

A rapid microscale procedure for the simultaneous preparation of cytoplasmic RNA, nuclear DNA binding proteins and enzymatically active luciferase extracts

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Here we describe a simple and rapid method which allows the simultaneous preparation of cytoplasmic RNA and nuclear binding proteins from only a small number of cells. In addition, the activity of transiently transfected luciferase reporter plasmids can be directly assayed. The procedure should be useful to investigators examining the transcription of endogenous genes under various conditions, along with associated changes in the DNA binding pattern of nuclear proteins. Furthermore, the transcriptional response of endogenous and exogenous templates can be compared in the same sample of cells, e.g. to assess the influence of chromosomal integration on the transcriptional regulation of viral genomes. In addition, its simplicity and speed allows the analysis of many cell samples at the same time and renders it suitable for kinetic studies.

5×10^5 HeLa cells were transfected with luciferase reporter constructs (1). Twenty four hours later cells were lysed directly on the culture dish in 1.6 ml cold RNA lysis buffer (0.6% NP40, 0.15 M NaCl, 10 mM Tris pH 7.9 and 1 mM EDTA), transferred into a 2 ml Eppendorf tube and incubated for 5 min on ice. The nuclei were pelleted (1250 g, 4°C, 5 min) and 100 µl of the supernatant were directly analysed for luciferase activity (2).

Cytoplasmic RNA was obtained from the supernatant after denaturing proteins in one volume 7 M urea and extracting once with 6 ml phenol/chlorofom/isoamyl alcohol (25:24:1) and once with 6 ml chloroform/isoamylalcohol (24:1). The RNA was subsequently precipitated in 9 ml 100% ethanol.

Nuclear proteins were extracted from the pelleted nuclei in 100 µl cold extraction buffer (10 mM HEPES pH 7.9, 0.1 mM EGTA, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.5 mM DTT, 0.5 mM PMSF and 25% glycerol) on ice for 20 min and cellular debris was removed by centrifugation for 5 min at 4°C and 1250 g. The supernatant containing nuclear proteins was stored at −70°C. Using this procedure intact cytoplasmic RNA can be isolated (Figure 1). Luciferase activities (Figure 3) are similar to values using a protocol optimized to improve the sensitivity of the luciferase assay (2). Furthermore, excellent quality DNA binding proteins comparable to the large scale method established by Dignam (3) were obtained (Figure 2).

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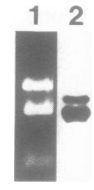

Figure 1. Analysis of cytoplasmic HeLa-RNA stained with ethidium bromide after separation in a 1% agarose gel (lane 1) and after Northern blot hybridization with ^{32}P-labelled HPV 18 probe (lane 2).

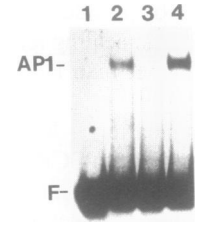

Figure 2. Gel retardation assay using an AP-1 binding site probe derived from the HPV 18 enhancer. Lane 1: probe without nuclear extract; lane 2: 3 µg nuclear extract prepared by the microscale procedure; lane 3: 3 µg nuclear extract competed with a 50 fold molar excess of an AP-1 binding site derived from the collagenase promoter; lane 4: 3 µg nuclear extract obtained by conventional large scale preparation (3). F, free probe.

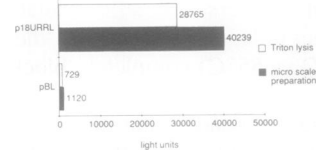

Figure 3. Luciferase activity in extracts obtained by the microscale preparation compared to an optimized Triton-lysis based extraction procedure (2). In plasmid p18URRL (4) the P. pyralis luciferase gene is under control of the HPV 18 upstream regulatory region. pBL parental plasmid lacking enhancer and promoter sequences (4).