IMMUNOPRECIPITATION (IP)

1. Grow cells up to OD600=1.0 (for haploid 1n strain), or =2.0 (for 2n strain) (log-phase). Pellet the cells at 3.500 rpm (Beckman). Resuspend cells in 10 ml of **IP-A buffer**, and spin one more time. Discard supernatant (SN) and freeze cells in liquid nitrogen. Store cell pellet at –80°C.

2. Resuspend 100 or more OD600 cells in 800 ul of **IP-A buffer+1mM PMSF** and transfer in a plastic tube with screw cap with 300 µl glass beads.

3. Disrupt cells with glass beads in FastPrep at setting "4" 4-5 times for 45 sec [60 to 90% cells broken). Put on ice for 5 min after first two cycles of disrtuption. After final disruption put on ice for 5 min and then centrifuge to clear the cell lysate at 13.000 for 10 min in Eppendorf Microcentrifuge in the cold room.

4. Transfer the cleared protein extract in fresh plastic tube and add the equal volume of **IP-B buffer**.

5. Add 0.2-0.3 ug of affinity-purified antibodies. Incubate with rocking at +4°C for 2-3 hours.

6. Add 30-40 ul of 50% (15-20 ul bed volume) suspension of ProteinG-Sepharose (prewashed 3 times with 1 ml of cold **IP-C buffer**) and rock at +4°C for 1 hour.

7. Spin down Protein G-Sepharose beads at 1800-2000 rpm for 5 minutes. Resuspend the beads in **IP-C buffer** and transfer to the fresh Eppendorf tube. Perform all steps on ice!

8. Wash the beads 4 times with 1 ml of **IP-C buffer** (spin down 2 min at 2000 rpm, discard SN, resuspend beads in 1 ml of **IP-C buffer** and repeat this cycle).

9. Resuspend beads with precipitated protein of interest in 20 ul of 2x Laemmli Loading Buffer and heat at 95°C for 10 min (mix 1-2 times during).
10. Load on the 9% SDS-PAGE gel.

IP Buffers:

IP-A buffer:	20 mM Tris pH7.5	per 400 ml:	8 ml of 1 M
	100 mM NaCl		8 ml of 5 M
	1mM EDTA		0.8 ml of 0.5 M pH 8.0

IP-A buffer + 1 mM PMSF: add 1 ml of 0.1 M PMSF (in isopropanol) per 100 ml of IP-A buffer.

IP-B buffer:	100 mM Tris pH7.5	per 100 ml:	10 ml of 1 M
	100 mM NaCl		2 ml of 5 M
	0.4% Triton X-100		0.4 ml

IP-C buffer:	50 mM Tris pH7.5	per 400 ml:	20 ml of 1 M
	100 mM NaCl		8 ml of 5 M
	0.2% Triton X-100		0.8 ml