Non-immunological precipitation of protein-DNA complexes using glutathione-S-transferase fusion proteins

Abraham Fainsod, Yael Margalit, Rebecca Haffner and Yosef Gruenbaum[1]
Department of Cellular Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem 91010 and [1]Department of Genetics, Hebrew University, Jerusalem 91904, Israel

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Recently, many genes have been isolated whose putative protein products appear to be able to bind DNA. In many instances these are DNA binding proteins whose target sequences are as yet unknown. Apart from a knowledge of their tissue distribution, it is important to identify and isolate the DNA sequence motif which they bind in order to determine their molecular function. Several methods have been described to identify and isolate target sequences, from either mixtures of oligonucleotides (1–3) or from genomic DNA (5–7). In addition in many instances it is necessary to produce the DNA binding protein in bacteria. Most of the methods described rely on the availability of antibodies in order to precipitate the protein-DNA complexes (1, 4, 6). In other instances, isolation of the complexes relied on the fact that the protein produced in bacteria was insoluble (5), alternatively the protein-DNA complex was isolated from gels (2, 3). We show a method to isolate protein-DNA complexes using fusion proteins between glutathione-S-transferase and DNA binding proteins. In addition to the ease in obtaining partially purified soluble fusion proteins in large quantities, the main advantage of this approach is that the protein-DNA complexes can be precipitated with commercially available glutathione-agarose beads thereby eliminating the need to produce antibodies for this purpose.

We constructed a fusion protein in the pGEX vector (7) that produces a glutathione-S-transferase protein fused to the chicken *CHox-cad* protein including the homeodomain (8). The fusion protein was partially purified from the *E.coli* lysate by binding to glutathione-agarose beads (Sigma # G-4510) as described (7). After washing, the beads with the bound fusion protein were resuspended in 50 mM Hepes (pH 7.5); 50 mM KCl; 5 mM $MgCl_2$; 10 μM $ZnSO_4$; 1 mM DTT; 50% glycerol and stored at −20°. Chicken genomic DNA was prepared for whole genome PCR by sonication or by restriction digest with AluI, HaeIII, Sau3A and RsaI according to the procedure of Kinzler and Vogelstein (4). For the first binding reaction, linkered genomic DNA was reacted with the fusion protein already bound to agarose beads. Binding and washing was performed as described (4) but instead of an antibody binding reaction after the DNA binding, the DNA-protein-bead complexes were purified by a brief centrifugation step. After PCR, the binding selection process was repeated three more times always using the products of the last PCR as targets. The PCR products of the fourth round of binding selection were cloned and tested in electrophoretic mobility shift assays (Figure 1). Six out of seven different genomic fragments tested for mobility shift formed a complex with the fusion protein.

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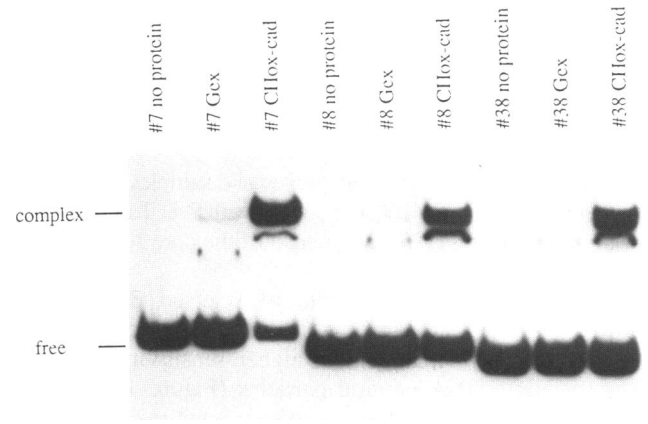

Figure 1. Eletrophoretic mobility shift assay of three isolated putative target sequences. Three independent clones (numbers 7, 8 and 38) isolated by precipitation of the fusion protein with glutathione-agarose beads were tested for specific binding by the mobility shift assay. Lanes labelled CHox-cad were treated with full *E.coli* extracts froin bacteria producing the GST-CHox-cad fusion protein. Lanes marked Gex were reacted with an *E.coli* extract harboring the pGEX vector as a negative control.