The pGEX System

Pharmacia; macplasmid #241

The pGEX system involves fusion of the C-terminus of Sj26, a 26 kDa glutathione S-transferase (GST) (Smith et al., 1988). The stable fusion proteins are soluble in aqueous solutions and can be purified from crude bacterial lysates under non-denaturing conditons by affinity chromatography on immobilized glutathione (Smith et al., 1988). The GST carrier can be cleaved from fusion proteins by digestion with site specific proteases thrombin or blood coagulation factor Xa (Pharmacia). The carrier and uncleaved fusion protein can be removed by absorption on glutathione-agarose. The pGEX vectors, pGEX-3X, pGEX-2T, contain the Ptac (trp/lac) hybrid promotor in which expression is induced by addition of IPTG. A fragment of the lac operon containing the over expressed lacIq allele of the lac repressor and part of the lac z gene are integrated to repress transcription from the tac promoter until IPTG is introduced. The disadvantages of this system include a long incubation period after IPTG addition (Pharmacia), activation of factor X by Russell's viper venom is required to convert it to the serine esterase (Fujikawa et al., 1972), and in some cases purification has been unsuccessful due to the insolubility of the fusion protein (Smith et al., 1988). Proteins with strongly hydrophobic regions, many charged residues, or that are larger than 100 kDa are unsuitable for this system (Smit et al., 1988).

References:

Smith et al,Gene 67:31 (1988)

Fujikawa et al, Biochemistry 11: 4892 (1972)