Chromatin immunoprecipitation

Chromatin preparation by method similar to that used by Jason Carroll's lab

-Trypsinize cells, wash in PBS and count.

-Need 5x10^7 cells per 1ml of chromatin.

-Take appropriate volume of cells, spin down and resuspend in 10ml PBS.

-Weigh out 23mg of EGS and dissolve in 100µl of DMSO.

-Add 40µl of EGS solution to 10ml of resuspended cells (final conc. of EGS is 2mM). Rotate at room temperature for 1hr.

- Add 270 µl 37% formaldehyde (final conc. 1%) and rotate at room temperature for 15 minutes.

-Add 1.5ml of 1M glycine to quench formaldehyde.

-Rotate at room temperature for 3 minutes.

-Spin at 2000rpm for 4 min at 4°C.

-Resuspend in 10ml LB1 (+1xPIC).

-Rotate at 4°C for 10 min.

-Spin 2000rpm for 4 min at 4°C.

-Resuspend in 10ml LB2 (+1x PIC).

-Rotate at 4°C for 5min.

-Spin 2000rpm for 4min at 4°C.

-Resuspend in appropriate volume of LB3 (+1x PIC). Use 1ml of LB3 per 5x10^7 cells.

-Divide into 1ml aliquots in 15 ml sonication tubes.

-Insert sonication bar/lid.

-Sonicate at 30secs on/off on high setting for 30 mins.

-If sonicating more than 1x1ml aliquots, re-pool identical samples together.

-Add 1/10 volume of 10% Triton x 100 made up in LB3 (e.g. 100ul 10% triton for 1ml chromatin). Mix by inversion.

-Spin at full speed at 4°C for 10 mins (if sample is greater than 1ml, need to re-divide into 1.5ml tubes).

-If sample is greater than 1ml, re-pool identical samples together.

-Aliquot and freeze.

Buffers

1M EGTA stock

19g EGTA (MW 380g/mol)
ddH2O to 90ml
adjust pH 7.5/8.0 with solid NaOH (>4g)
adjust volume to 100ml

Note: EGTA will not go into solution without NaOH. Once the pH has been raised sufficiently it dissolves quickly. For pH 7.5 the exact amount required is slightly above 4g. Add 3.5-4g immediately, then proceed carefully not to overshoot the desired pH.

LB1

	For 200ml
50mM HEPES KOH, pH 7.9	10ml of 1M
140mM NaCl	5.6ml of 5M
1mM EDTA	0.2ml of 1M
10% Glycerol	20ml of 100%
0.5% NP40	10ml of 10%
0.25% Triton x 100	5ml of 10%

LB2

	For 200ml
10mM Tris HCl, pH 8.0	2ml of 1M pH8.0
200mM NaCl	8ml of 5M
1mM EDTA	0.2ml of 1M
0.5mM EGTA	0.1ml of 1M

LB3

	For 200ml
10mM Tris HCl, pH 8.0	2ml of 1M, pH8.0
100mM NaCl	4ml of 5M
1mM EDTA	0.2ml of 1M
0.5mM EGTA	0.1ml of 1M
0.1% Na Deoxycholate	2ml of 10%
0.5% N-lauroylsarcosine	10ml of 10%

CHIP setup magnetic beads (purification using Zymogen columns for ChIPSEQ):

For each IP I use 100 µl of chromatin. Given that cells were lysed at a concentration of 5 x 10^7 cells per ml, 100 µl represents about 5 x 10^6 cells per IP (note that for Histone modification ChIP I used a lot less chromatin e.g. 1 x 10^5 cells per IP).

- For every IP to be performed, add 100 µl chromatin to 900 µl ChIP dilution buffer containing 1x protease inhibitor cocktail. If multiple IPs are to be performed, It is easiest to prepare a chromatin/buffer mastermix comprising enough material for required number of IPs

+1. e.g. If 5 IPs are required, mix 600 µl chromatin with 5400 µl ChIP dilution buffer containing 1x protease inhibitor cocktail.

- Per ChIP use 25uL magnetic beads (do ChIPs in 1.5mL LoBind tubes)
- Wash beads 3x 1ml 0.5% BSA/1xPBS → after last wash resuspend beads in 1mL 0.5% BSA/1xPBS and add required amount of antibody (2-5ug/CHIP)
- Incubate at 4C either overnight or at least 4hours (flying wheel/cold room)
- Place beads on magnetic → remove supernatant → wash beads 3x in 1mL 0.5% BSA/1xPBS
- Add 1mL of chromatin from (chromatin + ChIP dil buffer mastermix) → incubate with beads overnight/4C (Retain 100uL per Input)

- Wash beads (4min/ 4C, spin 1000xg/1min):
- 1x Low salt buffer
- 1x high salt buffer
- 1x LiCl buffer
- 2x TE buffer

- Resuspend beads in 100 µl elution buffer (1 % SDS, 0.1 M $NaHCO_3$) (made fresh).

- Vortex at room temperature for 30 minutes (or rotate if constant vortex isn't available).

- Transfer eluate to a fresh microcentrifuge tube.

- Reverse crosslink as per usual (Nacl/ RNase/protK)

i.e.: **E. DNA purification**

1. Take all 100 µl ChIP samples plus 100 µl Input sample (from Immunoprecipitation step 6).
2. To all samples (including input) add 4 µl 5 M NaCl.
3. Incubate at 65°C for 4 hours.
4. Add 2 µl RNase (Roche 11 119 915 001, 500 µg/ml) to each sample.
5. Incubate at 42°C for 1.5 hours.
6. Add 1 µl proteinase K (20 mg/ml) to each sample.
7. Incubate at 45°C for 1 hour.
8. We started using Zymogen columns for purifications as you achieve better recovery than Invitrogen MicroLink

Buffer	**Final concentration**	**Stock**	**Volume in 50 ml**
ChIP dilution buffer	1% triton-x100 1 mM EDTA	100% 0.5 M	500 µl 100 µl

	20 mM Tris-HCl (pH 8) 150 mM NaCl	1 M 5 M	1 ml 1.5 ml
Low Salt Buffer	0.1% SDS 1% triton-x100 2 mM EDTA 20 mM Tris-HCl (pH 8.1) 150 mM NaCl	10% 100% 0.5 M 1 M 5 M	500 µl 500 µl 200 µl 1 ml 1.5 ml
High Salt Buffer	0.1% SDS 1% triton-x100 2 mM EDTA 20 mM Tris-HCl (pH 8.1) 500 mM NaCl	10% 100% 0.5 M 1 M 5 M	500 µl 500 µl 200 µl 1 ml 5 ml
LiCl Buffer	0.25 M LiCl 1% NP-40 1% Deoxycholate 1 mM EDTA 10 mM Tris-HCl (pH 8.1)	1 M 100% 0.5 M 1 M	12.5 ml 500 µl 500 mg 100 µl 500 µl