

ChIP-Seq Protocol for YAP/TAZ, Zanconato *et al.*, 2015

1. CROSSLINK CELLS WITH FORMALDEHYDE

Culture cells in cell culture dishes to 80-90% confluence.

Add 1/10 volume of Formaldehyde solution (50 mM HEPES-KOH pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 11% formaldehyde) to culture medium and incubate 6-15 minutes at RT on an orbital shaker.

Add 1/20 volume of 2.5 M glycine and incubate 5 minutes at RT on an orbital shaker.

Discard the medium and wash cells twice with ice-cold PBS.

Harvest cells in 1 ml of ice-cold PBS (with protease inhibitors) by scraping.

Pellet cells and discard supernatant.

Proceed to lysis or store pellets at -80°C.

2. LYSIS AND SONICATION

Resuspend cells in Lysis Buffer 1 (50mM HEPES, pH7.5, 10mM NaCl, 1mM EDTA, 10% Glycerol, 0.5% NP-40, 0.25% Triton X-100, with protease inhibitors – 1 ml per 10-15 mln cells) for 10-30 min at 4°C, with gentle agitation.

Pellet cells and discard supernatant.

Resuspend cells in Lysis Buffer 2 (10mM Tris-HCl pH8, 200 mM NaCl, 1mM EDTA, 0.5 mM EGTA, plus protease inhibitors - 1 ml per 10-15 mln cells) for 10 min at RT.

Pellet cells and discard supernatant.

Resuspend nuclei in Lysis Buffer 3 (10mM Tris-HCl pH8, 200 mM NaCl, 1mM EDTA, 0.5 mM EGTA, 0.1% Na-deoxyxholate, 0.5% N-lautoysarcosine, plus protease inhibitors - 1 ml per 10-15 mln cells) and move nuclei suspension to a sonication tube.

Sonicate chromatin to obtain fragments in the range of 200-600 bp. We use a Branson Sonifier 4500D with a 1/8 inch microtip. Use a small aliquot of sheared chromatin to check the size of fragments (follow the instruction in part 6 to purify DNA).

3. IMMUNOPRECIPITATION

Set up the IP reaction in low retention tubes with:

50-100 mg sheared chromatin

3-5 mg antibody

Add Triton X-100 to 1% final concentration.

Set up also a negative control IP with aspecific IgGs.

Incubate overnight on a rotor at 4°C. Prepare an input (10% of IP volume) for each sample and save it for step 4.

Add 15-25 ml of Dynabeads Protein A (or G), pre-blocked overnight in 0.5% BSA/PBS.

Incubate for 2 hrs on a rotor at 4°C.

Wash beads in low salt wash buffer (20mM Tris-HCl pH8, 150mM NaCl, 0.1 % SDS, 1% Triton X-100 and 2mM EDTA), followed by high salt wash buffer (20mM Tris-HCl pH8, 2mM EDTA, 500mM NaCl, 0.1% SDS and 1% TritonX). Repeat. Rinse with TE + 50mM NaCl (wash beads by incubating them with the wash buffer for 5 min on a rotor at 4°C).

Remove the wash solution, and add 100 ml of elution buffer (50mM Tris-HCl pH8, 1%SDS, 1mM EDTA).

Incubate 20 min at 65°C on a thermomixer while shaking (or in a waterbath and vortex samples frequently). Move the supernatant to a new 1.5 ml tube.

4. DE-CROSSLINKING AND DNA PURIFICATION

- INCLUDE INPUT SAMPLES (add 1% SDS in a final volume of 100 ml) -

Incubate eluted chromatin at 65°C for 6hrs/overnight to reverse formaldehyde crosslinking.

Dilute chromatin 1:2 with TE, and add RNase A (0.2 mg/ml final concentration). Incubate 1h at 37°C.

Add Proteinase K (0.2 mg/ml final concentration). Incubate 1h at 55°C.

Purify DNA with QIAquick PCR Purification kit (Qiagen). Elute DNA in 100 ml of nuclease-free water.

5. qPCR

Perform qPCR in triplicate with 4 ml purified DNA. Include a standard curve with serial dilutions of the input. Determine the amount of immunoprecipitated DNA in each sample as fraction of input [amplification efficiency [^] (Ct_{INPUT} - Ct_{CHIP})].