



## **Chromatin Immunoprecipitation (ChIPs) Protocol (Breedon Lab)**

*This protocol has some minor modification to the protocol described in Strahl-Bolsinger S. et al. [1997, Gen & Dev 11, p83-93] and was obtained from Flick K. (The Scripps Research Institute).*

### **Protocol:**

1. Use 50 ml cells OD<sub>600nm</sub> = 0.7-1.0 per time point / sample
2. Add 1.35 ml 37 % Formaldehyde (endconcentration = 1 %), incubate 15 min at 25°C
3. Add 2.5 ml 2.5 M Glycine, incubate 5 min at 25°C
4. Spin down, wash once with 20 ml PBS
5. Transfer to 2 ml Eppendorf tube, wash again with PBS and freeze cells or proceed on
6. Resuspend in 200-400 µl ChIP lysis buffer, add an equal volume of glass beads
7. Shake for 30 min at 4°C on vortexer (maximum level)
8. Pierce tube bottom with needle and spin liquid into fresh tube
9. Resuspend extracts and sonicate for 30 sec level 2 (Branson, microtip probe)
10. Spin extract for 10 min, 10,000 rpm, at 4°C
11. Take supernatant and measure protein concentration (BioRad assay)
12. Use 1-5 mg protein per IP
13. Immunoprecipitate for 2 h to ON, 4°C



14. Wash immunoprecipitations pelleting the beads each time:
  - 2 x 1 ml ChIP lysis buffer
  - 2 x 1 ml ChIP lysis buffer (high salt)
  - 2 x 1 ml ChIP wash buffer
  - 2 x 1 ml TE
  
15. Elute immunoprecipitates: add 75  $\mu$ l elution buffer
  
16. Incubate for 10 min at 65°C
  
17. Spin, take supernatant, elute pellet again with 75  $\mu$ l elution buffer
  
18. Combine supernatants, incubate at 65° for 6 h to ON
  
19. Take 1/100 of the protein amount taken for the IP, add to 150  $\mu$ l elution buffer (INPUT control)
  
20. Incubate at 65°C for 6 h to ON
  
21. Add 750  $\mu$ l PB buffer (Qiagen PCR purification kit)
  
22. Purify DNA through Qiaquick column
  
23. Elute DNA into 50  $\mu$ l H<sub>2</sub>O
  
24. Use 0.5-1  $\mu$ l per 25  $\mu$ l PCR reaction:
  - 1 x 95 °C, 2 min
  - 21 x 95 °C, 30 sec; 60 °C, 30 sec; 72 °C, 1 min
  - 1 x 72 °C, 3 min

primers (1  $\mu$ M): 20-24 bp, 50 % GC, producing a 200-500 bp fragment
  
25. PCR products (10-15  $\mu$ l) are separated on 2 % agarose gels or 5-6 % non denaturing PAA gels



## **Buffers:**

### **2.5 M Glycine:**

For 500 ml: 93.84 g

### **PBS:**

For 1L: 8 g NaCl; 0.2 g KCl; 1.15 g Na<sub>2</sub>HPO<sub>4</sub> \* 7H<sub>2</sub>O; 0.2 g KH<sub>2</sub>PO<sub>4</sub>]

### **CHIP lysis buffer:**

50 mM HEPES pH 7.5, 140 mM NaCl, 1 % Triton X100, 0.1 % NaDeoxycholate, protease inhibitors. (for 500 ml: 25 ml 1 M HEPES pH 7.5, 18 ml 4 M NaCl, 50 ml 10 % Triton X100, 5 ml 10 % NaDeoxycholate, protease inhibitors)

### **CHIP lysis buffer (high salt):**

50 mM HEPES pH 7.5, 500 mM NaCl, 1 % Triton X100, 0.1 % NaDeoxycholate, protease inhibitors. (for 500 ml: 25 ml 1 M HEPES pH 7.5, 62.5 ml 4 M NaCl, 50 ml 10 % Triton X100, 5 ml 10 % NaDeoxycholate, protease inhibitors)

### **CHIP wash buffer:**

10 mM Tris pH 8.0, 250 mM LiCl, 0.5 % NP-40, 0.5 % NaDeoxycholate, 1 mM EDTA. (for 500 ml: 5 ml 1 M Tris pH 8.0, 5.3 g LiCl, 25 ml 10 % NP-40, 25 ml 10 % NaDeoxycholate, 1 ml 0.5 M EDTA)

### **elution buffer:**

50 mM Tris pH 8.0, 1 % SDS, 10 mM EDTA. (for 10 ml: 0.5 ml 1 M Tris pH 8.0, 1 ml 10 % SDS, 0.2 ml 0.5 M EDTA)