Native ChIP

This protocol is for native ChIP, which uses micrococcal nuclease (MNase) enzymes to fragment the chromatin. Certain parts of the protocol need to be optimized depending on the antibody and cell types used.
Please look at the separate protocol for library preparation if continuing to Illumina sequencing.

Make up stock solution of 25X PIC

Put 1 tablet Roche Complete EDTA-free PIC tablet into 2ml ddH20 (Milli-Q).
PIC stock solutions can be stored up to 1 week at 4°C after preparation.

- ❖ All solutions are made fresh prior to use.

Buffers to prepare:

IP Buffer				
Reagents	**Stock concentration**	**Final concentration**	**5ml**	**10ml**
Tris-HCl (pH 8.0)	1M	10mM	50ul	100ul
Triton X-100	10%	1%	500ul	1mL
Na Deoxycholate (DOC)	10%	0.10%	50ul	100ul
SDS	10%	0.10%	50ul	100ul
NaCl	5M	90mM	90ul	180ul
EDTA	0.5M	2mM	20ul	40ul
PIC	25X	1X	200ul	400ul
ddH2O (Milli-Q)			4.04ml	8.08ml

Hypotonic Lysis Buffer			
Reagents	**Stock concentration**	**Final concentration**	**5ml**
EDTA (pH 8.0)	0.5M	0.2mM	2ul
Benzamidine	1M	0.1mM	0.5ul
PMSF	200mM	0.1mM	2.5ul
DTT	1M	1.5mM	7.5ul
PIC	25X	1X	200ul
ddH2O (Milli-Q)			4.79ml

Douncing Buffer (DB)			
Reagents	**Stock Concentration**	**Final Concentration**	**1mL**
Tris-HCl (pH 7.5)	1M	10mM	10ul
$MgCl_2$	1M	4mM	4ul
$CaCl_2$	0.5M	1mM	2ul
PIC	25X	1X	40ul
ddH2O (Milli-Q)			944ul

ChIP Wash Buffer				
Reagents	**Stock concentration**	**Final concentration**	**10ml**	**20ml**
Tris-HCl (pH 8.0)	1M	20mM	200ul	400ul
SDS	10%	0.10%	100ul	200ul
Trixton-100	10%	1%	1ml	2ml
EDTA	0.5M	2mM	40ul	80ul
NaCl	5M	150mM	300ul	600ul
PIC	25X	1X	400ul	800ul
ddH2O (Milli-Q)			7.96ml	15.92ml

Final ChIP Wash Buffer				
Reagents	**Stock concentration**	**Final concentration**	**10ml**	**20ml**
Tris-HCl (pH 8.0)	1M	20mM	200ul	400ul
SDS	10%	0.10%	100ul	200ul
Trixton-100	10%	1%	1ml	2ml
EDTA	0.5M	2mM	40ul	80ul
NaCl	5M	500mM	1ml	2ml
PIC	25X	1X	400ul	800ul
ddH2O (Milli-Q)			7.26ml	14.52ml

Elution Buffer				
Reagents	**Stock concentration**	**Final concentration**	**2ml**	**4ml**
$NaHCO_3$	1M	100mM	200ul	400ul
SDS	10%	1%	200ul	400ul
ddH2O (Milli-Q)			1.6ml	3.2ml

I. Harvest the cells

1) Harvest 1 x 10^7 of cells from the culture plates.
2) Trypsin cells and collect the cells in a 15ml tube.
3) Centrifuge at 3000g for 4mins.
4) Remove the supernatant and wash the cell pellet with 10ml PBS.
5) Centrifuge and remove the supernatant.
   - ❖ If cells are not use immediately, flash freeze in liquid N_2 and store at -80°C until needed.

II. Micrococcal Nuclease (MNase) Digestion

6) Prepare hypotonic lysis buffer and chill douncing buffer on ice
   - ❖ All buffers should contain PIC
7) Add 250ul of ice-cold dounce buffer to each sample and transfer to 1.5ml tubes.
8) Homogenize the cells by pipetting up and down and passing the cells through a 1ml syringe with 25 5/8 gauge needles 20 times.
9) Incubate the homogenized sample with 150U/ml MNase** (Concentration required varies from batch to batch of MNase -check before starting) for 10mins at 37°C, 1000rpm on a thermomixer (optimize the incubation time for the cell type you are using).
10) Stop the reaction by adding 5ul of 0.5M EDTA (final concentration = 10mM)
11) Incubate on ice for 5mins.
12) Add 1ml of hypotonic lysis buffer and mix.
13) Incubate on ice for 60mins, with brief vortexing at every 10mins interval.
14) Centrifuge at 3000g for 5mins and transfer the supernatant to a fresh 1.5ml siliconized tube.
    - ❖ If not proceeding to IP after checking nuclei size, transfer 100µl of the chromatin in to a fresh 1.5ml tube and then flash freeze the rest using liquid N_2 and store in -80°C until needed.

III. Checking Nuclei Size

15) Purify 100uL of chromatin using a QIAquick PCR purification Kit or phenol:chloroform followed by ethanol precipitation.
16) Elute or re-suspend the pellet in 50ul of ddH_2O.
17) Run 5ul of the sample on 1.5% agarose gel to check the size.
    - ❖ Run at 100V, for 40 minutes using a 50bp ladder.
    - ❖ Nucleosome sized bands should be seen

IV. Preparation of IgG Dynabeads

18) Make PBS/BSA solution. Add BSA to a final concentration of 5mg/ml in 1x PBS and chill on ice.
19) Re-suspend the stock of anti-rabbit/anti-mouse IgG Dynabeads (depending on the host of primary antibody) fully by inverting the bottles several times.
20) Take out 11ul of beads per sample from the bottle (adjust the amount of beads you take depending on how many samples you will have)
21) Put the beads on the magnetic rack for 2mins to allow beads to pellet.
22) Remove the supernatant and add 150µl of PBS/BSA(5mg/ml) to the beads.
23) Wash by inverting several times.
24) Pellet the beads by placing on magnetic racks for 2 min or spin at 4000rpm for 2 min.
25) Remove the supernatant and avoid disturbing the beads when discarding supernatant.
26) Repeat the wash twice (2X) with 150µl of the PBS/BSA.
27) Resuspend the beads in 50uL 1X PBS/BSA per sample.
28) Aliquot 50uL per tube of the beads into new PCR tubes

V. IP

29) For each IP tube, add 3µg of antibody of interest
30) Rotate the tubes at 4°C for 2hrs.
31) Remove the tubes from the rotator and place the samples on magnetic rack.
32) Once the beads have collected, carefully remove supernatant from the tube.
33) Wash 3 times with cold IP buffer (+PIC).
34) After the final wash, add 100µl of IP buffer (+PIC) to each tube.
35) For each IP tube, add 120µl (1million cells equivalent) of the chromatin to make up a final volume of 220µl.
    - ❖ Save 120uL of the chromatin to a new 1.5mL tube and freeze at -20°C. This is the Input.
36) Incubate at 4°C on a rotator overnight.

VI. Washing

37) Spin down the beads or place the samples on magnetic rack. Remove the supernatant carefully without disturbing the beads.
    - ❖ All subsequent washes should be performed as described.
38) Add 150µl (Dynabeads) of ChIP wash buffer and vortex gently for 10s. Rotate the beads at 4°C for 3min.
39) Repeat washing the beads using the ChIP wash buffer and then a final wash with Final ChIP Wash Buffer.
40) Remove all the wash buffer before elution.

VII. Elution

41) Retrieve the Input from -20°C and add elution buffer to bring up the volume to 200ul final volume.
    - ❖ Input should be included in all subsequent steps
42) Add 100ul elution buffer to IP samples (just the beads after the final wash) and transfer to a fresh 1.5mL tube.
43) Add 0.5ul of 10ug/ul RNase to all samples.
44) Incubate all samples at 68°C for 2hrs. Gently vortex the tubes a few times during the 2hr of incubation period. (If using a Thermomixer, set to 700rpm to keep the beads in suspension).
45) For the IP samples, spin down at 4000rpm for 2mins/put on magnetic rack. Transfer the supernatant into fresh 1.5ml tubes.
46) Repeat the elution of beads by adding another 100ul of elution buffer to the beads. Incubate the samples at 68°C for 5mins.

47) Remove all the samples for the heat block. Pool supernatant of the same sample from both rounds of elution together. The sample volume should be around 200ul for all.

VIII. Purification of DNA

48) Purify DNA using the QIAquick columns.
49) Add 5 volume of PB buffer to the samples (for 200µl of samples add 1ml of PB buffer)
50) Check the color of the mixture. If buffer turns purple add 3M NaOAc pH5.5 until the buffer color changes back to yellow.
51) Transfer the samples to a QIAquick spin column and centrifuge for 1min at max speed. (Repeat is there are successive aliquot of samples.)
52) Discard the flow-through.
53) Add 0.75ml buffer PE to the column and centrifuge for 1min at max rpm.
54) Discard the flow-through and centrifuge the column for an extra 2mins.
55) Warm the elution buffer to 42°C.
56) Air-dry the column by sitting it on the bench for 1min.
57) Place the column onto a fresh 1.5ml tube and add 60ul of the pre-warmed elution buffer to the center of the membrane.
58) Let sit for 1min and centrifuge for 1min at max rpm.
59) The sample is ready for library preparation, qPCR or any other downstream experiments. Store at -20°C.