Mostoslavsky's Lab Protocols

Nucleosome immunoprecipitation

Materials

1. Lysis buffer: 10 mM HEPES pH 7.4, 10 mM KCl, 0.05% NP-40, 0.5 mM DTT and 1 mM PMSF. For 50 ml, mix 500 ml of 1 M HEPES pH 7.4, 500 ml of 1 M KCl, 25 ml of NP-40, 25 μl of 1M DTT and 100 μl of 0.5 M PMSF (See Note 1 and 2). Make up to 50 ml with water and store at 4°C.

2. Wash buffer: 20 mM HEPES pH 7.4, 20 mM KCl, 0.5 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF. For 50 ml, mix 1 ml of 1M HEPES pH 7.4, 1 ml of KCl, 50 μl of 0.5 M EDTA, 25 μl of 1M DTT and 50 μl of 0.5 M PMSF (See Note 3). Make up to 50 ml with water and store at 4°C.

3. Buffer A: 20 mM HEPES pH 7.4, 20 mM KCl, 0.4 mM EDTA, 0.4 mM DTT. For 50 ml, mix 1 ml of 1M HEPES pH 7.4, 1 ml of KCl, 40 μl of 0.5 M EDTA and 20 μl of 1M DTT.

4. 5 M NaCl. For 50 ml, dissolve 14.62 g of NaCl in 50 ml of water.

5. 2 M $CaCl_2$. For 50 ml, dissolve 11.1 g of $CaCl_2$ in 50 ml of water.

6. Microccocal nuclease (Roche, cat # 10107921001)

7. 0.5 M EGTA. For 50 ml, dissolve 9.5 g of EGTA in 50 ml of water.

8. 10% NP-40. For 50 ml, dilute 5 ml of NP-40 in 50 ml of water.

9. Agarose beads

10. Laemmli buffer: 200 mM Tris-Cl pH 6.8, 8% SDS, 40% Glycerol, 4% β-mercaptoethanol, and 0.08% bromphenol blue. For 20 ml, add 4 ml of 1M Tris-Cl, 8 ml 20% SDS, 8 ml Glycerol, 0.8 ml β-mercaptoethanol and 16 mg of bromphenol blue.

Methods

This protocol is adapted with modifications from Don W. Cleveland's laboratory.

1. Collect cells (15-30 x 10^6 per IP) and wash them with 1 ml of 1X PBS.

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

3. Incubate 20 min on ice.

4. Pellet nuclei at 300xg at 4°C, 10 min.

5. Wash the nuclei once in 1 ml of wash buffer and centrifuge at 1000xg at 4°C, 5 min.

6. Wash the nuclei once in 1 ml of wash buffer containing 300 mM NaCl and centrifuge at 1000xg at 4°C, 5 min.

7. Resuspend nuclei in 1 ml of wash buffer containing 300 mM NaCl and 3 mM $CaCl_2$. Digest chromatin for 1h at room temperature using 140 units/ml of microccocal nuclease (see Note 10).

8. Stop reaction by adding 10 μl of 0.5 M EGTA (5 mM final) and 5 μl of 10 % NP-40 (0.05 % final) and centrifuge at 10,000xg for 15 min at 4°C. Keep the supernatant, which contains the soluble chromatin (mainly mononucleosomes) (see Note 4).

9. Incubate chromatin extract with the antibody overnight at 4°C in an orbital rotator.

10. Add 40 μl of agarose beads (50 % slurry) and incubate 4h at 4°C in an orbital rotator.

11. Pellet the beads at 3000xg for 2 min at 4°C.

12. Wash the beads sequentially with 1 ml of buffer A, 1 ml of buffer A with 300 mM KCl and 1 ml of buffer A with 300 mM KCl, 1 mM DTT and 0.1 % Tween-20. Centrifuge at 3000xg for 2 min between washes to pellet the beads.

13. Elute the immunocomplexes by boiling the beads with 50 μl of Laemmli buffer for 10 min.

Notes

1. To prepare 1 liter of 1M HEPES pH 7.4, dissolve 238.3 g of HEPES in 800 ml of water, adjust pH at 7.4 and make up to 1 liter with water. To prepare 1 liter of 1M KCl, dissolve 74.55 g of KCl and make up to 1 liter with water.

2. To prepare 1 liter of 1M HEPES pH 7.4, dissolve 238.3 g of HEPES in 800 ml of water, adjust pH at 7.4 and make up to 1 liter with water. To prepare 1 liter of 1M KCl, dissolve 74.55 g of KCl and make up to 1 liter with water.

3. To prepare 1M solution of DTT, weigh and dissolve in 10 ml of water 1.54 g of DTT. Make aliquots of 0.5 ml and store at -20°C. To prepare 10 ml of 0.5 M PMSF, dissolve 0.87 g of PMSF in methanol and make up to 10ml. Make aliquots of 0.5 ml and store at -20°C. To prepare 1 liter of 0.5M EDTA, dissolve 146.12 g of EDTA and make up to 1 liter with water.

4. To confirm that most of the DNA is the mononucleosomal fraction, take an aliquot and purify the DNA as follows: add 1 volume of phenol/chloroform, mix well and centrifuge at 14,000 rpm for 3 min. Take aqueous phase (upper phase) and precipitate DNA with 1 volume of isopropanol. Centrifuge at 14,000 rpm for 5 min and discard the supernatant. Wash the pellet with 70% ethanol, centrifuge at 14,000 rpm for 3 min and air-dry the pellet. Resuspend DNA in water and run the samples in a 1.2 % agarose gel. The DNA present in mononucleosomes is about 147 bp in size.

References

1. Foltz, D.R., Jansen, L.E., Black, B.E., Bailey, A.O., Yates, J.R., 3rd, and Cleveland, D.W. (2006). The human CENP-A centromeric nucleosome-associated complex. *Nat Cell Biol 8*, 458-469.