mM NaCl
   1 mM EDTA
   5 mM $MgCl_2$
   1 mM DTT

10% glycerol

0.5 mM PMSF

2. Lysis buffer for ribosome IP [Panasenko and Collort, 2012]

   10 mM Tris-HCl (pH 7.5)

   100 mM NaCl

   30 mM $MgCl_2$

   0.1 % Triton X-100

   1 mM PMSF

   Note: All solutions contain 0.1 mg/ml of CHX, in case of keeping polysomes, or 25 mM of EDTA, in case of polysomes disruption.

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