IP and MS

This is the protocol for 6L of culture at ≈2 OD_{600} and low expressed protein

Everything (Buffers, tubes, incubations…) at 4ºC and washes in the cold room

Buffers and reagents

100mL UBA Buffer (at 4ºC)

60mL	H_2O
2.5mL	HEPES 1M pH7,5
15mL	KoAc 1M
1mL	$MgCl_2$ 1M
0,5mL	$CaCl_2$ 1M
1mL	NP40%
20mL	Glycerol

Magnetic Beads+Ab. For each sample:

1. Wash 200 ul Dynabeads Prot G (Invitrogen, 10009D) with 1.8mL PBS-T0,1% for 4min in the nutator at 4ºC
2. Wash 2 times more
3. Resuspend in 200ul of PBS-T0,1% and add the Antibody
   i.e. 25ul anti-Flag M2 mouse, Sigma F1804
   i.e. 15ul anti-HA rabbit, Sigma H6908
4. Incubate **4-6h** or **O/N** in the wheel
5. Wash 2 times with 1.8mL PBS-T0,1% for 4min in the nutator at 4ºC
6. Wash 2 times with 1.8mL UBA buffer for 4min in the nutator at 4ºC
7. Resuspend in 200ul UBA buffer

When I beads are concentrated (first, last step) is better to use commercial tips (you loose less, less binding to the wall of the tip)

Magnetic Beads.

Used for cleaning step. For each sample:

1. Wash 100 ul Dynabeads Prot G (Invitrogen, 10009D) with 900ul UBA buffer for 4min in the nutator at 4ºC
2. Wash 2 times more
3. Resuspend in 100ul of UBA buffer

When I beads are concentrated (first, last step) is better to use commercial tips (you loose less, less binding to the wall of the tip)

50mM NH_4HCO_3

Add 0.40 g of NH4HCO3 to 100 mL of H_2O.
Keep it at 4ºC

Benzonase and antibodies

Obtaining the lysates for the IP (5-6h)

1. Defrost the sample.
   It takes 1-2h at 4ºC. When it starts melting:
   - Add 1mL UBA Buffer + 1 pill of cOmplete Mini, EDTA-free PI Cocktail
   - Put together the powder for each strain
   - Add UBA Buffer (not much!) and resuspend it (vortex+wheel at 4ºC)
   - Add UBA Buffer up to 32,5mL
2. Add 7,5ul Benzonase (MPI homemade, 3 times more active than Merck one)
3. 1h in the wheel at 4ºC

Cool down centrifuges, rotors and tubes

4. Add UBA buffer up to 50mL and centrifuge max speed for 15min at 4ºC
5. Transfer "SN" to ultracentrifuge cold tubes
6. Ultracentrifugate 40K for 45min at 4ºC with Tfi45.Important to:
   - Use right tubes per your volume, to avoid tube collapse!
   - Weight and adjust volume with UBA buffer to have tubes properly balanced
   - Check that tubes and rotor have all the rubber rings
7. Transfer the supernatant into a new tube with a Pasteur Glass Pipette
8. Add UBA Buffer up to 45mL. *You can keep at -80ºC*

Immunoprecipitation

Samples go to MS, try to avoid keratin contaminants (always gloves!!!!)

9. Add 90ul of washed magnetic beads/sample
10. Incubate 20-30min in the wheel at 4ºC

    ➔ *good moment to wash the magnetic beads for control and the beads+Ab*

11. Put the tubes in the magnetic rack for 1min and transfer SN in a new tube
12. Take some 50-100ul as input
13. Add the washed beads+Ab
14. Nutate O/N at 4ºC (3h could be also possible)
15. Put the tubes in the magnetic rack for 1min and discard SN
16. Collect beads with UBA Buffer and transfer them into a 1,5mL tube
17. Wash for 4min in the nutator at 4ºC
18. Wash with 500ul UBA Buffer for 4min in the nutator at 4ºC
19. Wash 2 times with 500ul 50mM NH_4HCO_3
20. Resuspend with 500ul 50mM NH_4HCO_3, transfer into a new tube and wash
    You could take small aliquot (10-50ul) to run it and do a silver staining
21. Wash with 500ul 50mM NH_4HCO_3
    We are cleaning detergents, Beads will attach to the tube wall. That's good.
22. Discard SN. Keep the beads at 4ºC for an O/N or at -20ºC. Bring it to the Zfp