Embryo ChIP protocol (embryonic chromatin)

Jallow et al. (2004) Proc. Natl. Acad. Sci. USA., 101, 13525-13530
Version 6

Crosslinking

Rinse embryos (gastrula - tadpole stage) in tap water

Incubate 300 embryos in 5 ml tap water + 1% formaldehyde. Fix for 30 min. at RT.

> Formaldehyde stock: 37%, free from acid.
> 10% MMR can be used instead of tap water. The buffer should not contain Tris but Hepes. The free amine groups of Tris inhibit cross-linking. Use tap water only if the water is of excellent quality and does not contain significant levels of chlorine.

Transfer embryos to 25% MMR + 125 mM Glycine (stock 2.5 M). Incubate 30 min.

Wash 2x 15 min. in 25% MMR

Sonication

Homogenize embryos in 2 ml sonication buffer

Sonicate the sample (cooled by ice water) until DNA fragment size is 0.5 - 2 kb.

> Note: Proper sonication can achieved with different equipment:
> • Branson sonifier 250: 6 x 10 sec. at power 5, 30 sec. interval
> • Bioruptor 200: 30 x 30 sec. at high intensity, 30 sec. interval
> For the Bioruptor use only 15 ml BD Falcon tubes (cat. no. 352096)

Spin for 5 min. at max speed at 4ºC in eppendorf centrifuge, collect supernatant

Snap-freeze aliquots of supernatant (extract) in liquid nitrogen

__ Store at -80ºC (15 embryo equivalents (eeq) / 100 µl)

Immunoprecipitation (amounts per 15 eeq)

Dilute 100 µl extract two-fold with IP buffer (15 eeq / 200 µl)

Keep extract on ice or at 4°C at all steps, unless indicated otherwise

Add 1 to 5 µg of antibody, incubate 2 hours at 4ºC

During the antibody-extract incubation, prepare the beads as follows:

Block Protein A/G beads (SantaCruz, sc-2003): Incubate 1 h. in IP buffer + 1 mg/ml BSA, followed by 2x 1 ml IP buffer wash

Add 50 µl of 25% Protein A/G blocked beads slurry (corresponding to 12.5 µl of packed beads)

__ Incubate O/N at 4ºC on rotating mixer

> Note: The next step is washing of the beads to remove nonspecific interactions with protein-DNA complexes. For quantitation purposes it essential to have an input DNA control. This can be a fresh chromatin sample. To save ChIP chromatin it is also possible to keep 150 µl of the supernatant of a “beads-only” IP as input control (75%). If taken, keep this sample on ice until the reversal and extraction step.

Wash beads once with 1 ml of each of the following buffers (sequentially, everything on ice):

- ChIP1 buffer
- ChIP2 buffer
- ChIP3 buffer
- ChIP1 buffer.

Wash beads once with 0.5 ml TE (everything on ice)

Elute in 2x 200 µl $NaHCO_3$ pH 8.8 (0.1 M) / SDS (1%)

Note: Make fresh, at room temperature, 15 min per elution

Collect the supernatants in new tubes, keep at room temperature

> Use safe-lock tubes (Eppendorf). Otherwise the long incubation at 65°C in the next step may cause the lids to leak during subsequent phenol extraction

Reversal and extraction

Add 16 µl NaCl (5M) (and, optionally 0.5 µl proteaseK 10 mg/ml)

__ Vortex, incubate at 65ºC for 6 hours

Phenol-CI extraction

Ethanol precipitation:

Add 10 µg glycogen, 40 µl Na-acetate 3M, 1200 µl ethanol,

Incubate >60 mins. at –80ºC (or: o/n at –20ºC)

Spin 15 mins. at 4ºC at max. speed (eppendorf)

Retain and wash (visible) pellets in 70% ethanol, dissolve pellets in 200 µl TE

Quantify recovery of specific sequences by quantitative PCR

Make dilutions of input DNA (10%, 1%, 0.1%, 0.01%) to obtain a standard curve for quantitation (separately for each primer pair).

Test 5 µl of all samples in real time quantitative PCR

Calculate recoveries as % of input DNA

Sonication buffer

Low-salt whole cell extract (WCE-LS) buffer

20 mM Tris pH 8
70 mM KCl
1 mM EDTA
10 % Glycerol
5 mM DTT
0.125 % Nonidet P40
Protease inhibitors

IP buffer

50 mM Tris pH 8
100 mM NaCl
2 mM EDTA
1 mM DTT
1% NP40
Protease inhibitors

ChIP 1 buffer

IP buffer plus 0.1% deoxycholate

ChIP 2 buffer

IP buffer plus:

400 mM NaCl (extra, 500 mM final)
0.1% deoxycholate

ChIP 3 buffer

IP buffer plus:

250 mM LiCl
0.1% deoxycholate