µChIP

This protocol is modified from John Arne Dahl and Inkyung Jung, *et al*., 2016 to prepare ChIP samples from low cell numbers. Certain parts of the protocol need to be optimized depending on the antibody and cell types used. Please look at the original paper for further details.
Please look at the separate protocol for library preparation if continuing to Illumina sequencing

Crosslinking buffer				
Reagents	**Stock concentration**	**Final concentration**	**5 ml**	**10 ml**
Formaldehyde	16%	1.1%	343.75 µl	687.5 µl
PIC (protease inhibitor)	25X	1X	200 µl	400 µl
Sodium butyrate	0.5M	20 mM	200 µl	400 µl
PMSF	0.2M	1 mM	25 µl	50 µl
EDTA	0.5M	1 mM	10 µl	20 µl
PBS	---	---	4221.25 µl	8442.5 µl

NOTES:

- freshly prepared, store at RT
- formaldehyde waste should be disposed in separate container
- PIC, Sodium butyrate and PMSF should be added last
- PIC, Sodium butyrate and PMSF has 1 month shelf life
- Formaldehyde should be methanol free
- Dispose sodium butyrate once opened
- PIC pellet in 2mL is 25X

Crosslinking wash buffer				
Reagents	**Stock concentration**	**Final concentration**	**5 ml**	**10 ml**
Sodium butyrate	0.5M	20 mM	200 µl	400 µl
PIC	25X	1X	200 µl	400 µl
PMSF	0.2M	1 mM	25 µl	50 µl
EDTA	0.5M	1 mM	10 µl	20 µl
PBS	---	1X	4565 µl	9130 µl

- Prepare buffer right before use. Store at 4C

RIPA buffer				
Reagents	**Stock concentration**	**Final concentration**	**5 ml**	**10 ml**
Triton X-100	10%	1%	500 µl	1000 µl
NaCl	5M	140 mM	140 µl	280 µl
Tris-HCl (pH 8.0)	1M	10 mM	50 µl	100 µl
SDS	10%	0.1%	50 µl	100 µl
Na-deoxycholate (DOC)	10%	0.1%	50 µl	100 µl
EDTA	0.5M	1 mM	10 µl	20 µl
EGTA	0.5M	0.5 mM	5 µl	10 µl
ddH_2O (Milli-Q)	---	---	4195 µl	8390 µl

Lysis buffer				
Reagents	**Stock concentration**	**Final concentration**	**1ml**	**5ml**
SDS	10%	0.8%	80 µl	400 µl
Na butyrate	0.5M	20mM	40 µl	200 µl
PMSF	0.2M	1mM	5 µl	25 µl
PIC	25X	1X	40 µl	200 µl
PBS	---	---	835 µl	4175 µl

RIPA dilution buffer				
Reagents	**Stock concentration**	**Final concentration**	**5 ml**	**10 ml**
Triton X-100	10%	1.25%	625 µl	1250 µl
Sodium butyrate	0.5M	20 mM	200 µl	400 µl
PIC	25X	1 X	200 µl	400 µl
NaCl	5M	175 mM	175 µl	350 µl
Na-deoxycholate (DOC)	10%	0.125%	62.5 µl	125 µl
Tris-HCl (pH 8.0)	1M	10 mM	50 µl	100 µl
PMSF	0.2M	1 mM	25 µl	50 µl
EDTA	0.5M	1 mM	10 µl	20 µl
EGTA	0.5M	0.625 mM	6.25 µl	12.5 µl
ddH_2O (Milli-Q)	---	---	3646.25 µl	7292.5 µl

ChIP elution buffer				
Reagents	**Stock concentration**	**Final concentration**	**5 ml**	**10 ml**
SDS	10%	1%	500 µl	1000 µl
Tris-HCl (pH 7.5)	1M	20 mM	100 µl	200 µl
RNaseA	10 mg/mL	30 µg (per 150 µl)	100 µl	200 µl
NaCl	5M	50 mM	50 µl	100 µl
EDTA	0.5M	5 mM	50 µl	100 µl
ddH_2O (Milli-Q)	---	---	4200 µl	8400 µl

Note: Add RNaseA separately to individual tube and not to master mix.

I. Crosslinking of cells

1) Add 50 µl of PBS with 2% formaldehyde to 50 µl of cells to get a 1% final formaldehyde concentration and vortex (gently!), incubate at RT for 8 min, and vortex once more.
    - ❖ mESCs were prepared at 1 million cells per 50 µl
2) Add 5.3 µl of 2.5 M glycine stock (final conc. 125 mM), mix by gently vortexing, incubate at RT for 5 mins, and vortex once during the incubation.
3) Centrifuge the crosslinked cells at (centrifuge speed is dependent on cell type) at 4°C in a swing-bucket rotor with soft deceleration.
4) Wash the pellet twice with 400 µl ice cold PBS. Snap freeze after the last wash, store at -80°C.

II. Bead preparation

5) Transfer 2 µl of Dynabeads Protein A (per IP) to a 1.5 ml tube.
6) Place on a magnetic rack.
7) Remove excess buffer and wash with 500 µl RIPA buffer.

8) Resuspend beads in RIPA buffer to 100 µl per IP, move to new 200 µl tube.
9) Add 2 µg antibody to the tube.
10) Incubate at 40 rpm on rotator for at least 4 hrs at 4°C.

III. Chromatin preparation

11) Slowly thaw a tube(s) of frozen cell pellet to ice.
12) Add 100 µl of crosslinking buffer to the pellet.
13) Incubate at room temperature for 10 mins and vortex gently twice.
14) Add 3.5 µl of 2.5 M Glycine, vortex gently and incubate for 5mins.
15) Centrifuge for (centrifuge speed differs for cell type) at 4°C.
16) Wash the pellet twice with 400 µl of crosslinking wash buffer.
17) Discard the supernatant and resuspend in 120 µl lysis buffer.
18) Transfer to sonication tube.
19) Sonicate at optimized conditions.
    - For example, Covaris S220/E220 for tube of 1 million mESC

Peak Incident Power	175.0
Duty Factor	10.0
Cycle per burst	200
Time (seconds)	400
Water level	12.5
Temperature	2°C – 7°C
Volume	130 µl

20) Transfer lysate to new 1.5 ml tube, rinse sonication tube with 170 µl RIPA dilution buffer.
21) Centrifuge for 12,000 x g for 10 mins at 4°C.
22) Transfer supernatant to a new 1.5 ml tube.

IV. IP

23) Quick spin the pre-incubated beads and antibody complex to collect liquid at the cap.
24) Pellet on cold magnetic rack (keep on ice).
25) Remove the supernatant.
26) Wash in 130 µl RIPA buffer, vortex roughly.
27) Repeat the washing for a total of two washes.
28) Transfer appropriate volume of chromatin to each IP tube, keep the same volume as input. Also keep a small amount for size check.
    - Volume per of chromatin depends on how many marks you are doing and how you are splitting a tube of cells (ie. 1 mark and 1 input, roughly 65 µl)
29) Add 4 µg crosslinked recombinant histone octamers and 1.25 µg of non-immunized rabbit IgG to each ChIP reaction.
30) Top up IP volume to ~180 µl with RIPA dilution buffer.
31) Rotate the tubes at 4°C for at least 30hrs.

V. Washing

32) Wash the chromatin-antibody-bead complex 4 times with 100 µl cold RIPA buffer.
    - Concentrations of SDS and NaCl can be optimized for the antibody.
33) Wash the beads in 100 µl TE buffer and transfer to a new 1.5 ml tube.
    - Chill the TE before use

VI. Elution

34) Pellet on cold magnetic rack, remove the TE buffer and add 150 µl ChIP elution buffer.
35) Incubate at 37°C for 1hr, 1000 rpm on thermomixer.
36) Add 15 µl of Proteinase K (20 mg/ml) and incubate at 60°C for 1 hrs, 1000rpm, followed by 68 °C for 3hrs
37) Transfer eluate to a new 1.5 ml tube.
38) Add 150 µl fresh ChIP elution buffer to the beads and further elute for 5mins.
39) Pool the eluate from both elution.
40) Purify the DNA by phenol-chloroform isoamylalcohol extraction (ethanol precipitated with 10 µl acrylamide carrier) or use a kit such as QIAGEN MinElute PCR purification kit.
41) Dissolve or elute in 20 µl of EB (or 10 mM Tris-HCl).

References:

Dahl, J., Jung, I., Aanes, H. et al. Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* **537**, 548–552 (2016). https://doi.org/10.1038/nature19360