pDRIVE5s-mPGK

A plasmid with the native ubiquitous murine phosphoglycerate kinase promoter

Catalog # pDRIVE5s-mpgk

For research use only

Version # 11D20-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5s plasmid.
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, rspL (StrA), recA1, endA1 Δdcm ΔsbcC-sbcD.*
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

Shipping and storage:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20˚C. Bacteria are stable up to one year when properly stored.
- Store *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pDRIVE5s is an expression plasmid containing a native or composite promoter of interest. **pDRIVE5s** may be used to:

- Subclone a promoter of interest into another vector. Unique restriction sites are present at each end of the promoter allowing convenient excision. The 5' site is *Sda* I. *Sda* I is compatible with *Nsi* I and *Pst* I. The 3' restriction site is *BspH* I. *BspH* I is compatible with *Nco* I and *Bsp*LU11 I.

- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5s promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (*Bsp* HI and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Murine PGK-1 (gene : Phosphoglycerate Kinase) promoter

Complete Promoter size: 1440bp

Specificity: Ubiquitous

Pgk-1 is an X-linked gene encoding 3-phosphoglycerate kinase, an enzyme necessary in every cell for glycolysis. The promoter region of the pgk-1 gene is rich in G and C nucleotides and contains five copies of the hexadeoxynucleotide, GGGCGG, a potential binding site for the Sp1 transcription factor, a CCAAT sequence, but no TATA box[1]. This promoter can efficiently drive high levels of expression of reporter genes (i.e. SEAP, LacZ and GFP) and therapeutic genes, such as tumor-associated antigenes[2, 3]. Furthermore, in contrast to the CMV promoter, the PGK promoter yields sustained expression[2].

1. Adra CN. et al. 1987. Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. Gene 60(1):65-74.
2. Gerolami R. et al. 2000. Gene transfer to hepatocellular carcinoma: transduction efficacy and transgene expression kinetics by using retroviral and lentiviral vectors. Cancer Gene Ther 7(9):1286-92.
3. Lizee G. et al., 2004. Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells. Hum Gene Ther. 15(4):393-404.

PLASMID FEATURES

• **SEAP gene** encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **EM2K** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

• ***Zeo*** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE5s plasmid.

***Note:* Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.**

METHODS

Growth of pDRIVE5s-transformed bacteria:

Use sterile conditions to do the following:

1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.

2- Streak bacteria taken from this suspension on a zeocin LB agar plate prepared with the *E. coli* Fast-Media® Zeo agar provided (see below).

3- Place the plate in an incubator at 37˚C overnight.

4- Isolate a single colony and grow the bacteria in TB supplemented with zeocin using the Fast-Media® Zeo liquid provided (see below).

5- Extract the pDRIVE5s plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* Fast-Media Zeo:

E. coli Fast-Media® Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

E. coli Fast-Media® Zeo can be ordered separately (catalog code fas-zn-l, fas-zn-s).

Method:

1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.

2- Add 200 ml of distilled water to the flask

3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**

4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**

5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

6- Let agar medium cool to 45˚C before pouring plates. Let liquid media cool to 37˚C before seeding bacteria.

***Note:** Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

TECHNICAL SUPPORT
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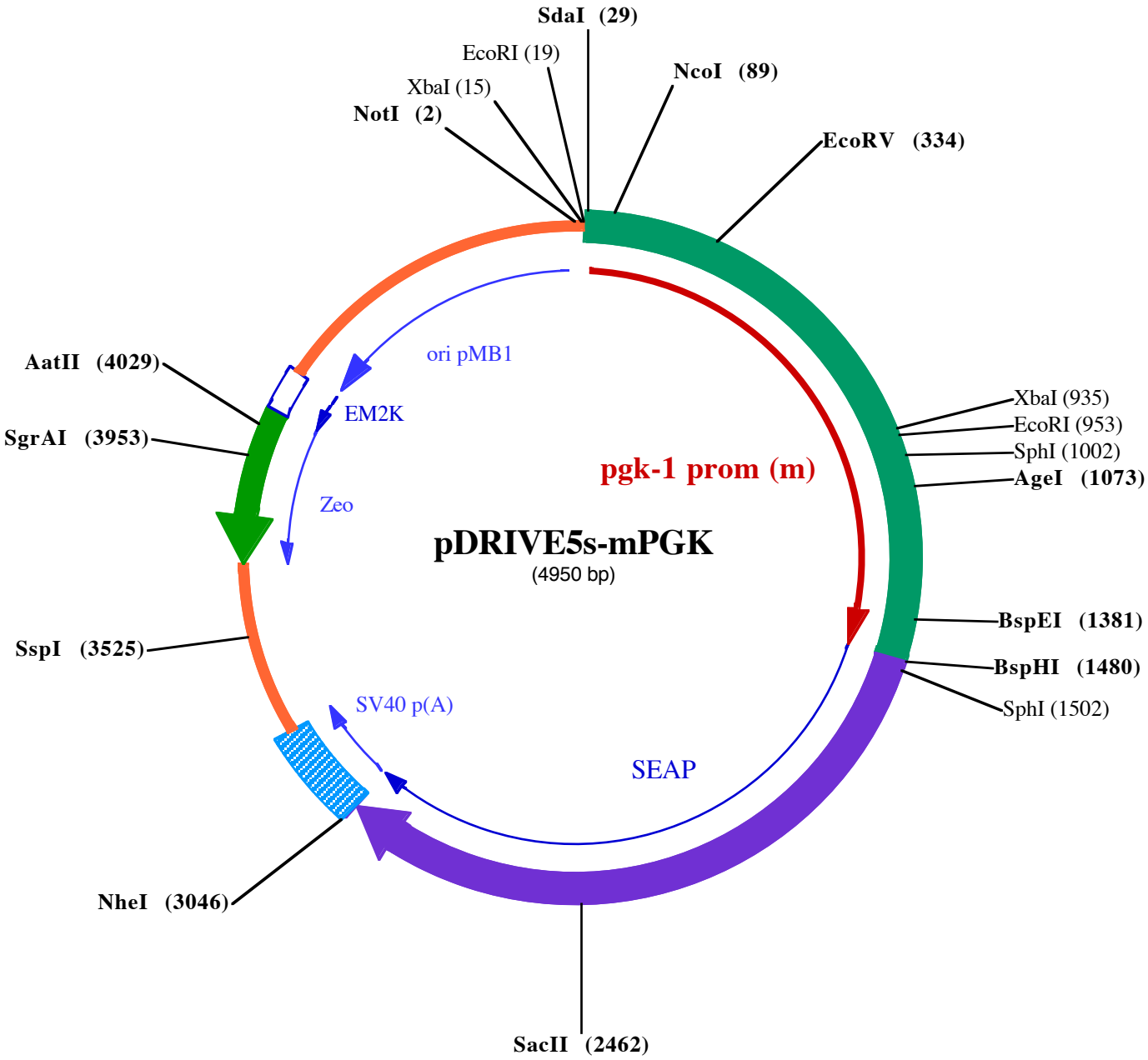
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EcoRI (19)
NcoI (89)
XbaI (15)
NotI (2)
EcoRV (334)
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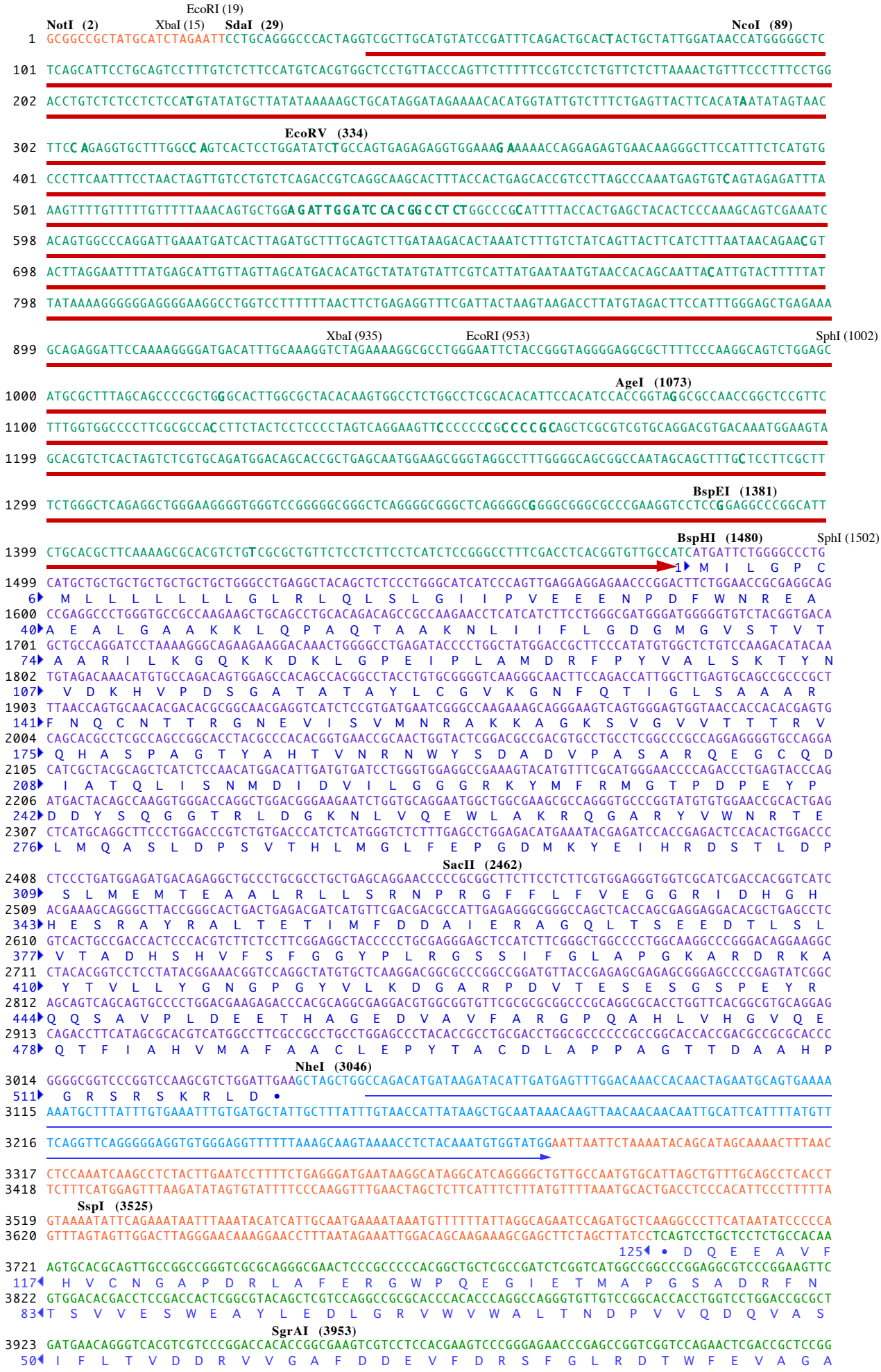
AatII (4029)
EM2K
SgrAI (3953)
XbaI (935)
EcoRI (953)
SphI (1002)
AgeI (1073)
pgk-1 prom (m)
Zeo
pDRIVE5s-mPGK
(4950 bp)
BspEI (1381)
SspI (3525)
BspHI (1480)
SphI (1502)
SV40 p(A)
SEAP
NheI (3046)
SacII (2462)

InvivoGen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AgeI (1073)
BspEI (1381)
BspHI (1480)
SphI (1502)
SacII (2462)
NheI (3046)
SspI (3525)
SgrAI (3953)


AatII (4029)
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4630 ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT
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