**Xie Lab 2016/07/11**

**STAR ChIP-seq protocol**

1. Each sample is lysed in 20ul lysis buffer (0.5% NP-40, 0.5% Tween, 0.1% SDS and proteinase inhibitor), pipette samples up and down several times and put them on ice for 5 minutes. (Cultured cells can be directly lysed in MNase working buffer plus 0.2% Triton X-100 and proteinase inhibitor)
2. Add 20ul MNase working buffer(100mM Tris-HCl ph8.0, 2mM CaCl2). Mix gently.
3. Add 5ul diluted MNase(Sigma, N3755-200UN) and flick tube to mix, then put in 37℃ block for 5 minutes. (MNase concentration should be tested beforehand in order to get best digestion result. Here 0.5U is a recommended working concentration. MNase storage buffer: 20mM HEPES-KCl (PH7.4), 50mM KCl, 50% Glycerol) Terminate reaction by adding 2ul Stop buffer(500mM EDTA and 500mM EGTA).
4. Add 50ul cold “2xRIPA buffer”(1% Triton X-100, 280mM NaCl, 0.1% SDS, 0.2% DOC 5mM EGTA supplemented with 2 ul proteinase inhibitor) to be brought to RIPA conditions. Vortex thoroughly.
5. Spin at 12000rpm in 4℃ for 10 minutes.
6. Transfer supernatants to new tubes and keep them on ice.
7. Before adding antibody, each chromatin sample is supplemented with 70 ul RIPA buffer (10mM Tris-HCl ph8.0, 140mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% DOC, 1mM EDTA). IP sample is incubated with 1~1.5ug histone antibody(1.5 ul K27ac antibody; 4 ul K4me3 antibody; 2 ul K36me3; 2ul K27me3), 4℃ overnight.
8. Each IP will require 300 ug (10 ul x 30 ug/ul) protein A/G dynabeads(K27ac K4me3 with protein A, K27me3 and K36me3 with protein G)(Life technologies).
9. In the next day, Wash the appropriate amount dynabeads with RIPA buffer for three times. Resuspend them in 200ul RIPA buffer. Add 300ug beads to every IP sample and incubate 3 hours with rotation at 4℃.
10. Prepare LiCl wash buffer(250 mM LiCl, 10mM Tris-HCl ph8.0, 1mM EDTA, 0.5% NP-40, 0.5% DOC). Place samples on a magnetic rack to discard unbound chromatin. Wash the beads 5 times in 180ul RIPA buffer and once in 180ul LiCl buffer with rotation at 4℃ (all for 5 min each time).
11. Spin tubes briefly and use a pipette to remove LiCl buffer completely. For each IP sample, resuspend beads with 27ul ddH2O plus with 1ul 10x ExTaq buffer(TaKaRa). Add 1ul proteinase K(less than 10000 cells; 2ul pK for more than 10000 cells)(Roche) to elute DNA. Incubate with shaking at 1400rpm in a 55℃ block for 2-3 hrs (longer with more cells).
12. Spin tubes briefly and place them on magnetic rack. Transfer supernatant to a new tube and put in a 72℃ block for 40minutes to inactive proteinase K.
13. Add 1ul rSAP to dephosphorylate 3’ end of DNA and incubate in a 37℃ block for 1 hour. Inactive rSAP at 65℃ for 10 minutes.
* At this stage, samples can be stored at -20 ℃ .
1. There is no need to purify the 29ul sample. Go directly for TELP library preparation. Add 1ul 1mM dCTP for TdT reaction. The following processes are the same as previously described. PCR was performed to amplify the library for 14-16 cycles for about 500 cells.