

Glutathione S-transferase fusion proteins as an affinity reagent for rapid isolation of specific sequence directly from genomic DNA

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ABSTRACT

We describe a DNA binding assay for isolation of specific sequence(s) recognized by protein of interest directly from genomic or cosmid DNA. In our assay, the protein is fused to the glutathione-S-transferase and bound to glutathione-Sepharose beads. Then the immobilized fusion protein can be used to search for DNA fragment(s) that interact specifically with the protein of interest. As an example of such an approach, we identified and cloned a few prokaryotic *oriC* regions using the initiator DnaA protein fused to the glutathione-S-transferase.

Protein–DNA interactions are intrinsic to virtually every cellular process. Proteins expressed in *Escherichia coli* as fusions with glutathione S-transferase (GST; 1,2) have proven to be useful for analysis of protein–protein (3,4) and protein–DNA interactions (5,6). We have developed a DNA binding assay for isolation of specific sequence(s) recognized by protein of interest directly from genomic or cosmid DNA. In this assay the protein or the DNA binding domain is fused to the glutathione-S-transferase. The GST fusion protein bound to glutathione-Sepharose beads can function as an affinity reagent to bind selectively the DNA fragment(s). The outline of the technique is presented in Figure 1.

As an example of such an approach, we identified the prokaryotic *oriC* region using the GST–DnaA–Sepharose beads. The DnaA protein is the key protein in the initiation of DNA replication in bacteria. It interacts with the DnaA boxes of the *oriC* region and forms the initial replication complex (7,8). The *oriC* region was isolated from two *Streptomyces* species: *S.lividans* and *S.chrysomallus*. The DNA binding domain of the *S.lividans* initiator protein DnaA was fused to the C-terminus of GST protein. Plasmid pLEXDnaA (9) which encodes the entire *dnaA* gene was partially digested with *XhoII* and completely digested with *NheI*. A 432 bp *XhoII*–*XhoII*–*NheI* fragment encoding the DNA binding domain of DnaA protein was subcloned into the *BamHI*- and *NheI*-restricted pGEX-3X-6His. The GST–DnaA(BD) fusion protein overexpressed in *E.coli* was bound directly from bacterial extract to the glutathione-Sepharose beads (1,2). The DNA digested with restriction endonuclease was

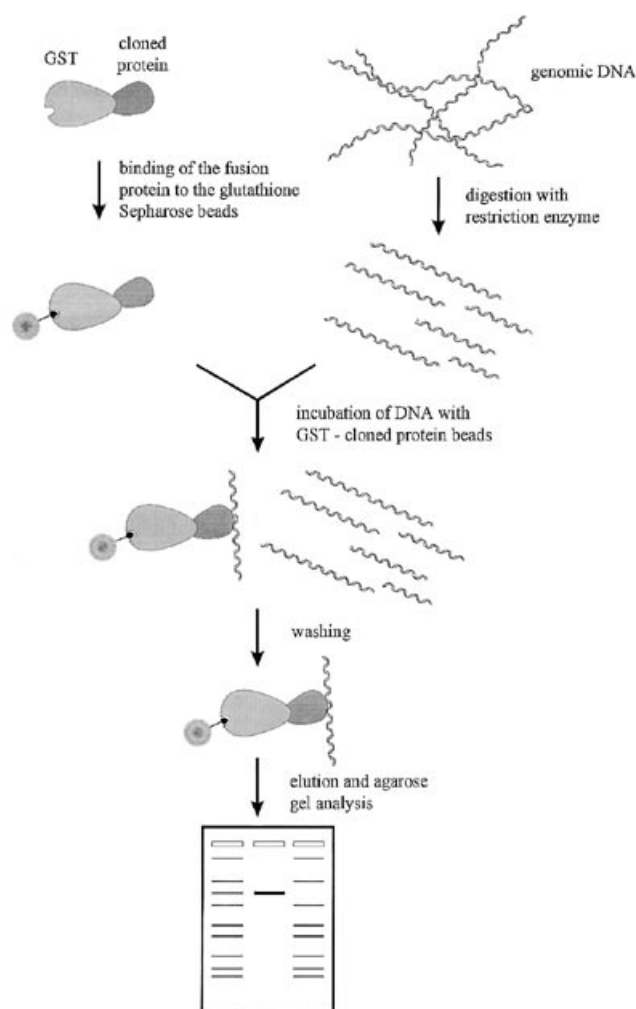


Figure 1. Outline of the specific DNA binding assay.

loaded on the glutathione-Sepharose–GST–DnaA(BD) column equilibrated with 'low' salt buffer (20 mM Tris, 100 mM NaCl,

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