BIORUPTOR® PROTOCOL

TAP-TAG: PROCEDURE FOR THE PURIFICATION OF A CHROMATIN PROTEIN WITH BIORUPTOR®

The Tandem Affinity Purification (TAP) is a general procedure for the purification of protein complex. The fusion of the TAP tag to the protein of interest allows the rapid purification under a native environment. Molecular complexes can then be isolated and used for various applications for the identification of partners. Slimane AIT-SI-ALI and his "epigenetic and cell fate" team from the University Paris Descartes combined the TAP tagging with the Bioruptor®. This combination is essential for the efficient purification of a chromatin protein.

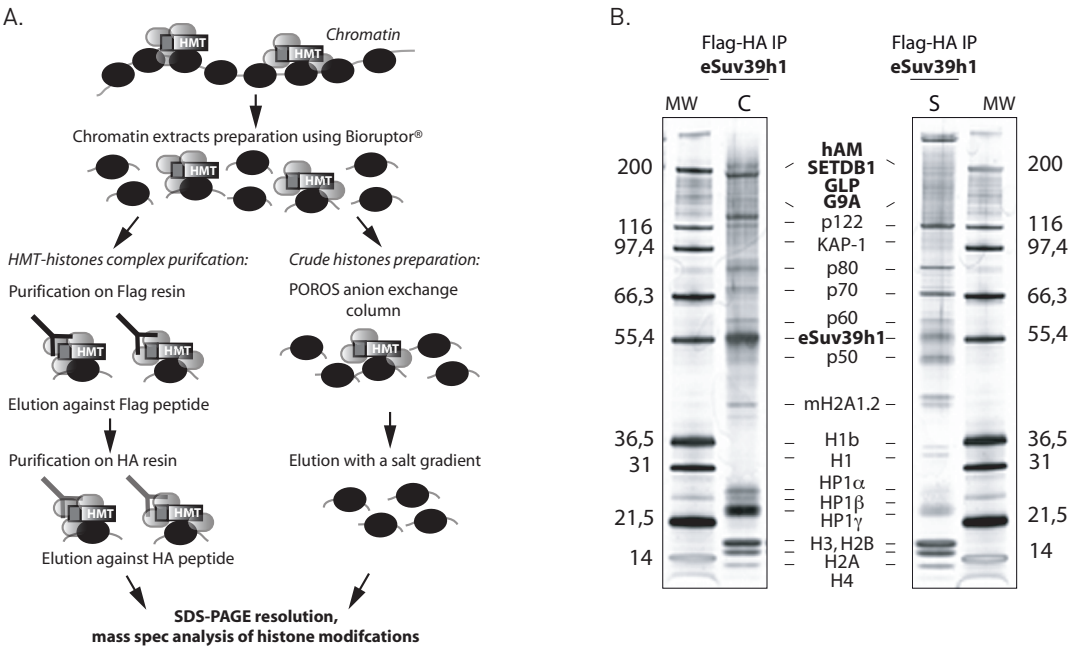

Figure: Protocol for the purification of histone or chromatin protein complexes developed by Lauriane Fritsch form the laboratory of Slimane AIT-SI-ALI. A) Schematic representation of the protocols used for the purification prior mass spectrometry analysis of histone complexes and crude histones (adapted from © 2008 Robin et al.; licensee BioMed Central Ltd. - http://creativecommons.org/licenses/by/2.0/#). B) Immunopurification of eSuv39h1 complex from nucleosome-enriched extracts (C) and nuclear soluble fraction (S) obtained using the Bioruptor®. Silver staining of 4%-12% SDS-PAGE gel unveiled complexes and endogenous interactions of Suv39h1 (Figure 1A from Fritsch, L., Robin, P., Mathieu, J.R.R., Souidi, M., Hinaux, H., Rougeulle, C., Harel-Bellan, A., Ameyar-Zazoua, M., and Ait-Si-Ali, S. (2010). A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex. Mol Cell 37, 46–56 ; Copyright © 2010 Elsevier Inc.)

1. Material required and buffer preparation

- Bioruptor Plus (Cat. No. B01020001) with 15 ml tube holder and 15 mL sonication probes (Cat. No. B01200013) or 1.5 ml tube holder
- 15ml TPX tubes (Cat. No. C30010009) or 1.5 ml TPX tubes (Cat. No. C30010010)
- 50 ml tubes
- Silver stain kit
- 4-12% precast gel
- Mnase (Sigma-Aldrich, Cat. No. n3755), Reconstitute in ultra pure water at 0,5U/uL.
- 4 ml dounce tissue grinder
- Ultracentrifuge (reaching 40,000 rpm)
- Anti-flag M2 affinity gel (Sigma, A2220)
- Monoclonal Anti-HA Agarose Conjugate Sigma resin anti-HA (Sigma, A2095)
- Sigma spin column (Sigma, S 0185)
- Rotation wheel

- Flag peptide (Sigma, F4799)
- HA-peptide (Sigma, I2149)
- Buffer composition (store at 4°C):
  Add Protease Inhibitor and 1mM DTT just prior use.

Hypotonic buffer	TEGN
10mM Tris pH7.65	20mM Tris pH7.65
1.5mM MgCl2	0.1mM EDTA
10mM KCl	10% glycerol
	150mM NaCl
	0.01% NP40

Tampon sucrose	High salt buffer
20mM Tris pH7.65	20mM Tris pH7.65
60mM NaCl	0.2mM EDTA
15mM KCl	25% glycerol
0.34M sucrose	900mM NaCl
0.15mM spermin	1.5mM MgCl2
0.15mM spermidin	

2. Procedure

Day 1

- Use 2 g of cells / purification. If starting from cell pellets, thaw quickly in a water bath at 37 °C.
- Resuspend in 2ml of hypotonic buffer.
- Load a 4mL dounce (2ml of pellet + 2ml of hypotonic buffer) and perform 20 back and forth to break cytoplasmic membranes.
- Clean the dounce with 1/3 of the volume of sucrose buffer.
- Centrifuge for 7 min at 9,000 rpm at 4°C.
- Collect the supernatant containing the cytoplasmic fraction.
- Resuspend the pellet in 1 ml of sucrose buffer. Add drop by drop while vortexing the Hight salt buffer to reach a final concentration of 300 mM of NaCl.
- Incubate 30 min on ice and, occasionally roll over.
- Add 1/3 of the volume of sucrose buffer.
- Centrifuge for 10 min at 10,000 rpm at 4°C.
- Collect the supernatant (with the nuclear fraction containing soluble proteins) and the pellet containing proteins strongly interacting with the chromatin.
- Centrifuge the nuclear soluble fraction for 30 min at 40,000 rpm at 4°C to remove cell debris.
- Resuspend the pellet containing porteins strongly interacting with the chromatin by adding 1 volume of sucrose buffer.
- Add CaCl2 at final concentration of 1mM.
- Heat for 1 min at 37°C and add MNase to a final concentration of 0.0025 U / ul.
- Incubate for 12 min at 37°C, occasionally vortex.
- Transfer samples into Diagenode 15 ml or 1.5ml TPX tubes and add EDTA to a final concentration of 4 mM and incubate on ice for 5 min.
- Sonicate with Bioruptor® for 5 cycles at HIGH power (sonication cycle: 1 min ON, 1 min OFF).
- Centrifuge for 30 min at 40,000 rpm and collect supernatant (the chromatin fraction containing the nuclear proteins strongly associated with chromatin).
- Suggested procedure for the IP:
  - Wash anti-flag M2 affinity gel with TEGN buffer at maximum 2,000rpm.
  - Add anti-flag M2 affinity gel to supernatant and incubate on a rotation wheel overnight at 4°C.

Day 2

- Wash 7 times anti-flag M2 affinity gel incubated with the supernatant with TEGN buffer for 2 min on a rotation wheel.
- Transfer in 1.5 ml eppendorf and add flag peptide.
- Add TEGN buffer to tubes.
- Incubate on a rotation wheel overnight at 4°C.

Day 3

- Wash a sigma spin column (trash resin, wash with water to remove all resin trace and centrifuge to discard any trace of water).
- Transfer flag resin in a sigma spin column.
- Centrifuge for 1 min at 8.000 rpm at 4°C and keep the flow trough.
- Add HA-resin.
- Incubate on a rotation wheel overnight at 4°C.

Day 4

- Wash 7 times HA-resin with TEGN buffer for 2 min on a rotation wheel.
- Transfer in 0.5 ml eppendorf and add HA peptide.
- Add TEGN buffer.
- Incubate on a rotation wheel overnight at 4°C.

Day 5

- Wash a sigma spin column (discard resin, wash with water to remove all resin trace and centrifuge to discard any trace of water).
- Transfer flag resin in sigma spin column.
- Centrifuge for 1 min at 8,000 rpm at 4°C and keep the flow trough.
- Use a 4-12% Bis-tris gel and run about 1h in MES buffer at 180V.
- Stain the gel with a silver stain kit.

3. Selected publications

1. Robin P, Fritsch L, Philipot O, Svinarchuk F, Ait-Si-Ali S. **Post-translational modifications of histones H3 and H4 associated with the histone methyltransferases Suv39h1 and G9a.** *Genome Biol. 2007;8(12):R270.*
2. Fritsch L, Robin P, Mathieu JRR, Souidi M, Hinaux H, Rougeulle C, et al. **A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex.** *Mol Cell. 2010 Jan 15;37(1):46–56.*

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