Protocol : Co-immunoprecipitation (Co-IP)

Material :

60 N2 plates (not starved)
M9

Lysis buffer (for 1 Co-IP : 20ml)

2.5ml	25 mM HEPES NaOH pH7.4	5ml of 100mM
0.3ml	150 mM NaCl (5M)	3ml of 1M
10µl	1mM DTT (1M)	200µl of 100mM
0.25ml	0.5% Triton X-100 (20%)	1ml of 10%
20µl	1mM EDTA NaOH (0.5M)	40µl of 0.5M
1	protease inhibitor tablet	2
+ water → 10ml		→ 20ml

Wash buffer :

10ml	lysis buffer
100µl	NaCl 5M

G sepharose beads
TCA + NaOH 5M
Cold Acetone (-20°C)
10% deoxycholate Na solution
2x Sample Buffer

Protocol :

First day :

- Prepare the lysis buffer (fresh solutions) and keep it on ice. Add protease inhibitor tablet just before use: Mini-complete tablets have a half-life of only 30min !!!
- Wash the plates with M9 and collect the mixture into 2 Falcons 15ml (minimum 30 min for 60 plates)
- Centrifuge 2000 rpm during 2 min
- Wash 3x with M9 (with ± 5-10ml M9). Centrifuge, discard supernatant (s/n) …
- After the last wash, let incubate the worms into the M9 during 30 min at RT
- Centrifuge 2000 rpm during 2 min and discard the s/n
- Rinse the worms with 1 ml H_2O and transfer into ED (use 2µl ED, more place). 100-150µl of worms are needed / tube. With 60 plates → 2 ED

- Centrifuge 2000 rpm during 2 min and discard the maximum of s/n
- From this step : Always ON ICE !!!
- Add 800µl of lysis buffer to worm pellet and put on ice
- Prepare the Polytron with the 5mm tige
- Homogenize 30'000 rpm during 15 sec
- Put 1 min on ice and repeat 5x the homogenization. It is important that the solution do not heat !!!
- Centrifuge the carcasses 14'000 rpm during 15 min at 4°C (there is many worms in the pellet → normal).Remove the s/n carefully and put in a new ED. Re-centrifuge 5 min to be sure that there is no more carcasses (or do pre-cleared with beads)
- Put the s/n together (2x800 = 1,6 ml) to homogenize them
- Divide the s/n in 3 parts (if there is more, distribute the rest into the tube)

  - 500µl for **Total extract** : keep at 4°C for TCA precipitation (the next day)

  - 500µl for **control-IP** (with IgG or mock or both)

  - 500µl for the **IP**

- Calculate the amount of antibodies (AB) to obtain **1µg** of AB
- Add 1µg of IgG / preimmune (2.5µl IgG Santa Cruz) into the **control-IP** and 1µg of AB for the **IP**. Rotate the ED at 4°C during min. 1 hour on the wheel
- During this time, prepare the G sepharose beads

  - Take 150µl protein G sepharose beads (50%) (need 50µl per co-ip, beads are expensive !!!)

  - Add 600µl of lysis buffer

  - Centrifuge 30'' 2000 rpm, rotate the ED for 180° and centrifuge 30'' 2000 rpm. Remove the s/n

  - Repeat the wash 3 x

  - After the last wash, remove the s/n and estimate the volume of the beads

  - To obtain a 50% slurry solution of beads, add the same volume of lysis buffer. Keep on ice

- After minimum 1 hour, add 50µl of 50% beads to each ED containing the AB and lysate. Rotate O/N at 4°C

- Add the NaCl into the lysis buffer to obtain the wash buffer. Keep at 4°C for the next day. Prepare the gel and keep at 4°C

Second day :

- If the gels are not made, do it now. Work at 4°C. Centrifuge the control-IP and the IP 2000 rpm, 30'', rotate 180°C, 2000 rpm 30'' at 4°C

- Remove the s/n and transfer it into new ED. Keep the beads on ice

- Add TCA 100% to total extract, s/n control-IP and s/n IP to obtain a conc of 20%. Keep on ice during 1 hour.

- Continue with the beads : Wash step : add 500µl of wash buffer, let incubate 5 min on the wheel at 4°C

- Centrifuge 2000 rpm, 2 x 30'' (rotation between), discard the s/n. Repeat the wash once again

- In a separated falcon, prepare wash buffer + 0.1% SDS and 0.1% deoxycholate (need 500µl pro tube). Wash with this solution, centrifuge and discard the s/n

- Wash once again with normal wash buffer. After centrifugation, discard the maximum of of s/n.

- Add 30µl of 2x SB, boil 5 min at 95°C and keep on ice (till loading the gel possible to keep at –20°C)

- Continue with the TCA precipitation : Spin the ED at max speed at 4°C for 15 min. Carefully remove the supernatant.

- Add ± 300 µl cold acetone (do not resuspend the pellet) and spin 5 min at 4°C.

- Remove the s/n and dry the pellet. Resuspend samples in 2x SB. The solution can become yellow. Add 1µl NaOH 5M to obtain a blue solution (add 1µl more if it's still yellow). Boil 5 min at 95°C.

- Perform a normal SDS-PAGE migration (!!! gel concentration !!!) with 20-25µl of sample. (It is better to load TCA extract on another gel because it migrates most of time not vertical)

- Perform a normal Western Blot and detect with the right AB