Fractionation of nuclear or cytoplasm proteins

1. Twenty million transfected cells were harvested and washed twice with ice-cold PBS followed by resuspending the cell pellet in a hypotonic buffer A (10mM HEPES-K^+ pH7.5, 10mM KCl, 1.5mM $MgCl_2$, 0.5 DTT) in the presence of protease inhibitor cocktail (PIC: 1mM PMSF, 10μg/ml aprotinin, 10μg/ml leupeptin, 10μg/ml pepstatin A, 10μg/ml phenanthroline and 16μg/ml benzamidine).
2. Cells were pelleted by spinning at 1000×rpm 5 min. The cells were lysed in ice-cold 0.5% NP-40 containing buffer A with PIC on ice for 10 min.
3. The nuclei were pelleted at 3,000 rpm 2 min, 4°C centrifugation.
4. The supernatant (cytoplasm protein) harvested and frozen at –80°C for use.
5. The nuclear pellets were washed with buffer A (without NP-40), followed by resuspending in buffer C (20mM HEPES-K^+ pH7.9, 420mM NaCl, 0.2mM EDTA, 1.5mM $MgCl_2$, 0.5 DTT, 25% Glycerol) with PIC.
6. Nuclei were incubated on ice for 30 min, and vortex periodically.
7. Supernatant containing nuclear protein were collected by spinning at 14,500rpm for 10 min at 4°C and then snap frozen for further use.
8. Antibodies against nuclear protein Sp1 (1C6, Santa Cruz Inc., Santa Cruz, CA) and cytoplasm protein Hsp70 (BD transduction laboratory, San Jose, CA) were used as markers.