Worm Lysates Immunoprecipitation Protocol
(RCC/Meyer lab)

All steps performed at 4 °C.

1. Thaw embryo lysates, total of 3 mg per IP.
2. Pre-clear lysate against IgGsorb for 30 minutes on a nutator. Use 100 µL packed IgGsorb beads per mL of lysates.
3. Spin in a microfuge for 2 min at ~ 2,000 x g to pellet the IgGsorb.
4. Transfer the supernatant to a new microfuge tube and spin in a microcentrifuge at top speed for 10 min. Use the supernatant for IP.
5. Add approximately 5 µg of affinity purified antibodies to 3 mg total protein. Add enough ChIP buffer, if needed, to make final protein concentration 5 mg/mL. (For a typical IP 600 uL final volume.
6. Nutate for 2 hrs.
7. Pellet non-specific precipitates by spinning in a microcentrifuge at top speed (approximately 16,00 x g) for 10 min.
8. Remove the supernatant and transfer to a new microfuge tube with 25 µL of Protein A Sepharose (packed beads). Nutate for 30 min.
9. Pellet the antibody-antigen complexes captured on the Protein A Sepharose beads by spinning in a microcentrifuge for 2 min. at ~ 2,000 x g. Remove the supernatant.
10. Wash the beads by adding 1 mL of ChIP buffer and spinning in a microcentrifuge for 2 min. at ~2,000 x g. Remove the wash buffer. Repeat this step for a total of four washes.
11. Elute by boiling the beads with 1 x SDS sample buffer and loading directly onto an SDS-PAGE gel.
    Alternatively, incubate the beads with 200 µL of 0.1 M glycine (pH 3.0) at room temp. Mix a few seconds, then pellet beads as described above. Remove and save the supernatant. Repeat elution one more time. Precipitate the eluate with TCA.
    Third alternative, if the antibody used was raised against a peptide antigen, you can use 0.4 mg/mL peptide solution to elute native protein complexes.

Materials

IgGsorb, The Enzyme Center/Charm Sciences #IgSL10x10. Reconstitute as directed on label. Stored as 10% cell suspension at 4°C. (Need 1 mL for 100 uL packed pellet.)

Protein A Sepharose CL-4B, Amersham #17-0780-01, 1.5 g dry powder.
Before use, swell a small amount in water (beads expand a lot), wash 2x in PBS, spin between washes at 2000g. Store as 50% suspension in PBS/azide at 4°C.

ChIP buffer
50 mM HEPES-KOH, pH 7.6
1 mM EDTA
140 mM KCl
0.05% NP-40, store at 4°C