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Chip seq (fta Modified)

Day 1--Harvest, fix, wash cells, sonication, and start IP

1. Wash with PBS 2 times, crosslink with 10ml 1% formaldehyde in PBS for 10-15min at RT.
2. Stop crosslinking with Glycine. Wash cells twice with Glycine in PBS (50ml PBS+1ml 2.5M Glycine). At the second wash, keep cells in glycine for 1 minute.
3. Wash twice with PBS. Scrape off cells in PBS and spin down.
4. Resuspend cells in 5-6ml Buffer A2.
5. Sonicate in Bioruptor, 30s on, 30s off for 14 cycles.
6. Set aside 20µl cell extract after sonication as genomic Input control and store at -20°C.
7. Use dynabeads M-280 sheep anti-mouse IgG for mouse antibody IP. 1µg Ab/13-14µl beads. Use Protein G dynabeads for Goat antibody IP. 1µg Ab/5µl beads. Use dynabeads M-280 sheep anti-rabbit IgG for rabbit antibody IP. 1µg Ab/13-14µl beads.
8. Vortex beads to fully mix the beads. Remove original dynabeads solution using a magnetic rack, and then add PBS.
9. Wash beads with PBS 3 times using a magnetic rack.
10. Dilute antibody in PBS+0.5%BSA and add to beads. Incubate on rotating rack at 4°C for 4hr.
11. After sonicated chromatin are prepared, get beads from incubation and add sonicated chromatin. Incubate o/n in the cold room at 4°C.

Day 2--Wash IP, reverse cross-linking and DNA clean up

1. Wash IP 3 times with 800µl RIPA Wash buffer using a magnetic rack. Each wash is 30s.

(If you don't have a magnetic rack, you can centrifuge in between washes).

2. Wash once with 1X TE +50mM NaCl
3. Return to RT. From now on, barrier tips are used to avoid DNA contamination from pipettes.
4. Add 150 µl elution buffer +150 µl 1x TE to both IP sample and Input.
5. Add 4µl RNase(10mg/ml, final =0.2µg/µl)to every sample. Incubate on 37°C with shake at 950rpm for 2hr.
6. Add 2µl Protease K (Invitrogen 20mg/ml) to samples.
7. Incubate for 2hr at 55°C for protease K digestion and 6hr at 65°C for at least 6hr for reverse crosslinking. This can be done in a hybridization oven or in the PCR machine (6 h 65°C and then a 4°C hold).

   (Use tubes designed "not-to-pop-open". "Easy-open" tubes can result in dried out beads)
8. Extract once/w phenol:chl:IA. (Centrifuge at 12,000 rpm for 5 min at RT. The interphase contains protein, the upper phase contains DNA. Remove upper phase to a new tube, and discard old tube in phenol waste.)
9. Transfer to new microfuge tube containing 12 ul of 5M NaCl ([final] = 200 mM) and 2ul glycogen (30ug)
10. Precipitate with 750 ul cold (4°C) EtOH . **You can freeze here if needed.
11. Mix well and keep in -80°C for 30 min-1hr.
12. Spin 20min at 4°C at max speed and decant EtOH. (Make sure you see a pellet before decanting)
13. Vortex and spin another 5min at 4°C at max speed and decant EtOH
14. Add 800 µl cold 70% EtOH, wash by vortexing and spin 5 min at 4°C, decant, spin briefly at RT and remove remaining liquid with a pipette.
15. Dry briefly and resuspend pellets in 55 µL water. (Avoid over-drying of pellets and resuspend well, e.g. by putting the tubes in 37°C heat block for 5 min) **You can freeze here if needed.
16. Perform QPCR here.

Q-PCR set up for 20µl system

SybrGreen mix	10µl
Primers(Forward+Reverse) 2µM	5µL
Samples after dilution	5µl

*sample dilution: IP sample(PolII IP) 5µl+ 17µl H_2O

Input sample 2µl+ 100µl H_2O

250 ml Buffer A2 (w/o PI)

3.75 ml	1M HEPES pH 7.5	(final 15 mM)	
7 ml	5 M NaCl	(final 140 mM)	(e.g. Sigma S5150)
0.5 ml	0.5M EDTA	(final 1 mM)	
250 ul	0.5M EGTA	(final 0.5 mM)	
2.5 ml `	Triton X100	(final 1%)	(e.g. Sigma T8787)
2.5 ml	10% sodium deoxycholate	(final 0.1%)	(e.g. Sigma D6750)
2.5 ml	10% SDS	(final 1%)	

12.5 ml 10% N-lauroylsarcosine (or 1.25 g) (final 0.5%)

ddH_20 ad 250 ml

Filter-sterilize and store at 4°C.

500 ml Wash Buffer (RIPA)

25 ml	1M HEPES pH 7.5	(50 mM final)	
1 ml	0.5M EDTA	(1 mM final)	
35 ml	10% sodium deoxycholate	(0.7 % final)	
50 ml	10% NP-40 (IGEPAL CA-630)	(1% final)	(e.g. Sigma 18896)

31.25 ml 8M LiCl (0.5 M final) (e.g. Sigma L7026)
ddH_2O ad 500 ml
Filter-sterilize and store at 4°C.

100 ml Elution buffer

5 ml	1 M Tris pH8	(final 50 mM)
2ml	0.5 M EDTA	(final 10 mM)
10ml	10% SDS	(final 1%)

Filter-sterilize and store at RT.

100 ml TE

1 ml	1 M Tris pH8	(final 10 mM)
200ul	0.5 M EDTA	(final 1 mM)

Filter-sterilize or autoclave. Store at RT.

2.5M Glycine (75.07 g/mol)

9.4 g per 50 ml ddH2O

Store at RT.

10% N-lauroylsarcosine

10 g N-lauroylsarcosine sodium salt (e.g. Sigma L5125) in 100 ml ddH2O

Store at RT.

100 ml 1M dithiothreitol (DTT)

Dissolve 1.545 g DTT in 10 ml ddH2O
Store at -20°C.

(References)

1. Tonh Ihn Lee, Sarah E Johnstone & Richard A Young. Chromatin immunoprecipitation and microarray-based analysis of protein location. Nature protocols, 13 July 2006; doi:10.1038/nprot2006.98