酵母染色体沉淀分析方法 Yeast Chromatin Immunoprecipitation (ChIP) Assay

ABSTRACT

This protocol describes a method for the detection of proteins bound to specific regions of chromatin in yeast. There are many variations of this assay.

MATERIALS

Reagents

Antibody of choice (see Step 21)

DOC buffer

Tris-Cl (10 mM, pH 8.0)

LiCl (0.25 M)

NP-40 (0.5%)

Deoxycholate (DOC) (0.5%)

EDTA (1 mM)

Dry ice

Ethanol (100% [ice cold], 70%)

Glycine (2.5 M)

Bring to pH 8.0 with NaOH so that it will dissolve.

Glycogen (20 mg/ml)

Growth medium appropriate for yeast strains

NaOAc (sodium acetate, 3 M)

Paraformaldehyde for yeast ChIP (prepare fresh)

9.4 g paraformaldehyde (PFA) (stored at 4oC)

50 µl NaOH (10 N)

15 ml phosphate-buffered saline (PBS)

Using a hotplate, heat to 65°C until PFA is dissolved; this takes ~20 minutes. Bring to a final volume of 25 ml with PBS, and filter through a 0.45 µM filter. The final concentration of PFA is 37.5%.

Phenol:chloroform

Phosphate-buffered saline (PBS, ice cold)

137 mM NaCl

2.7 mM KCl

10 mM Na2HPO4

2 mM KH2PO4

To prepare 1 liter of PBS(Phosphate-buffered Saline), dissolve 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4, and 0.24 g of KH2PO4 in 800 ml of distilled H2O. Adjust the pH to 7.4 (or 7.2 if required) with HCl. Add H2O to 1 liter. Dispense the solution into aliquots and sterilize them by autoclaving for 20 minutes at 15 psi (1.05 kg/cm 2) on liquid cycle or by filter sterilization. Store the buffer at room temperature. If necessary, PBS may be supplemented with 1 mM CaCl2 and 0.5 mM MgCl2. Can be made as a 10x stock.

Protein A agarose (50%, pre-swollen)

Protein G agarose (50%, pre-swollen)

Proteinase K (10 mg/ml)

RNase

TE buffer 10X (use at 1X)
100 mM Tris-Cl (desired pH)
10 mM EDTA (pH 8.0)

TES yeast buffer

Tris-Cl (50 mM, pH 8.0)

EDTA (10 mM)

SDS (1%)

Yeast ChIP lysis buffer, ice cold

HEPES/KOH (50 mM, pH 7.5)

NaCl (500 mM)

EDTA (1 mM)

1% Triton X-100 (1%)

DOC (0.1%)

SDS (0.1%)

Pefabloc (0.4 mg/ml)

Leupeptin (10 µg/ml)

Pepstatin (10 µg/ml)

Aprotinin (5 µg/ml)

Add protease inhibitors fresh each time. For washes, instead of adding individual inhibitors, use Complete Protease Inhibitor Cocktail Tablets (Roche: 1 tablet per 25 ml of buffer).

Yeast cultures

Equipment

BioSpec bead beater (set up in a cold room)

Centrifuge (benchtop) at 4ºC

Centrifuge tubes (15 ml)

Flasks (250 ml) for yeast cultures

Glass beads (cold, acid-washed, 500-μm diameter)

Ice bucket

Incubator preset to appropriate temperature for yeast (usually 30°C)

Microcentrifuge tubes (2-ml screw-cap; labeled, one for each culture)

Microcentrifuge tubes (snap-cap; two sets, labeled)

Needles (21-gauge [hot] and 26-gauge)

Rotator

Siliconized microcentrifuge tubes (seven sets with caps; one set without caps; labeled)

Sonicator with microtip

Tubes (50-ml conical Falcon; labeled, one for each culture)

Vacuum desiccator

Water bath preset to 65°C

METHOD

Growing Cells

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

The night before cells are to be fixed, inoculate 50-ml cultures (250-ml flask) of selective medium with three 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 hours at the appropriate temperature.

At least one culture should be at an OD between 0.5 and 1.0 after this period of time.

Fixing Cells

Select cultures that are between OD600 0.5 and 1.0. Place appropriate cultures on a rotating platform at room temperature.

With cultures rotating, add 1.5 ml of paraformaldehyde for yeast ChIP dropwise to each culture. Continue to rotate for 15 minutes.

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

Transfer cell suspensions to 50-ml conical tubes. Centrifuge at top speed for 5 minutes at 4°C.

Wash cell pellets twice in 50 ml of ice-cold PBS.

After the final wash, transfer cell pellets (in 1 ml of PBS) to 2-ml screw-cap microcentrifuge tubes. Place the tubes on ice until ready to proceed with lysis (but do not wait too long).

Lysing Cells

Collect the cells by centrifugation. Resuspend each sample in 400 µl of ice-cold yeast ChIP lysis buffer.

Add 500 µl of cold, acid-washed, glass beads (500 µM diameter) to each sample. Close the tubes.

Place the tubes (in pairs, so that machine is balanced) into the BioSpec bead beater, set up in the cold room. Lyse cells with four 40-second pulses, with the bead beater set to "homogenize." Wait 30 seconds between pulses.

Puncture the bottom of each 2-ml screw-cap tube with a hot 21-gauge needle. Place each tube on top of a siliconized 1.5-ml microcentrifuge tube (with no cap), and place both into a 15-ml centrifuge tube.

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

Remove the top tubes, and transfer the lysate (not pellet) to new siliconized 1.5-ml

microcentrifuge tubes. Keep the tubes on ice.

Shearing Chromatin

Keeping each tube on ice, sonicate at a medium setting with a microtip for 10 seconds (count to 11). Return the tubes to ice for a minimum of 60 seconds. Repeat this procedure, for a total of 20 seconds of sonication.

This time is calibrated for a specific sonicator, and designed to produce DNA fragments between 500 and 1 kb in length. Other cross-linking conditions and machines may require different sonication times.

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

Immunoprecipitation

Prepare Protein A/G agarose mix by combining 30 µl of Protein A agarose per sample with an equal volume of Protein G agarose. Wash three times in ice-cold lysis buffer, and resuspend in 1 volume of ice-cold lysis buffer.

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

Centrifuge at 2000 rpm for 5 minutes at 4°C. Transfer the supernatants to fresh tubes.

Remove 50 µl from each sample to a fresh siliconized microcentrifuge tube labeled "Input." Freeze the tubes at -20°C until ready to reverse cross-links (see Step 30).

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.

Rotate for 3 hours at 4°C.

Prepare Protein A/G agarose mix as in Step 17.

Centrifuge the samples briefly. Add 50 µl of Protein A/G agarose mix to each sample. Incubate for 1 hour at 4°C to collect immune complexes.

Washing IP

Centrifuge the tubes at 3000 rpm for 2 minutes. Remove the supernatant. Wash the beads at room temperature as follows:

Twice for 5 minutes each in 1 ml of Yeast ChIP lysis buffer.
Once for 5 minutes in 1 ml of DOC buffer.

Once for 5 minutes in 1 ml of TE.

Always use separate tips for washing!

At the last wash, transfer the pellets in TE to fresh tubes. Centrifuge. Remove the supernatant with a 26-gauge needle. Proceed immediately to Step 27.

Reversing Cross-Links

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

Centrifuge at top speed for 5 minutes in a microcentrifuge. Transfer the supernatant to fresh tubes.

Add 150 µl of TES to the beads. Mix. Centrifuge. Transfer the supernatant to the tubes from Step 28.

Label one set of siliconized microcentrifuge tubes as "Input." Add 150 µl of TES to these tubes.

Incubate all tubes overnight at 65°C.

Centrifuge the tubes briefly. Transfer the supernatant to microcentrifuge tubes containing 25 µl of 10 mg/ml Proteinase K and 200 µl of TE. Incubate for 2 hours at 37°C.

Extract the samples with 400 µl of phenol:chloroform. Transfer the supernatants to fresh tubes, each containing 44 µl of 3 M NaOAc and 1 µl of glycogen (20 mg/ml).

Add 1 ml of ice-cold ethanol to each sample and precipitate in a dry ice ethanol bath. Collect DNA by centrifugation and wash in 70% ethanol. Vacuum dry (briefly). Resuspend in 40 µl of TE containing (1:200) RNase. Store at -20°C until PCR is performed.