

ULTIMATE YEAST ChIP ASSAY

This assay is for detection of proteins bound to specific regions of chromatin. There are many variations of this assay; this one works well for us.

A. GROW CELLS.

1. Inoculate 5 ml cultures of each yeast in appropriate media. Grow to saturation at appropriate temperature (usually 30°C).

2. Inoculate 50 ml cultures (250 ml flask) of selective media with 3 different amounts of the original starter culture. For cultures growing at 30°C, use 10, 30, and 100 μl of the original starter; for cultures growing at 25°C, use 30, 100, and 300 μl of the original culture. Grow for ~16 hr at appropriate temperature. The idea is to have at least one culture the next day that is at OD 0.5-1.0.

B. FIX CELLS.

Prepare:

25 ml of 37.5% PFA fresh by mixing:

9.4 g paraformaldehyde (stored at 4°C).
50 μl 10N NaOH
15 ml PBS

Heat to 65°C until PFA is dissolved (7 on hotplate; takes ~20 minutes). Take to 25 ml final volume with PBS; filter through 0.45 μM filter.

Ice-cold PBS

Benchtop centrifuge at 4 degrees

1. Select cultures that are between OD_{600} 0.5 and 1.0. Place culture on rotating platform at room temperature.

2. With culture rotating, add 1.5 ml of 37% PFA dropwise to each culture. Continue to rotate for 15 minutes.

Prepare:

Labeled 50 ml falcon tubes, one for each culture

Labeledl 2 ml screwcap tubes, one for each culture

3. Add 3ml of 2.5M glycine to each culture. Continue to rotate for 5 minutes at room temperature.

4. Transfer cell suspension to 50 ml conical tube. Spin top speed for five minutes at 4°C.

5. Wash cell pellet twice in 50 ml ice-cold PBS.

6. After the final wash, transfer cell pellet (in 1 ml PBS) to a 2 ml screw-cap microfuge tube. Place on ice until ready to proceed with lysis (but don't wait too long).

C. LYSE CELLS.

Prepare:

Ice-cold lysis buffer + freshly-added inhibitors (450 μl per sample)

21G needles

Labeled siliconized microfuge tubes (no caps)

Labeled siliconized microfuge tubes (caps)

1. Pellet cells. Resuspend in 400 μl of ice-cold LYSIS BUFFER.

2. Add 500 μl cold, acid-washed, glass beads (500 μM diameter). Close tube.

3. Place tubes (in pairs, so that machine is balanced) into the BioSpec bead beater, set up in the cold room. Lyse cells with 4 x 40 second pulses, with the bead beater set to 'homogenize'. Leave 30 seconds between pulses.

4. Puncture the bottom of the 2 ml screwcap tube with a hot 21G needle. Place this tube on top of a siliconized 1.5 ml eppendorf tube (with no cap), and place both into a 15 ml centrifuge tube.

5. Spin the tubes for 5 minutes at top-speed in the benchtop centrifuge.

6. Remove the top tube, and transfer the lysate (not pellet) to a new siliconized 1.5 ml eppendorf tube. Keep tube on ice.

D. SHEAR CHROMATIN.

Prepare:

Check sonicator, clean tip.

2 sets of label siliconized eppendorf tubes.

1. Keeping each tube on ice, sonicate (setting 5; microtip) for 10 seconds (count to 11), then return to ice for a minimum of 60 seconds. Repeat this procedure 2 times, for a total of 20 seconds sonication [NB: this time is calibrated for a specific sonicator, and designed to give DNA fragments

between 500 and 1 kb in length. Other crosslinking conditions and machines may require different sonication times].

2. Spin all tubes at top speed in microcentrifuge. 5 minutes at 4°C. Expect to see a small pellet. Transfer supernatants to fresh siliconized tubes. Store on ice until immunoprecipitation.

E. IMMUNOPRECIPITATION.

Prepare:

2 sets of labeled siliconized microfuge tubes; one labeled 'INPUT'

Ice-cold lysis buffer + freshly-added inhibitors (50 μl per sample)

Protein A agarose/Protein G agarose. Mix 30 μl of protein A agarose per sample with an equal volume of protein G agarose. Wash three times in lysis buffer and resuspend in 1 volume of lysis buffer (+inhibitors).

1. To each sample, add 50 μl of Protein A/G agarose. Rotate at 4°C for 1 hr.

2. Spin 2000 rpm for 5 minutes at 4°C. Transfer S/N to fresh tube.

3. Remove 50 μl from each sample to fresh tubes labeled INPUT. Freeze at –20°C until step G.

4. To each sample, add appropriate antibody. For 12CA5, use 10 μl of ascites diluted 1:10 in lysis buffer. For 9E10, use 10 μl of purified Ab-1 from Oncogene Sciences.

5. Rotate at 4°C for three hours.

Prepare:

Protein A agarose/Protein G agarose. Mix 30 μl of protein A agarose per sample with an equal volume of protein G agarose. Wash three times in lysis buffer and resuspend in 1 volume of lysis buffer (+inhibitors).

6. Spin briefly. Add 50 μl of protein A/G agarose. Incubate at 4°C for 1 hr to collect immune complexes.

F. WASHING IP.

Prepare:

LYSIS Buffer + Complete Inhibitor. Prepare 3 ml per IP. Dissolve 1 Complete tablet per 25 ml.

Labeled siliconized microfuge tubes.

1. Spin tubes 3000 rpm for 2 minutes. Remove supernatant. Wash beads as follows:

2 x 5 min (room temp) in 1 ml LYSIS Buffer

1 x 5 min (room temp) in 1 ml DOC Buffer

1 x 5 min (room temp) in 1 ml TE

Always use separate tip for washing!

2. At last wash, transfer pellets in TE to a fresh tube. Spin down. Remove supernatant with 26G needle. Proceed immediately to step G.

G. REVERSE CROSSLINKS.

Prepare:

Labeled siliconized microfuge tubes.

65°C waterbath

1. Add 50 μl of TES to the beads, and incubate at 65°C for 10 minutes.

2. Spin 5 minutes at top speed in microfuge. Transfer supernatant to fresh tube.

3. Add 150 μl of TES to the beads. Mix. Spin. Transfer supernatant to the tube in step 2.

4. Add 150 μl of TES to the INPUT tubes.

5. Incubate all tubes at 65°C overnight.

Prepare:

2 sets of Labeled SNAPCAP microfuge tubes.

6. Spin tubes briefly. Transfer to snapcap tube containing 25 μl of 10 mg/ml proteinase K and 200 μl of TE. Incubate at 37°C for 2 hours.

7. Extract with 400 μl of phenol/chloroform. Transfer supernatant to a fresh tube containing 44 μl of 3 M NaOAc and 1 μl of glycogen (20 mg/ml).

8. Add 1 ml ice-cold ethanol and precipitate in dry-ice ethanol bath. Pellet DNA, wash in 70 % ethanol, vacuum dry (briefly), and resuspend in 40 μl TE containing (1:200) RNase. Store at –20°C until PCR.

SOLUTIONS

2.5 M GLYCINE

(take to pH8.0 with NaOH to get to dissolve)

ICE-COLD PBS

LYSIS BUFFER

50 mM HEPES/KOH (pH 7.5) _____

500 mM NaCl _____

1 mM EDTA _____

1% Triton X-100 _____

0.1% DOC _____

0.1% SDS _____

PLUS INHIBITORS ADDED FRESH

0.4 mg/ml Pefabloc	1:100 of 40 mg/ml stock
10 μg/ml Leupeptin	1:1000 of 10 mg/ml stock
10 μg/ml Pepstatin	1:500 of 2 mg/ml stock
5 μg/ml Aprotinin	1:2000 of 10 mg/ml stock

TES

50 mM Tris-Cl (pH 8.0) _____

10 mM EDTA _____

1 % SDS _____

DOC Buffer

10 mM Tris.Cl (pH 8.0) _____

0.25M LiCl _____

0.5% NP-40 _____

0.5% DOC _____

1 mM EDTA _____