ID	1
Name	Chromatin immunoprecipitation (ChIP)
Model system	S. cerevisiae
Origin	---
Obtained from	Marston lab
Solutions	ChIP diluent 2x FA lysis buffer TBS ChIP wash buffers 1-4

Procedure

Mitosis: OD(600) of around 0.5 for a 100 ml culture (SCC2-FLAG) or 50 ml (SCC1-6HA) is enough

Meiosis: start with at least 60 ml yeast culture in sporulation medium at OD(600) of around 1.9 (take 50 ml sample of each culture for ChIP

Preparation of crosslinked chromatin

1. Crosslink cells by adding 5 ml of freshly made 11% formaldehyde (1.5 ml 37% formaldehyde + 3.5 ml ChIP diluent) to 50 ml culture of cells.

2. Incubate for 2 hours at room temperature swirling at 90 rpm.

3. Pellet cells by spinning for 3 minutes at 3000 rpm at 4˚C. Wash twice with 10 ml ice-cold TBS and once with 10 ml freshly made, ice-cold FA lysis buffer + 0.1% SDS.

4. Decant and resuspend liquid remaining in the tube. Transfer to a chilled 2 ml screwcap fastprep tube. Pelet again and remove the supernatant. Snap freeze in liquid nitrogen and store over night at -80˚C.

Lysing, sonicating and setting up the IP

5. Resuspend the pellet in 1 volume (0.2-0.5 ml) ice-cold FA lysis buffer + 0.5% SDS + 1mM PMSF + 1x protease inhibitors (PIs).

6. Add an equal volume of silica beads. Lyse in fastprep machine at 4˚C twce for 30 seconds at 6.5 setting, with a 10 minute waiting period on ice in between.

7. Wipe the tube dry and tap on bench to move contents towards the cap. Puncture a hole in the bottom of the tube with a thin needle and collect the lysate in a new fastprep tube. In order to do so, place the new fastprep tube (without lid) into a 15 ml Falcon and put the tube with the sample in it on top. Centrifuge the falcon tube at 2500 rpm for 3 minutes at 4˚C.

8. Transfer the lysate (both pellet and supernatant) to new, cold 1.5 ml eppendorf. Spin at 14000 rpm for 5 min at 4˚C. Aspirate the supernatant and add 1 ml ice-cold FA lysis buffer + 0.1% SDS + 1 mM PMSF + 1x PI. Resuspend with a tip or plastic inoculating loop. Spin again. Remove the supernatant (note the presence of a glass-like transparent top layer).

9. Thoroughly resuspend the pellet in 500 µl ice-cold FA lysis buffer + 0.1% SDS + 1mM PMSF + 1x PI using a tip or plastic inoculating loop.

10. Use the sonicating waterbath (bioruptor) at setting HIGH for 28 cycles of 30 seconds right waterbath, 30 seconds left waterbath (30 cycles of meiosis). The waterbath needs to be switched on 15 min before using it so that it can cool down to 4˚C.

11. Pellet the debris by centrifugation at 14000 rpm for 5 min at 4˚C. Transfer the supernatant into a new, cold eppendorf containing 500 µl ice-cold FA lysis buffer + 0.1% SDS + 1mM PMSF + 1x PI. Mix well by inversion. Centrifuge at 14000 rpm for 15 min at 4˚C.

12. Transfer 1 ml of supernatant into a new cold eppendorf tube and add 300 µl ice-cold FA lysis buffer + 0.1% SDS.

Immunoprecipitation, decrosslinking and DNA extraction