iChIP / enChIP to purify genomic DNA

Originally developed by Toshitsugu Fujita on September 18, 2012
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1. Crosslinking of cells

(1) Culture target cells. Use 2 x 10^7 cells (e.g. Ba/F3, DT40) for chromatin preparation.

(2) Add 37% formaldehyde to 1% final concentration into the culture medium with cells. Incubate at 37°C for 5-10 min (usually 5 min).

Cell volume	30 ml
37% formaldehyde	810 µl

(3) Stop crosslinking by adding 1.25 M Glycine solution to 127 mM final concentration. Incubate at room temperature for 10 min.

Cell volume	30 ml
1.25 M Glycine	3.05 ml

1.25 M Glycine	Glycine MW: 75.07
Glycine	18.8 g / 200 ml

(4) Collect cells by centrifugation (1,300 rpm, 4°C for 5 min).

(5) PBS wash twice. Collect the pellet (cells). The cells can be stored at -80°C.

2. Preparation of chromatin

(1) Suspend the fixed cells in 10 ml of CLB. Incubate on ice for 10 min.

Cell Lysis Buffer (CLB) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% IGEPAL CA-630, 1 x protease inhibitors

	40 ml
1 M Tris-HCl (pH 8.0)	400 µl
0.5 M EDTA	80 µl
IGEPAL CA-630	200 µl
Complete-Mini	4 tablets
DDW	39.32 ml

(2) 2,000 rpm, 4°C for 8 min. Discard carefully the supernatant.

(3) Suspend the pellet in 10 ml of NLB. Incubate on ice for 10 min. Vortex every 2-3 min.

Nuclear Lysis Buffer (NLB) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.5% lauroylsarcosine sodium salt, 1 x protease inhibitors

	40 ml
1 M Tris-HCl (pH 8.0)	400 µl
0.5 M EDTA	80 µl
5 M NaCl	4 ml
Triton X-100	400 µl
10% sodium deoxycholate	2 ml
30% lauroylsarcosine sodium salt	666 µl
Complete-Mini	4 tablets
DDW	32.46 ml

10% sodium deoxycholate

sodium deoxycholate 1 g / 10 ml

(4) 2,000 rpm, 4°C for 8 min. Discard carefully the supernatant.
(5) Suspend the pellet in 10 ml of PBS. 2,000 rpm, 4°C for 10 min. Collect the pellet as the chromatin fraction. The chromatin fraction can be stored at -80°C after immediate freezing in liquid nitrogen.

3. Sonication of chromatin

(1) Suspend the collected chromatin fraction in 800 µl of MLB3. Transfer the suspension into a 1.5 ml microtube.

Modified Lysis Buffer 3 (MLB3) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 150 mM NaCl, 0.1% sodium deoxycholate, 0.1% SDS, 1 x protease inhibitors

	10 ml
1 M Tris-HCl (pH 8.0)	100 µl
0.5 M EDTA	20 µl
0.1 M EGTA	50 µl
5 M NaCl	300 µl
10% sodium deoxycholate	100 µl
10% SDS	100 µl
Complete-Mini	1 tablet
DDW	9.33 ml

(2) Sonication of the chromatin by using Ultrasonic disruptor UD-201 (TOMY SEIKO). Condition is as follows:

Output: 3
Duty: 100% (continuous)
Time: Free
10 - 18 cycles of sonication for 10 sec and cooling on ice for 20 sec
2 min on ice after 5 - 6 cycles

Keep the position of the tip of the sonication bar approximately 0.5 cm away from the tube bottom.
(3) 13,000 rpm, 4°C for 10 min. Collect the supernatant (800 µl). The supernatant can be stored at -80°C after immediate freezing in liquid nitrogen.

4. Reverse crosslinking (Evaluation of fragmentation of chromatin)

(1) Suspend 10 µl of the fragmented chromatin in 85 µl of distilled water.
(2) Add 4 µl of 5M NaCl. Incubate at 65°C overnight.
(3) Add 1 µl of 10 mg/ml RNase A. Incubate at 37°C for 45 min.
(4) Add 2 µl of 0.5M EDTA (pH 8.0), 4 µl of 1M Tris-HCl (pH 6.8), and 1 µl of Proteinase K (Roche). Incubate at 45°C for 1.5 h.
(5) Pick up 10 µl for electrophoresis in 1% agarose gel without staining dye. 100 V for 30 min.
(6) Gel staining with staining dye for 0.5-1 h.

5. Preparation of Dynabeads conjugated with antibody

(1) Transfer 30 µl Dynabeads-Protein G (Invitrogen) in a new 1.5 ml tube.
(2) Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting.
(3) Add 1 ml PBS with 0.01% Tween-20. Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting.

PBS	10 ml
10% Tween-20	10 µl

(4) Repeat the step (3).
(5) Add 300 µl PBS with 0.01% Tween-20 and 0.1% BSA.

PBS	10 ml
10% Tween-20	10 µl
7.5% BSA	133 µl

(6) Add 3 µg antibody (e.g. anti-FLAG antibody Sigma F1804, control IgG). Rotate at 4°C overnight.
(7) Spin down briefly. Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting.
(8) Add 300 µl PBS with 0.01% Tween-20. Invert several times and spin down briefly. Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting.
(9) Repeat the step (8), twice. The Dynabeads are ready for the next step.

6. Chromatin immunoprecipitation

(1) Transfer 160 µl of the fragmented chromatin, which corresponds to chromatin extracted from 4 x 10^6 cells, into a new 1.5 ml tube.
(2) Add 340 µl of MLB3 1.47% Triton X-100 (final 1%).

MLB3	1 ml
Triton X-100	14.7 µl

(3) Transfer all (500 µl) of the chromatin solution into the tube, in which the Dynabeads conjugated with control IgG were prepared at the step 5-(9). Rotate 4 °C for 1h.
(4) Put the tube on a magnet stand and wait for 2 min.
(5) Transfer the supernatant into the tube, in which the Dynabeads conjugated with specific antibody (e.g. FLAG antibody) were prepared at the step 5-(9). Rotate 4 °C overnight.
(6) Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting.
(7) Wash 1: Add 1 ml of LSB. Rotate 4 °C for 10 min. Put the tube on a magnet stand and wait for 2 min. Discard the supernatant by pipetting. Repeat wash with LSB.

Low Salt Buffer (LSB) 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 0.1% SDS

	20 ml
1 M Tris-HCl (pH 8.0)	400 µl
0.5 M EDTA	80 µl
5 M NaCl	600 µl
Triton X-100	200 µl
10% SDS	200 µl
DDW	18.52 ml

(8) Wash 2: Repeat the step (7) with HSB x 2.

<u>High Salt Buffer (HSB)</u> 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% SDS

	20 ml
1 M Tris-HCl (pH 8.0)	400 µl
0.5 M EDTA	80 µl
5 M NaCl	2 ml
Triton X-100	200 µl
10% SDS	200 µl
DDW	17.12 ml

(9) Wash 3: Repeat the step (7) with LiCl buffer x 2.

<u>LiCl Buffer</u> 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.25 M LiCl, 0.5% IGEPAL CA-630, 0.5% sodium deoxycholate

	20 ml
1 M Tris-HCl (pH 8.0)	200 µl
0.5 M EDTA	40 µl
8 M LiCl	625 µl
IGEPAL CA-630	100 µl
10% sodium deoxycholate	1 ml
DDW	18.035 ml

(10) Wash 7 and 8: Repeat the step (7) with **TBS with 0.1% IGEPAL-CA-630** x 2.

<u>TBS (50 mM Tris-HCl (pH 7.5), 150 mM NaCl) with 0.1% IGEPAL CA-630</u>

(11) Elution: Add 150 µl of 500 µg/ml 3xFLAG peptide (Sigma, F4799) in TBS with 0.1% IGEPAL CA-630. Incubate at 37 °C for 20 min. Put the tube on a magnet stand and wait for 3 min.

3xFLAG peptide (5 mg/ml)	50 µl	**66 µl**
TBS w/ 0.1% IGEPAL CA-630	450 µl	**594 µl**

(12) Repeat the elution step. Total 300 µl.

(13) Add

5 M NaCl	12 µl	final	200 mM
0.5 M EDTA	0.6 µl		1 mM

(14) 65°C, overnight.

(15) Add **3 µl** of 10 mg/ml RNase A. Incubate 37°C for 1 hr.

(16) Add

10% SDS	**16 µl**
Proteinase K	**10 µl**

(17) Incubate at 45°C for 2 hr.

(18) Purify DNA using ChIP DNA Clean & Concentrator kit (Zymo Research, D5205). Add 1.5 ml of ChIP DNA Binding Buffer.

(19) Transfer mixture into a Zymo-Spin Column in a Collection Tube.

(20) 15,000 rpm, 30 sec.

(21) Discard flow-through.

(22) Wash with 200 µl of Wash Buffer x 2.

(23) Elute DNA with 50 µl of Elution Buffer.