**ChIP protocol**

**Step 1: Cell collection and fixation (after trypsinization)**

* Fix 10 million cells in 1% for 10min (need to be optimized according to different cell type) at RT.
* Quench with 125mM glycine at RT for 10 min.
* Wash cells in ice-cold PBS.
* Store cells in -80°C.

**Step 2: Cell lysis and Chromatin shearing**

* Resuspend the cell pellet in 1ml Hypotonic buffer and incubate the samples at 4 °C for 10 min.
* Centrifuge the hypotonic slurry at 1700g for 5 min at 4°C to collect the nuclei.
* Discard the supernatant and resuspend the pellet in 100ul Lysis buffer and incubate samples at 4°C for 10 min.
* Sonicate samples until the desired lengths of DNA fragments are achieved (100-500 bp)(check the fragments size on 1.5% agarose gel or bioanalyzer).
* Centrifuge the samples for 10 minutes at maximum speed at 4°C and transfer the supernatant to a clean dry microcentrifuge tube.
* Dilute samples 10 times using ChIP dilution buffer ( so the final concentration of SDS will be 0.1%, this concentration is safe for antibody binding)

**Step 3: Chromatin immunoprecipitation**

* Thoroughly resuspend the protein A(or protein G) beads in the solution and transfer 50μl of beads to 1.5ml tubes. Magnetize beads and discard supernatant.
* Wash protein A beads with 1ml PBST(0.1% Tween-20) and discard the supernatant, 2×.
* Resuspend beads with 200 μl ChIP dilution buffer.
* Add 3-5μg antibody to beads with ChIP dilution buffer and rotate at RT for 1 hour.
* Magnetize beads and discard supernatant and wash quickly with RIPA buffer.
* Add chromatin lysate to beads/antibody
* Rotate for 1hr at RT(or overnight at 4°C).
* Magnetize beads and discard supernatant.
* Wash with 500μl ChIP wash buffer 3X at RT(5 minutes each time).

Note: Before the last wash, transfer the resuspended beads to a new tube.

* Resuspend beads with 175 μl elution/reverse-crosslinking buffer.
* Perform RNase A digestion at 37°C for 30min and then add 20ul 10% SDS to final concentration of 1% and 5ul proteinase K(final: 0.5mg/ml) at 65°C for additional 4hr.
* Purify eluted DNA using column or phenol chloroform.

**Hypotonic buffer**

10mM Tris-HCl

10mM NaCl

1mM EDTA

0.2% Triton X-100

**Lysis buffer**

50mM Tris-HCl pH8.0

10mM EDTA

1% SDS

**ChIP dilution buffer**

0.01% SDS

1.1% Triton X 100

1.1mM EDTA

20mM Tris-HCl

167mM NaCl

**Wash buffer**

20mM Tris-HCl

500mM LiCl

1% NP40

1% deoxycholate

1mM EDTA

**Elution/reverse-crosslinking buffer**

10mM Tris-HCl

10mM EDTA

300mM NaCl

100ug/ml RNase A

**RIPA buffer**

0.1% deoxycholate

0.1% SDS

1% Triton X-100

10mM Tris-HCl

1mM EDTA

140mM NaCl