Upload image

Version 2

Aug 11, 2020

Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation V.2

Georgios I Laliotis[1], Philip N. Tsichlis[1]

[1]The Ohio State University

Georgios I Laliotis: Corresponding Author

1 Works for me dx.doi.org/10.17504/protocols.io.bjj6kkre

George Laliotis

DOI

dx.doi.org/10.17504/protocols.io.bjj6kkre

PROTOCOL CITATION

Georgios I Laliotis, Philip N. Tsichlis 2020. Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjj6kkre

CREATED

Aug 11, 2020

LAST MODIFIED

Aug 11, 2020

PROTOCOL INTEGER ID

40286

Chemicals Required 1d

1 1. Cytosolic Lysis Buffer (200 ml) ; 5 mM PIPES (pH 8.0), 85 mM KCl, 0.5% NP40 + PI

PIPES 0.3 g =>Adjust pH 8.0
2M KCl 8.5 ml
10% NP40 10 ml

2. Nuclear Lysis Buffer (50 ml) ; 50 mM Tris (pH 8.0), 10 mM EDTA, 0.5% SDS

1 M Tris (pH 8.0) 2.5 ml
0.5 M EDTA 1 ml
20% SDS 1.25 ml

3. IP Dilution Buffer (250 ml) ; 16.7 mM Tris (pH 8.0), 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, 0.01% SDS

1 M Tris (pH 8.0) 4 ml
0.5 M EDTA 0.6 ml
5 M NaCl 8.5 ml
20% Triton X-100 12.5 ml
10% SDS 250 ul

Citation: Georgios I Laliotis, Philip N. Tsichlis (08/11/2020). Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation. https://dx.doi.org/10.17504/protocols.io.bjj6kkre

4. Low Salt Wash Buffer (250 ml); 20 mM Tris(pH8.0), 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 0.1% SDS

1 M Tris (pH 8.0)	5 ml
0.5 M EDTA	1 ml
5 M NaCl	7.5 ml
20% Triton X-100	12.5 ml
10% SDS	2.5 ml

5. High Salt Wash Buffer (250 ml); 20 mM Tris(pH8.0), 2 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% SDS

1 M Tris (pH 8.0)	5 ml
0.5 M EDTA	1 ml
5 M NaCl	25 ml
20% Triton X-100	12.5 ml
10% SDS	2.5 ml

6. NS Buffer ; 50 mM Hepes, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS

7. LiCl Wash Buffer (250 ml); 10 mM Tris (pH8.0), 1 mM EDTA, 250 mM LiCl, 1% NP40, 1%(w/v) deoxycholic acid

1 M Tris (pH 8.0)	5 ml
0.5 M EDTA	0.5 ml
1 M LiCl	72.5 ml
10% NP40	50 ml
Deoxycholic acid	0.25 g

8. Miscallenous

1.25 M Glycine	9.38 g / 100 ml	
1 M NaHCO3	8.4 g / 100 ml	=> aliqute (0.5 ml) and keep at -20 °C

Cross-linking and Lysis of the cells 6h

2

- **For cell lines** (20-50x10^6 cells)

For each Ab to be tested, 3 plates for each cell line must be used.

1. Add 270 ul of 37% Formaldehyde into 10 ml culture media (P100)
2. Incubate at 37 °C for 15 min
3. Add 1 ml of 1.25 M Glycine and incubate 5 min at RT
4. Wash with cold PBS (x2)
5. Wash with 1 ml of cold PBS (+ PI)
6. Scrap cells and collect into a new tube
7. Tip : Scrap each plate and place it in a different tube
8. Centrifuge at 14000 rpm for 1 min at 4 °C to remove PBS
9. Remove supernatant
10. Incubate lysis buffer in 37 deg. For a few minutes to dissolve precipitants
11. Add fresh 1x proteinase inhibitor-10ul from 100x stock
12. Add/Resuspend 1 ml of nucleic lysis buffer (+PI) per 3 P100 plates ==> 200ul for 1x10^6
13. Manually shake tube to break pellet
14. Split the 1ml lysate in 2 tubes with 500ul-Helps the sonication process
15. Incubate on ice for 10'
16. Sonication : 15 sec on/45 sec off (60 sec x 6-7 times = 6-7 min on/each sample)
    at 30% duty
17. Centrifuge at 14000 rpm for 15 min at 4 °C
18. Transfer supernatant into a new single tube
    * can be stored at -80 °C

Citation: Georgios I Laliotis, Philip N. Tsichlis (08/11/2020). Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation. https://dx.doi.org/10.17504/protocols.io.bjj6kkre

* keep 10% lysate (50ul-10% of 500ul Ab solution) for input-you can dilute it to 1% I put for qPCR analysis

Immunoprecipitation of Crossliked protein/DNA complex 1d 6h

3 1. Dilute 5 fold of lysate volume with IP dilution buffer in a 15 ml tube
2. Add 1x protease inhibitors in the IP dilution buffers
3. Dilute to 5ml from initial 1ml using 4ml IP dilution buffers
4. Split in 2x tubes for Ab and 2 for normal IgG

- **Caution!Each tube is HALF REACTION**
  => final buffer concentration) 0.1% SDS of nucleic lysis buffer

5. Pre-cleaning : Add 30 ul of protein G bead / Ab reaction for pre-clearing per tube
6. Incubate for 1 hr at 4 °C with rotation
7. Centrifuge at 3000 rpm for 2 min at 4 °C
8. Transfer supernatant into a new 2 ml tube
9. Add 2 ug of antibody or normal IgG per tube (for Normal Rabbit IgG add 2ul from 1mg/dL. In order to make that add 8.77ul from 11.4mg/dl stock and 92.23 ddH20)
10. Incubate overnight at 4 °C with rotation
11. Add 25 ul of protein G agarose bead / Ab reaction per tube
12. Incubate for 2 hrs at 4 °C with rotation
13. Centrifuge at 3000 rpm for 2 min at 4 °C
14. Place beads at least 5 minutes in magnetic rack
15. Remove supernatant carefully with WB long tips gradually
16. Add/Resuspend beads 500 ul cold low salt wash buffer/tube and combine tubes in 1
17. Rotate for 5' at 4 deg
18. Wash with wash buffer

Order : cold low salt wash buffer (x2)
cold high salt wash buffer (x2)
cold LiCl wash buffer (x2)
cold TE buffer (x3)

19. Centrifuge at 3000rpm for 2'
20. Place on magnetic rack for 5'
21. Add 500ul of buffer
22. incubate for 5 min at 4 °C with rotation
23. Centrifuge at 3000 rpm at RT (or 4 °C) for 1 min
24. Place on magnetic rack for 5'
25. Remove supernatant
26. Wash with next one etc.
27. Add 200 ul elution buffer (0.1M $NaHCO_3$and 1% SDS)

For 1.5ml :

1,2ml H20 first
then, 150ul 10x SDS
finally, 150ul 1M NaHCO3

28. Incubate for 15 min with shaking heating block (900 rpm) at 23 °C
29. Centrifuge at 3000 rpm for 3 min
30. Put in magnetic rack for 5'-Transfer supernatant into a new tube
31. Repeat #16-19 (Total elution vol. will be 400 ul)

Reverse Crosslinking and Elution of Protein/DNA complexes 6h

4 32. Add 20 ul of 5M NaCl and 1 ul of 20 mg/ml RNase / 200 ul tube (remember the volume is 400ul)

* Don't forget input sample (10% lysate + 350ul Elution buffer)!!

Citation: Georgios I Laliotis, Philip N. Tsichlis (08/11/2020). Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation. https://dx.doi.org/10.17504/protocols.io.bjj6kkre

33. Mix well manually before putting into the cycler
34. Incubate at 65 °C in Thermo cycler for 5 hrs or overnight with 450rpm rotation to prevent precipitation
35. Add 10 ul of 0.5M EDTA, 20 ul of 1M Tris (pH 8.0), and 2 ul of 10 mg/ml Proteinase K/ tube
36. Incubate at 45 °C for 1 hr

QIAGEN PCR extraction kit (Or use P/C/I extraction method)

37. Add 5 vol. of PBI buffer (QIAGEN PCR extraction kit) and mix well-450ul ==> 2,25ml Final volume
    * Check PH => add 10 ul 3M sodium acetate if #23 turn orange or violet color.

38. Add sample into a column-max 800ul
39. Centrifuge at 14000 rpm for 1 min
40. Add 750 ul of PE buffer
41. Centrifuge at 14000 rpm for 1 min and remove liquid from a tube
42. Centrifuge at 14000 rpm for 1 min again
43. Add 60 ul of EB buffer
44. Centrifuge at 14000 rpm for 1 min
45. Store DNA elution at -20 °C
46. Carry out qPCR using 2 ul elution/reaction

Citation: Georgios I Laliotis, Philip N. Tsichlis (08/11/2020). Effective identification of DNA-bound protein complexes using Chromatin Immunoprecipitation. https://dx.doi.org/10.17504/protocols.io.bjj6kkre