

# Chromatin IP (lsw1/2)

**References:** Dudley et al, Genes Dev 13: 2940-2945, 1999. Also, [http://staffa.wi.mit.edu/cgi-bin/young\\_public/navframe.cgi?s=12&f=ChIPArray](http://staffa.wi.mit.edu/cgi-bin/young_public/navframe.cgi?s=12&f=ChIPArray)

## Reagents:

Fix soln: 11% formaldehyde, 0.1 M NaCl, 1 mM EDTA, 50 mM Hepes-KOH pH 7.6. **Freshly** prepared. Do not store in glass bottles.

2.5 M glycine.

TBS: 20 mM Tris pH 7.6, 150 mM NaCl.

Breaking buffer: 100 mM Tris pH 8.0, 20% glycerol, 1 mM PMSF (added fresh).

Acid washed glass beads 425-600  $\mu$  (Sigma G-8772).

FA buffer: 50 mM Hepes-KOH pH 7.6, 150mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate. NaCl conc must be determined for each combination of tagged factor and epitope tag. (optinal) Add protease inhibitors (100x) just before use.

FA-HS buffer: same as FA buffer but contains 500 mM NaCl.

RIPA buffer: 10 mM Tris pH 8.0, 0.25 M LiCl, 0.5% NP40, 0.5% sodium deoxycholate, 1 mM EDTA.

3XFLAG peptide (Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys. 5 mg / ml stock in Buffer H 0.1.)

Dynabeads Protein G (Dynal, cat# 100.04).

FLAG M2 antibody (Sigma).

Ig G free BSA (Sigma A-9085).

2x Stop buffer: 20 mM Tris pH8.0, 100 mM NaCl, 20 mM EDTA, 1% SDS.

## Beads Prep

This needs to be started the night before IP (if you are planning to do IP on the day of chromatin prep, you need to prep beads the day you inoculate yeast culture).

1. Take 20  $\mu$ l of Pro G beads/sample (IP reactions) into a siliconized eppi tube.
2. Concentrate the beads on a concentrator (MPC) and remove sup. To avoid clamping, do not leave beads on MCP for more than a couple of minutes.
3. Add 1 ml of 5 mg/ml BSA, freshly prepared in PBS.
4. Rotate at room temp for 5 min. Concentrate on MPC and remove sup.

5. Repeat step 4 twice (total 3 washes).
6. Add 100  $\mu$ l/sample of 5 mg/ml BSA in PBS, suspend beads well, and add 1  $\mu$ l (4  $\mu$ g) anti-FLAG M2 antibody/sample.
7. Rotate overnight at 4 °C.
8. Just before chromatin samples become ready, wash beads twice with 1 ml of 5 mg/ml BSA in PBS, and suspend beads in 30  $\mu$ l/sample of 5 mg/ml BSA in PBS. Keep beads on ice.

## Chromatin Prep

1. On the day before chromatin prep, inoculate yeast so that the culture will be OD<sub>600</sub>=0.5 at the time you want to start chromatin prep. I normally use 300 ml culture/rep.
2. At OD<sub>600</sub>=0.5, add 1/10 volume of Fix soln to culture, and shake slowly @ room temp for 5-60 min. Optimum time for fixation must be determined for each epitope tag and tagged protein.
3. Add 18 ml of 2.5 M glycine to every 100 ml of culture to terminate fixation. Shake culture for 5 min at room temp.
4. Pellet cells (5,000 rpm 5 min in JA-10), and wash twice with ice cold TBS (10 ml ea) in 14 ml tubes.
5. Re-suspend cells in breaking buffer (500  $\mu$ l buffer for 300 ml culture) and transfer to flat bottom 2 ml microfuge tubes.
6. Add one scoop (500  $\mu$ l PCR tube) of glass beads and vortex in cold room at full speed for 40 min.
7. Puncture one hole each in the bottom then top of the tube by a needle, and put tubes on Falcon 2059 (14 ml snap cap) or equivalent tubes. Centrifuge @ 1,000 for 2 min to recover cell lysates.
8. Add 1 ml FA buffer to lysates, transfer to 2 ml microfuge tubes, and spin at 14,000 rpm for 1 min at 4 °C and discard sup.
9. Suspend ppt in 1 ml FA buffer by gentle pipetting. Repeat step 8 twice to partially purify chromatin.
10. Suspend chromatin in 300  $\mu$ l FA buffer and sonicate for 10 seconds (setting 1.5-2 with micro tip) 12-16 times. Take 2 min intervals between each sonication (tubes on ice) to keep samples cold. \*
11. Alternatively, you can sonicate up to 4 samples in a sonicator with a cup hone (Branson Sonifier 450). In this case, add a scoop of glass beads (in Toshi's drawer) per tube and sonicate at setting 3, 10% duty cycle for 20 minutes three times. Before each cycle, take out some water from a cup, and add ice up to the line. If you have less than 4 samples, make sure to use dummy tubes so that every other hole of tube holder (total 4) is occupied.
12. Add 1.2 ml FA buffer to chromatin, mix gently and centrifuge in 2 ml microfuge tubes at 14,000 rpm for 30 min twice (turn tubes in between) at 4 °C. The sup is the chromatin sample. Go to IP reaction, and/or freeze chromatin in liquid N<sub>2</sub>. Store at -80 °C. \* You can freeze samples and stop at steps 4 (freeze cell ppt after washes). Also, if yeast cells grow at different rates, you can keep washed cells on ice after step 4 for a couple of hours to wait for other samples to catch up.

## IP reaction

1. Thaw chromatin in room temp water and take the amount needed. I usually take 250  $\mu$ l (200  $\mu$ l for IP and 20  $\mu$ l each for DNA and western analyses).

2. Spin chromatin at 14,000 rpm for 15 min. Filter sup through Millipore Ultrafree-MC 0.45 µm unit (14,000 rpm 1 min).
3. Save 20 µl each of filtrate for DNA and western blotting ("input" sample). Add 20 µl of 2 x Stop buffer or 2 x SDS/PAGE sample buffer to DNA and western samples, respectively.
4. Add 200 µl of chromatin from step 2 to 30 µl of Protein G Dynabeads as prepared above in siliconized microfuge tubes. Rotate at room temp for 90 min for binding.
5. Concentrate beads on MPC. Save 20 µl of sup for western ("unbound" fraction) and discard the rest.
6. Add 1 ml FA buffer and rotate 5 min at room temp. Concentrate on MPC and aspirate off the buffer.
7. Do washing (step 6) for total 3 times with FA buffer, 2 times with FA-HS buffer and 1 times with RIPA buffer.
8. Elute by adding 50 µl of x2 Stop buffer and incubating at 65 °C for 15 minutes with occasional vortexing. Repeat elution once and combine them.
9. Save 10µl of eluate for western from each eluate ("bound" fraction). Then, add 70 µl of 2x Stop buffer to the eluates (total 160 µl).
10. For western blotting, incubate samples for 30 min on 95 °C heat block. Determine how much of lsw1/2 was precipitated by comparing signals for "input", "unbound" and "bound".
11. For all DNA samples, add 1 µl of 20 mg / ml glycogen per tube. For "input" samples, add 120 µl of 2x Stop buffer (total 160 µl). Incubate @ 75 °C for at least 6 hrs, then add 2 µl of 10 mg / ml proteinase K. Incubate at 50-55 °C for several hrs to overnight. Phenol-chloroform extract twice and EtOH ppt DNA (40 µl 10 M NH<sub>4</sub>OAC and 250 µl EtOH).

(Optional: Purify DNA through Qiagen PCR purification kit).

## PCR

1. Take up DNA in 50 µl of TE. Take 3µl of "input" samples, treat with RNase A and run on 1.0-1.3 % agarose gel to check size of DNA. You should see smears centered around 300-500 bp.
2. For control, I usually use 5-fold serial dilution of "input" sample. Try 10, 50, and 250-fold dilution. For "bound", use undiluted sample.
3. Typical PCR reaction (20 µl).
  - o 2 µl DNA sample
  - o 1X buffer
  - o 1.5 mM MgCl<sub>2</sub>
  - o 20 pmole each primers
  - o 200 µM each 4dNTP

95 °C 2 min--[95 °C 20 sec - 59 °C 40 sec - 72 °C 30 sec] x 26-28 cycles--72 °C 5 min (RoboCycler).

4. Run on 2 % agarose in 0.5 x TBE.

\* : You may need to try different number of cycles for different primers/samples. For lsw1/2, I adjust amount of DNA so that 26 cycles would be sufficient to see signals. Higher numbers of cycles are not recommended because reactions tend to go out of linear range.

\*\* : For radioactive PCR for quantitation (strongly recommended), I add 0.5 µl [ -32 P] dCTP

(3,000 Ci/mmol, 10  $\mu$ Ci/ $\mu$ l) per 100  $\mu$ l PCR reaction. Run DNA on 2% agarose in 0.5 x TBE.

\*\*\* : For CHIP on DNA microarray, I follow Rich Young lab's protocol [http://staffa.wi.mit.edu/cgi-bin/young\\_public/navframe.cgi?s=12&f=ChIPArray](http://staffa.wi.mit.edu/cgi-bin/young_public/navframe.cgi?s=12&f=ChIPArray) after step 1.