CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

Steps

Starting from egg-plates

1. If you want egg-laying, young worms, probably it's best to start harvesting about 29-30 hours post-plating, with incubation at 25°C.
2. Add about 20ml water or M9 on top of each 14cm plate and wash off all the worms. A second wash doesn't harm. From here on, try to keep everything on ice, to stop the development.

Collect the worms

1. Collect the worms in 50ml conicals. Pellet them in the clinical centrifuge: 800rpm (about 100xg) for 1 min, stop brake when about 150rpm (alternatively, you can set the Heraeus clinical to disable the brake once at 100 or 200rpm). Remove the supe. Distribute the worms evenly between conicals. Pool them together if you want. Try to end up with 1-2 conicals. Repeat 2x.

Sucrose float the worms

1. after last wash add some M9 or water on top of the pellet, such that you are about 20-25ml total volume.
2. Add an equal volume of ice-cold 60% Sucrose on top of this.
3. Centrifuge 5' at 500rpm (50xg) and then (without stopping!) 5' at 2400 rpm (1000xg). Collect the upper layer with a wide-bore pipette.
4. Wash the worms at least 2x with H-100+10% Glycerol.
5. Start the ultracentrifuge cooling system (2-4°C).

Worm lysis

1. after the last wash, add a pellet volume of H-100+10% Glycerol+0.05% NP-40. [if you want, you can supplement with NP-40 up to 0.1% final concentration]
2. add 1 tablet of Complete (EDTA-free) PI inhibitors (Roche) to 5ml of worm/lysis buffer [it will solve within first 10sec of sonication]
3. Probe sonicate on ice-water bath: on the Branson sonicator, small probe, 60% output, 4-6 cycles of 15" sonication + 55" pause.
4. Check evolution by taking a small sample with a cut-off yellow tip and observing under 10x mag on a DIC microscope.**Save a CRUDE sample**

Centrifugation

1. Transfer the crude extract in an ultracentrifuge tube. Spin at 16,500xg for 15min. Set DECEL to 5 (5000rpm to stop in 3 min).**Remove a LSS sample**
2. Transfer the supernatant in a new tube and spin 20-30' at 135,000xg. **Remove a HSS sample.**

IP

1. Split the HSS in two samples: IP and Control. Try to perform the next steps in Eppendorf tubes.
2. Add about 5µg antibody (or random IgG) per 5-10 mg total protein. Incubate 1h in the cold room, best on the wheel.
3. In the meantime wash the Protein-A beads with H-100+Gly+NP-40 about 4-5 times. Equilibrate them in Lysis buffer. You will need to start with at least 11µl beads per 5µg antibody.
4. After the 1h incubation, add the beads to the HSS-antibody mixture. Incubate 1h in the cold room (4°C) on the wheel.
5. Gently pellet the beads (or even let them settle in 2-3min) and wash them with H-100+Gly+NP (no protease inhibitors). Repeat with prayer and fasting (5-8 times).

Elution

1. After last wash, centrifuge the beads hard.
2. Remove as much supernatant as you can.
3. Add 0.1M Glycine pH 2.3. [Keep volume as small as you can, but reasonably big; 10-50µl should do; take in account that i) you will have to add sample buffer and ii) you want the sample to fit the well].
4. Incubate 2-3min (eventually with gentle shaking).
5. Spin down hard, remove as much supernatant as you can. Note down the final volume.[alternatively, you could use an empty spin column, put the slurry in there and recover the flow-through]
6. Add 6x loading buffer w/DTT. If the sample turns yellow or brownish try blowing some NH4OH vapors over the sample with a P1000, until it turns back blue. Boil the sample and load on gel. Treat the beads similarly if you are not sure the elution was effective.

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Solutions & Reagents

1. 60% Sucrose, ice-cold (about 100ml)
2. Affiprep Protein-A beads (Bio-Rad, Cat# 156-0006)
3. H-100: 50 mM HEPES, pH 7.4, 1 mM EGTA, 100 mM KCl, 1 mM MgCl2
4. H-100+10% Glycerol
5. H-100+10% Glycerol+0.05% NP-40
6. Lysis buffer: H-100+10% Glycerol+0.05% NP-40; just before use add 1tablet of Complete/5ml
7. 0.1M Glycine, pH 2.3
   NH_4OH (29%, normal commercial solution)