pSG5 Vector

INSTRUCTION MANUAL

Catalog #216201
Revision #111001a

For In Vitro Use Only

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pSG5 Vector

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MATERIALS PROVIDED

Material Provided	Quantity
pSG5 vector	20 µg
AG1 strain*, glycerol stock	1 tube

* *recA1, endA1, gyrA96, thi-1, hsdR17,* (r_K-, m_K+), *supE44, relA1*, (uncharacterized mutation improves transformation efficiency)

STORAGE CONDITIONS

pSG5 vector: –20°C
AG1 bacterial glycerol stock: –80°C

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VECTOR FEATURES

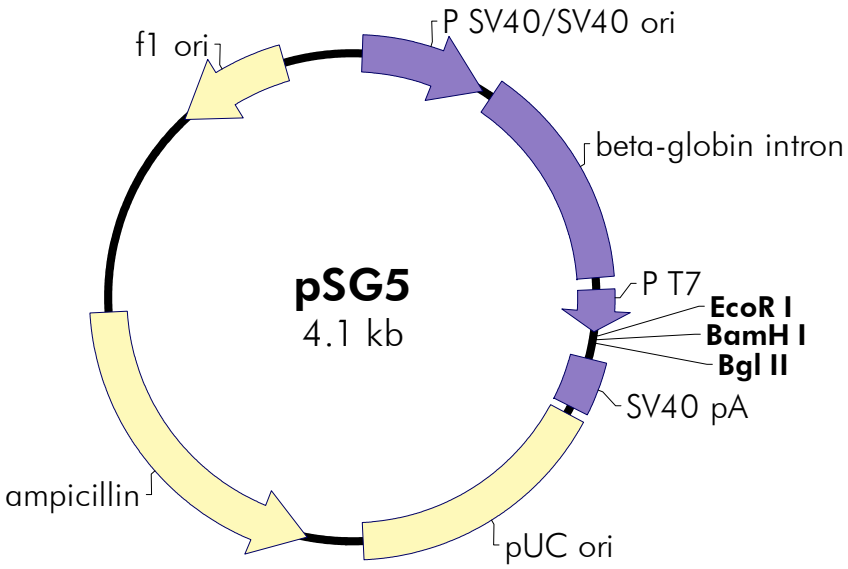
The pSG5 Vector is a eukaryotic expression vector constructed by combining pKCR2 and Stratagene's pBS vector. Because of the high copy number of this plasmid, large quantities of double-stranded DNA are obtained.

Applications

The pSG5 is useful for both in vitro and in vivo expression. Expression in vivo is achieved via transient expression in a variety of cell lines (highest level of expression is obtained following transfection of a cell line expressing the T antigen). The f1 origin allows rescue of ssDNA for use in mutagenesis and sequencing. The SV40 early promoter and polyadenylation signal promotes expression in vivo, and the T7 bacteriophage promoter facilitates in vitro transcription of cloned inserts. The β-globin intron II allows splicing of expressed transcripts.

To ligate the gene of interest into the pSG5 vector, use the unique restriction sites *Eco*R I, *Bam*H I, and *Bg*l II (downstream from the promoter).

The pSG5 Vector



Feature	Nucleotide Position
SV40 promoter and SV40 origin of replication	28–366
β-globin intron	395–967
T7 promoter	1022–1040
EcoR I	1043
BamH I	1049
Bgl II	1055
SV40 polyA signal	1069–1202
pUC origin of replication	1342–2009
ampicillin resistance (*bla*) ORF	2160–3017
f1 origin of ss-DNA replication	3587–3893

Figure 1 Circular map and features of the pSG5 vector. The complete sequence and list of restriction sites is available at *www.stratagene.com*.

PREPARATION OF HOST STRAIN

The host strain has been sent as a glycerol stock. For the appropriate media and plates, please refer to the following table:

Bacterial strain	Plates for bacterial streak	Media for glycerol stock
AG-1	LB	LB

On arrival, prepare the following from the glycerol stock:

Note *Do not allow the contents of the vial to thaw. The vials can be stored at –20 or –80°C, but most strains remain viable longer if stored at –80°C.*

1. Revive the stored cells by scraping off splinters of solid ice with a sterile wire loop.

2. Streak the splinters onto an LB plate.

Restreak the cells fresh each week.

Preparation of a –80°C Glycerol Stock

1. In a sterile 50-ml conical tube, inoculate 10 ml of the appropriate liquid media with one or two colonies from the plate. Grow the cells to late log phase.

2. Add 4.5 ml of a sterile glycerol–liquid media solution (5 ml of glycerol + 5 ml of liquid media) to the bacterial culture from step 1. Mix well.

3. Aliquot into sterile centrifuge tubes (1 ml/ tube).

This preparation may be stored at –20°C for 1–2 years or at –80°C for more than 2 years.

PREPARATION OF MEDIA AND REAGENTS

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Add deionized H_2O to a final volume of 1 liter
Adjust to pH 7.0 with 5 N NaOH
Autoclave

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)