

A new fast and easy method for the oligonucleotide affinity purification of DNA binding proteins

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Usually purification of DNA binding proteins is a time consuming process.We have developed a rapid small scale one step oligonucleotide affinity chromatography batch process that is capable of purifying DNA binding proteins from crude ^{35}S-Methionine labelled nuclear extracts to near homogeneity. We are interested in factors that bind to intragenic regions of the Adenovirus 12 E1A gene (1).

The C_{del} oligonucleotide (see table) was 5'-aminoalkylated with N-MMT C6 aminomodifier (Clonetech) using an oligonucleotide synthesizer (Pharmacia) and coupled to tresylactivated Sepharose CL-4B (tresyl: 2,2,2,-trifluorethanesulfonyl chloride). Typically, 20 μg of oligonucleotide were coupled per ml resin in 8 h at 25°C in 0.1 M $NaHCO_3$, 500 mM NaCl, pH 7.5. The resin was equilibrated with buffer A (10 mM HEPES, 15% glycerol, 1 mM EDTA, 4 μg/ml E1B oligonucleotide as unspecific competitor (see table), 0.1 μg/ml ovalbumin, 2 μg/ml aprotinin, pH 7.9).

Nuclear extracts from ^{35}S-Methionine labelled HeLa cells were prepared as described by Dignam (2) with some modifications (1). For the following batch procedures mini spun columns with a PTFE diaphragm (Mobitec) were used and non bound or eluted material was recovered by centrifugation at 450×g for 5 min. All following procedures were carried out at 4°C: $3-4\times10^7$ cpm of these extracts (about 60−100 μg of total protein) were preincubated with 1.8 ml of buffer A with Tris-blocked Sepharose CL-4B in the spun columns by rotation to remove unspecific Sepharose binding proteins. The unbound eluate was then incubated for 2 and 4 hours (Figure 1) or 1.5 hours (Figure 2) with 150 μl C_{del} DNA affinity resin, washed with buffer A plus 0.05% Triton-X-100 and different amounts of NaCl for either 20 min (Figure 1) or 90 min (1 step) and subsequently 20−30 min (all following steps; Figure 2). Specifically bound proteins were eluted with 400−750 mM NaCl in buffer A resulting in 2−3 bands in SDS-polyacrylamide gel electrophoresis as shown in Figures 1 and 2.

Obviously, a significant decrease of background was achieved by reduction of the incubation time and extension of the first washing step (Figure 2). The 70 kD protein was shown to be responsible for a specific band found in gel shift assays with crude nuclear extracts (1). The proteins at about 84 and 100 kD have not been identified yet.

Our method, that was successfully extended to protein purification from Vero cell nuclear extracts, is a time saving analytical procedure for initial characterization of DNA binding proteins.

REFERENCES

1. Kirch,H.-C., Krüger,H. and Schulte Holthausen,H., submitted for publication.
2. Dignam,J.D., Lebovitz,R.M. and Roeder,R.G. (1983) *Nucl. Acids Res.* **11**, 1475.

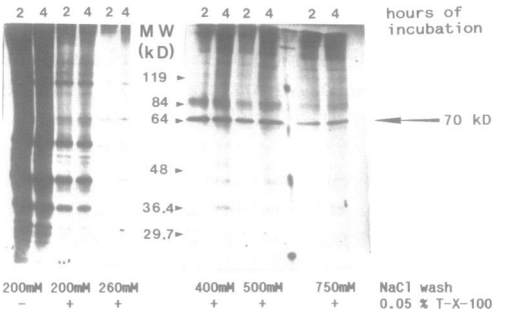

Figure 1. SDS-polyacrylamide gel electrophoresis (separation-gel: 10.5%) of oligonucleotide affinity purified C_{del} binding proteins (see text for details).

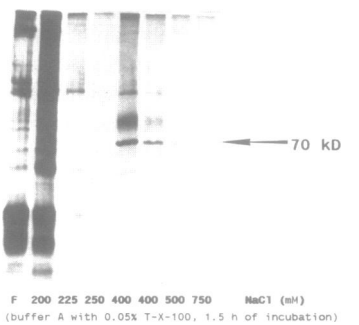

Figure 2. SDS-polyacrylamide gel electrophoresis of oligonucleotide affinity purified C_{del} binding proteins as in Figure 1 with different binding and washing conditions (see text).

Table: Oligonucleotides used in the purification procedures:
C_{del}: oligonucleotide
5'-(GGGGGG)ATTGTGCTACGACGCAGCTGCTGCTGCCGCT-3'
3'-TAACACGATGCTGCGTCGACGACGACGGCGA-5'

E1B random oligonucleotide:
5'-(GGG)CGCAGATGATAGAGATAAGCAGGAAAAAAA-3'
3'-GCGTCTACTATCTCTATTCGTCCTTTTTTT-5'