BIORUPTOR® PROTOCOL

ACTIVITY TEST: PROCEDURE FOR IMMUNOPRECIPITATION OF NATIVE CHROMATIN PROTEINS.

Proteins play role in almost all cellular processes, and the study of their activity is crucial for understanding these mechanisms. Slimane AIT-SI-ALI and his "epigenetics and cell fate" team from the University Paris Descartes developed a protocol allowing the immunoprecipitation of chromatin proteins prior the analysis of their activity. They used the Bioruptor® in the initial step of the procedure to release this specific protein, which is advantageous in preserving protein quality.

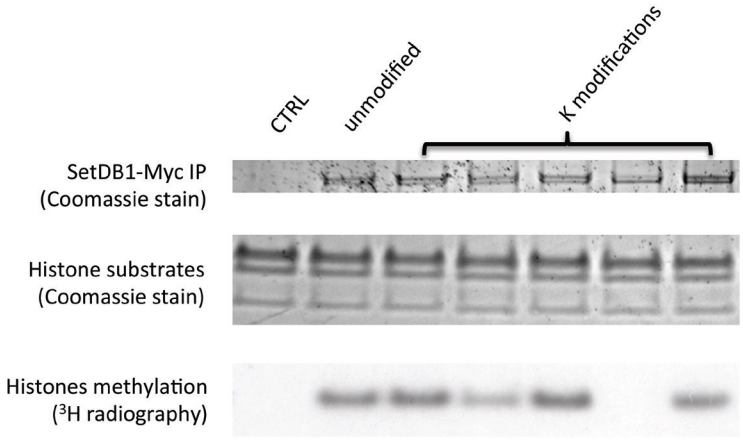

Figure: Protocol for activity testing of chromatin protein developed by Lauriane Fritsch at the Epigenetics and Cell fate group (Paris Diderot University). Coomassie blue staining of gel loaded with protein samples obtained by myc IP performed on cells over-expressing the Histone-lysine N-methyltransferase SETDB1 tagged with Myc (top panel). Histone methyltransferase activity measurement by incorporation of tritium (bottom panel).

1. Material required and buffer preparation

- Bioruptor® Standard (Cat. No. B01010001) or Bioruptor® Plus (Cat. No. B01020001) with 1.5ml tube holder
- 1.5ml TPX tubes (Cat. No. C30010010) and 1.5ml Eppendorf tubes
- 0.1M CaCl2
- Mnase (Sigma-Aldrich, catalog number n3755). Reconstitute in ultra pure water at 0,5U/µl.
- Rotation wheel
- Protein A (or G) agarose beads
- UltraLink beads (Life technologies)
- sssDNA 5ug/µl
- 10ug/µl BSA
- Buffer composition (store at 4°C):

⚠ Add Protease Inhibitor and 1mM DTT immediatly before use.

Lysis buffer (300mM NaCl)	*Helping hand for 50ml buffer preparation*
50mM Tris pH7.5	2.5mL Tris 1M pH 7.5
300mM NaCl	3 mL NaCl 5M
0.5% NP40	2.5 mL NP40 10%
10mM MgCl2	500 µl de MgCl2 1M
10% glycérol	5mL glycérol stock

Dilution buffer (NaCl free)	
50mM Tris pH7.5	2.5mL Tris 1M pH 7.5
0.5% NP40	2.5 mL NP40 10%
2.5mM CaCl2	250 µl de CaCl2 0,5M

IP buffer (140mM NaCl)	
50mM Tris pH7.5	2.5mL Tris 1M pH 7.5
140mM NaCl	1.4mL NaCl 5M
0.4% NP40	2mL NP40 10%
10mM MgCl2	5mL MgCl2 100µM

2. Procedure

Day 1

- Pellet cells and add 1x volume of lysis buffer. Transfer samples to 1.5ml TPX tubes and incubate for 30 min on ice.
- Add 0.0025U/µl Mnase and CaCl2 to a final concentration of 1mM.
- Install tubes in the Bioruptor®.
- Sonicate at High Power for 3-4 cycles (sonication cycle : 15 sec ON, 1 min OFF).
- Centrifuge at 40,000 rpm for 30 min at 4°C.
- Collect supernatant in a new 1.5 ml eppendorf tube and add 1 volume of dilution buffer.
- Pre-clearing :
  - Wash 30 µL protein G (or A) agarose beads with 1 ml IP buffer (spin at 1000 x g for 3 min at 4°C). Repeat 2 times.
  - Add beads slurry to the lysate.
  - Incubate for 90 to 120 min at 4°C under gentle agitation or rotation.
  - Collect supernatant by centrifugation at 1000 x g for 5 min at 4°C.
- Immunoprecipitation :
  - Add relevant antibody to the supernatant. We recommend the use of 5ug antibody / 500-1000ug proteins.
  - Incubate the lysate with the antibody overnight under gentle rotation at 4°C.
  - Prepare UltraLink beads for the next day :

    Gently shake UltraLink beads stock solution and pipet 7.5 µl per IP.

    Add 1ml of IP buffer and wash 3 times by centrifugation at 1,200 rpm for 2 min at 4°C.

    Add 1ml IP buffer containing 7.5 µl sssDNA and 2 µl of 10ug/µl BSA.

    Incubate overnight at 4°C on a rotation wheel.

Day 2

- Rinse UltraLink beads 2 times with 1ml IP buffer by centrifugation at 1,200 rpm for 2 min at 4°C and add 800 µl of IP buffer after the last wash.
- Collect supernatant from lysate/antibody mixture after a 10 min centrifugation at 13,000 rpm at 4°C and place in a new tube.
- Add 50 µl of rinsed UltraLink beads and incubate for 120 min at room temperature on a rotation wheel.
- Perfom 5 washes with 1ml IP buffer for 5 min at room temperature (centrifuge at 1,200 rpm for 2 min).
- Proceed with Western blot experiment.

Using Bioruptor® in a creative way?

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