A versatile *in vivo* and *in vitro* eukaryotic expression vector for protein engineering

Stephen Green, Isabelle Issemann and Elisabeth Sheer

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg, France
Submitted October 27, 1987

The ability to engineer specific modifications within the coding region of a structural gene is a powerful tool with which to study the relationship between protein structure and function. The process of site-directed mutagenesis involves a) mutation of the target gene, b) verification of the mutation by sequencing and c) expression of the mutated gene. We describe here a vector, pSG5 (Fig.1A), in which each of these steps may be performed. pSG5 was constructed essentially by combining the eukaryotic expression vector, pKCR2 (1), and the high copy plasmid vector Bluescribe M13+ (Stratagene). The principle features of pSG5 are a) unique EcoRI and BamHI restriction enzyme sites for insertion of cDNAs, b) production of single stranded DNA containing the antisense strand of the structural gene for mutagenesis and sequencing, c) high yields of double stranded DNA, d) *in vivo* expression from the SV40 early promoter and e) *in vitro* expression from the T7 promoter situated just upstream of the cDNA insertion site.

Expression from pSG5 was tested after insertion of a cDNA encoding the human oestrogen receptor (2) (ER) into the EcoRI site to produce pSG5-ER. Fig.1B shows an SDS polyacrylamide gel of the 66Kd ER protein synthesised in a rabbit reticulocyte cell-free translation cocktail programmed with mRNA transcribed from the T7 promoter of either Bluescribe-ER (BSM-ER (3)) or pSG5-ER. The ER when expressed in HeLa cells stimulates transcription from oestrogen-responsive 'reporter' genes (4) (vitellogenin-TK-globin (VTG) in Fig. 1C). Using quantitative S1 nuclease analysis with an internal reference gene (REF in Fig.1C), the ER expressed from either pSG5-ER or pKCR2-ER stimulates gene transcription equally well indicating that equivalent levels of ER are expressed *in vivo* using either of these two vectors.

![Diagram](https://example.com/diagram.png)

References: