

## Kim hyun Lab – ChIP assay

Original ChIP protocol (Sooyoung) - cell (60 mm dish – 3 tubes, IP, 100 mm dish – 5 tubes IP) Brain tissue – glycine add

Generally, DNA, 5, 10, or 20 ug,  
H3, H4, 2ug, 2hr, 5 ul PCR -> result good  
Generally, DNA 20 ug, 2 ug antibody, ON

- 1) Washing-Cold PBS washing (x2)
- 2) Fixation-1% formaldehyde, 10 min RT (3 ml), longer time -> bad sonication yield
- 3) Washing-RT PBS washing (X2) (1ml)
- 4) Cell harvest- 100 mM TrisHCl (pH9.4), 10 mM DTT (fresh buffer), (500 ul, do within 5 min)
- 5) Centri – 5000 rpm, 3min
- 6) Washing – PBS, clear I, clear II buffer (each 5000 rpm 3min), careful pipetting  
Clear I ---0.25 Triton X-100, 10 mM EDTA, 0.5mM EGTA, 10 mM HEPES, pH6.5  
Clear II--- 200 mM NaCl, 1 mM EDTA, 10mM HEPES, pH6.5
- 7) Lysis buffer 15min, staining  
Lysis buffer --- 1% SDS, 10 mM EDTA, 50mM Tris-HCl, pH8.1
- 8) Sonication- 30%- 15 sec, 4 times (45 sec rest)
- 9) 100 ul (working) into E tubes, input 10ul (working 1/10), check OD
- 10) Dilution buffer – 900ul

Dilution buffer --- 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl pH 8.1

- 11)Preclearing – protein sepharose (G/A) 50% slurry 40 ul+4 ug sperm DNA/ One tube (1 hr, 4'C, 16 roter)
- 12)Centri – 3000 rpm 2 min (important step)
- 13)Supernatant (SNT), Antibody add (2-4 ug usually)
- 14)1hr to ON, 4'C
- 15)Protein sepharose G/A (same to precleaning)
- 16)1 hr, 4'C
- 17)3000 prm, 2 min
- 18)Washing, -TSEI, TSEII, buffer III – each 10 min (4'C, last buffer, RT)  
TSE I--- 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20 mM Tris-HCl, pH 8.1, 150mM NaCl  
TSE II --- 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20 mM Tris-HCl, pH 8.1, 500mM NaCl  
Buffer III --- 0.25M LiCl, 1% NP-40, 1% deoxycholate, 1mM EDTA, 10 mM Tris-HCl pH8.1
- 19)Washing-TE (2min, x3)
- 20)Extract (each, 100 ul, 15 min, X3)  
Extraction buffer --- NaHCO3 (100mM), 1% SDS
- 21)65'C, at least 15 min, (Nacl add, final 250mM- increase yield)
- 22)Elution (Quiagen)