Chromatin preparation from small cell populations

Cross-linking

1. Spin down cells at max speed for 10s at RT then aspirate supe.
2. Re-suspend cell pellet in 500 µl PBS and chop with fine scissors if necessary.
3. Add 500 µl 2.22% formaldehyde/PBS.
4. Rotate 10 min at RT for Transcription factor or histone modification ChIP, or 15 min at RT for co-activator ChIP.
5. Stop cross-linking by adding 59 µl 2.5 M glycine to final concentration 0.14 M.
6. Rotate 5 min at RT.
7. Spin down cells at max speed for 10s at RT then aspirate supe.
8. Add 1mL PBS to wash, spin down cells, and aspirate supe.
9. Bring up pellet in 200-800 µl cold ChIP Whole Cell Lysis Buffer.
10. Homogenize with small plastic pestle by hand and incubate on ice for 10 min.

Sonication and Input Prep

11. Sonicate using BioRuptor :
    a. High setting, water cooler on, 15 min, cycling 30s on- 30s off
12. Spin in microfuge at max RPM at 4°C for 15 minutes.
13. Take off 10 µl as Input, and add it to 90 µl PBS. Add 3.5 µl 5 M NaCl. Heat this at 65 °C overnight.
14. Freeze remaineder in liquid nitrogen.
15. The next day, add 4 µl 1M Tris-HCL (pH 7.5), 2 µl 500mM EDTA, and 1 µl 1-mg/mL Proteinase K to the input sample.
16. Incubate 1 hour at 45 °C.
17. Purify un-crosslinked chromatin using Qiagen PCR purification kit. Elute in 50µl EB.
18. Check for appropriate shearing using the Bioanalyzer or running on a gel.

Buffers

1. 2.22% formaldehyde/PBS (make fresh)
   a. For 1 mL : 60 µl 37% formaldelhyde + 940 µl PBS
2. 2.5 M glycine (make fresh)
   a. 190 mg in 1ml H_2O
3. ChIP Whole Cell Lysis Buffer:
   (10 mM Tris-Hcl, pH 8.0, 10 mM NaCl, 3 mM $MgCl_2$, 1% NP-40, 1% SDS, .5% DOC)

1M Tris-Hcl (pH 8.1)	10 µl
5M NaCl	2 µl
1M $MgCl_2$	3 µl
10% NP-40	100 µl
10% SDS	100 µl
10% DOC	50 µl
50X Protease Inhib.	20 µl
H_2O	715 µl
	1 mL