Restriction Enzyme Accessibility Protocol in Mammalian Cells

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[Abstract] This protocol describes a method to indirectly assess chromatin structure by using restriction enzyme in mammalian cells, modified from Current Protocols.

Materials and Reagents

1. Tris
2. NaCl
3. $MgCl_2$
4. DTT
5. EDTA
6. SDS
7. Chloroform
8. PBS
9. NP-40
10. 70% EtOH
11. NaAc
12. Phenol/chloroform
13. Digest buffer (NEB)
14. Restriction enzyme (NEB)
15. TE (Tris 10 mM, EDTA 1 mM) buffer
16. Proteinase K (Promega corporation, catalog number: V3021)
17. NP-40 (United States biological corporation, catalog number: N3500)
18. Lysis buffer (see Recipes)
19. Wash buffer (see Recipes)
20. Stop solution (see Recipes)

Equipment

1. Type B dounce homogenizer
2. Centrifugation
3. Falcon 2059 tube

Procedure

1. Harvest cells (1 x 10^8 cells for 4 conditions) by centrifugation for 10 min at 1,000 rpm 4 °C.
2. Wash cells once in x 20 pellet vol. with ice cold PBS by gentle resuspension and transfer to microfuge tube.
3. Spin for 6 min at 1,000 rpm 4 °C.
4. Resuspend cells in 1x 1.5 pellet vol. of cell lysis buffer. Add 5 mM DTT immediately prior to use.
5. Lyse cells with 10 “extremely gentle strokes” of type B Dounce homogenizer (if sample vol. is >500 µl) and transfer to microfuge tube or Falcon 2059 tube (if sample vol. is <500 µl resuspend gently in cell lysis buffer and incubate for 5 min on ice).
6. Collect nuclei by centrifugation for 5 min tomy/2,500 rpm/4 °C.
7. Wash nuclei by gentle resuspending in x1.5 pellet vol. of wash buffer.
8. Collect nuclei by centrifugation for 4 min, 2,000 rpm Tomy.
9. Resuspend washed nuclei in (1x) digest buffer NEB#2(+5 mM DTT).
10. Depending on how many conditions resuspend nuclei in desired amount and divide into 200 µl aliquots.
11. Add enzyme (0, 50, 100,200, 400 units).
12. Incubate 45 min 37 °C.
13. Stop rxn with stop sol (300 µl) and put on ice; Vortex briefly after adding stop solution.
14. Add proteinase K at 0.4 mg/ml from 20 mg/ml stock. Vortex to mix.
15. Incubate at 37 °C for at least 6 h (or O/N).
16. Transfer to Falcon 2059 tube and dilute to 2 ml with TE (Tris 10 mM, EDTA 1 mM) buffer.
17. Extract with freshly equilibrated phenol/chloroform. Spin 8,000 rpm SS-34. Repeat extraction 3-4 times until you see nothing at the interface.
18. Do 1-2 chloroform extraction.
19. Precipitate DNA with NaAc/EtOH. Rock the tube gently side to side to have DNA tangle form. If DNA is more than 100 µg you should see white precipitate. If less than 100 µg freeze at -70 °C for 10 min. spin down the precipitate.
20. Wash DNA with 2 ml of 70% EtOH.
21. Air dry DNA ~20 min in the hood (make sure you don’t over dry it).
22. Resuspend in ~200 µl TE. May increase the vol. depending on the recovery of DNA.
23. Carefully determine the concentration of gDNA. The solution is very sticky. I typically take 8-10 µl in 800 µl of TE to determine the concentration.
24. Digest DNA with 2nd restriction enzyme. Typically 100-200 µl digest with 5-10 fold excess of enzyme for overnight. Add 10 µg tRNA and purify the digested DNA by one extraction with freshly equilibrated phenol/chloroform followed by one chloroform extraction. Air dry and resuspend in 30 µl TE buffer.
25. Run gel with 15 µg of genomic DNA/lane. 10 µg is often ok, but try to use more.

26. Perform southern blot analysis.

Notes

Do not stop in the middle during steps 1-18.

Recipes

1. Lysis buffer
   10 mM Tris (pH 7.5)
   10 mM NaCl
   3 mM $MgCl_2$
   0.2% NP-40
   10 mM DTT
2. Wash buffer
   10 mM Tris (pH 7.5)
   10 mM NaCl
   3 mM $MgCl_2$
   10 mM DTT add right before use.
3. Stop solution
   10 mM Tris
   25 mM EDTA
   1% SDS

References

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2. Tack, L. C., Wassarman, P. M. and DePamphilis, M. L. (1981). Chromatin assembly. Relationship of chromatin structure to DNA sequence during simian virus 40 replication. *J Biol Chem* 256(16): 8821-8828.

