Mostoslavsky's Lab Protocols

Chromatin extraction

Materials

1. Lysis buffer: 10 mM HEPES pH 7.4, 10 mM KCl, 0.05% NP-40. For 50 ml, mix 500 ml of 1 M HEPES pH 7.4, 500 ml of 1 M KCl and 25 ml of NP-40 (See Note 2). Make up to 50 ml with water and store at 4°C. Prior to use, add protease, phosphatase (1 mM sodium ortovanadate) and deacetylase (5 mM TSA) inhibitors.

2. Low salt buffer: 10 mM Tris-HCl pH 7.4, 0.2 mM $MgCl_2$. For 50 ml, mix 500 ml of 1 M Tris-HCl pH 7.4 and 100 ml of 1 M $MgCl_2$ and make up to 50 ml (See Note 3). Store at 4°C. Prior to use, add protease, phosphatase (1 mM sodium ortovanadate) and deacetylase (5 mM TSA) inhibitors.

3. 0.2 N HCl: for 10 ml, dilute 165.3 μl of 12.1 N HCl in 10 ml of water.

4. 1 M Tris-HCl pH 8: for 10 ml, dissolve 1.21 g of Tris in 5 ml of water and adjust the pH to 8. Make up to 10 ml with distilled water.

Methods

This protocol is adapted with modifications from Junjie Chen's laboratory.

1. Collect cells and wash them in 1X PBS.

2. Resuspend cell pellet in 2-5 volumes of lysis buffer.

3. Incubate 20 min on ice.

4. Centrifuge at 14,000 rpm at 4°C, 10 min. The **supernatant** contains the **cytoplasmatic proteins** and the pellet contains the nuclei.

5. Wash the nuclei once with lysis buffer and centrifuge at 14,000 rpm at 4°C, 10 min. If nucleoplasmatic proteins are desired go to step 6, otherwise go to step 8.

6. OPTIONAL: Resuspend the nuclei with 2-5 volumes of Low Salt Buffer+1% Triton-X 100 and incubate 15 min on ice.

7. Centrifuge at 14,000 rpm at 4°C, 10 min. The **supernatant** contains the **nucleoplasmatic proteins,** and the pellet contains the chromatin.

8. Resuspend chromatin with 2-5 volumes of HCl 0.2N and incubate 20 min on ice.

9. Centrifuge at 14,000 rpm at 4°C, 10 min.

10. Keep and neutralize SN (contains the acid soluble proteins) with the same volume of 1M Tris-HCl pH 8. This would be the chromatin fraction.

References

Huang, J., Huen, M.S., Kim, H., Leung, C.C., Glover, J.N., Yu, X., and Chen, J. (2009). RAD18 transmits DNA damage signalling to elicit homologous recombination repair. *Nat Cell Biol 11*, 592-603.