Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells

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Octamer binding proteins (oct-1, oct-2A/B) are transcription factors which regulate the expression of immunoglobulin heavy chain genes and various housekeeping genes (reviewed in 1). Here we present an extremely simple protocol, modified from that of Lee et al. (2), which allows one to make nuclear extracts from as few as 5 x 10^5 cells or from lymphocytes isolated from 2 ml of peripheral blood (3). At the same time RNA can be isolated from the cytoplasmic fraction and further analysed, e.g. by Northern blotting. Detection of the DNA binding proteins is performed by bandshift assay. Many different cell lines can be quickly screened for the presence of octamer-binding and other transcription factors. Moreover, "factor-induction" experiments can be conveniently performed in small scale cultures. In addition, this technique will allow clinical investigations using bioptic material or blood mononuclear cells, e.g. lymphocytes purified from 2 ml of peripheral blood (3).

Typically, 0.5 -1 x 10^6 cells from tissue culture, from homogenized mouse spleen (about 0.5 g) or peripheral blood lymphocytes are collected, washed with 10 ml TBS (Tris buffered saline) and pelleted by centrifugation at 1500 x g for 5 min. The pellet is resuspended in 1 ml TBS, transferred into an Eppendorf tube and pelleted again by spinning for 15 sec in a microfuge. TBS is removed and the cell pellet is resuspended in 400 µl cold buffer A (10 mM HEPES pH 7.9; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM DTT; 0.5 mM PMSF) by gentle pipetting in a yellow tip. The cells are allowed to swell on ice for 15 min, afterwhich 25 µl of a 10 % solution of Nonidet NP-40 (Fluka) is added and the tube is vigorously vortexed for 10 sec. The homogenate is centrifuged for 30 sec in a microfuge. The supernatant containing cytoplasm and RNA is transferred to a fresh tube containing 400 µl buffer B (10 mM Tris pH 7.5; 7 M urea; 1% SDS; 0.3 M NaAc; 20 mM EDTA) and 600 µl phenol/chloroform (1:1), mixed immediately and stored at - 20 °C until it is convenient to further purify the RNA according to (4). The nuclear pellet is resuspended in 50 µl ice-cold buffer C (20 mM HEPES pH 7.9; 0.4 M NaCl; 1 mM EDTA; 1 mM EGTA; 1 mM DTT; 1 mM PMSF) and the tube is vigorously rocked at 4 °C for 15 min on a shaking platform. The nuclear extract is centrifuged for 5 min in a microfuge at 4 °C and the supernatant (ca. 55 µl) is frozen in aliquots at -70 °C. Usually, 1-2 µl of this extract (ca 2-4 µg protein) is used for a bandshift assay in the presence of 3 µg poly dI dC as described in (5).

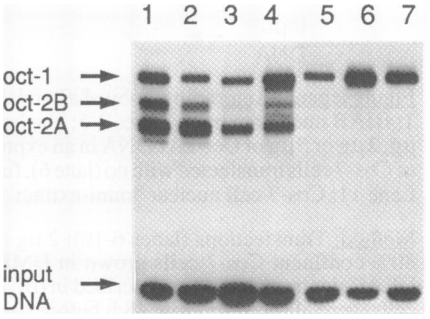

Figure legend: Autoradiography of a bandshift experiment with a radiolabelled octamer probe (5) and various "mini-extracts"; arrows designate nuclear proteins oct-1, oct-2A and oct-2B lane 1: conventionally prepared nuclear extract (6) of BJA-B lymphocytes; lane 2-7 mini-extracts: lane 2: BJA-B lymphocytes; lane 3: mouse spleen; lane 4: human peripheral lymphocytes; lane 5: HeLa cells; lane 6: HUT 78 T-cells; lane 7: MLA144 T-cells.

References:

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