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pDRIVE5Lucia-mPGK
A plasmid with the native ubiquitous murine phosphoglycerate kinase promoter
Catalog # pdrive5l-mpgk
For research use only
Version # 14C04-MM

PRODUCT INFORMATION

Content:
- 20 µg of pDRIVESLucia-mPGK provided as lyophilized DNA
- 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)

Shipping and storage:
- Products are shipped at room temperature.
- Lyophilized DNA is stable for at least 12 months when stored at -20°C.
- Resuspended DNA is stable for 6 months when stored at -20°C. Avoid repeated freeze-thaw cycles.
- 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.

GENERAL PRODUCT USE
pDRIVE is an expression plasmid containing a native or composite promoter of interest. pDRIVE 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 which includes the ATG start codon, and is compatible with Nco I and BspLU11 I.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE promoter drives the expression of the Lucia luciferase reporter gene which allows for testing of the promoter’s activity in transient transfection experiments. Furthermore, the Lucia luciferase gene is flanked by restriction sites (BspH I and Nhe I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS
Murine PGK-1 (gene : Phosphoglycerate Kinase) promoter
Complete promoter size: 1440 bp
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 hexadoxynucleotide, GGCGGG, a potential binding site for the Sp1 transcription factor, a CCAAT sequence, but no TATA box. This promoter can efficiently drive high levels of expression of reporter genes (i.e. LacZ and GFP) and therapeutic genes, such as tumor-associated antigens.

Furthermore, in contrast to the CMV promoter, the PGK promoter yields sustained expression.


PLASMID FEATURES
- Lucia luciferase is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.
- ORF size (from the ATG to the stop codon): 634 bp

Lucia luciferase activity can be evaluated using QUANTI-Luc™, an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.
- SV40 pA: 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 pDRIVE plasmid.

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

METHODS

Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:
Plasmid amplification and cloning can be performed in E. coli GT116 or other commonly used laboratory E. coli strains, such as DH5α.

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 microwavable, 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-1, 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.
5- Reheat the medium for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. Be careful, the bottle and media are hot.
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.
3401  CCGACAGGACTATAAGATACCAAGGCGTTCTCCCTGGAAGCTCTCCTGTGCGTCTCTCTGTTCCGCCCTACGGATACCTGTCCGCCTTTC
3501  TCCCTTGAGGAAGCTGGCGCTTTCTCTATAGCTACGCCTGTTAGTTCTAGTTGCCGCTTCGTTCCAAAGCTGGGCTGTCAGAACCCTCC
3601  CGTTGAGCCCCGCCGCTGCCTTTATCGGTAATAGCTGCTTGAAGTCCAACCCGGTGAAGACAGACCTTATGCGCAGCTGGCAGCAGCAGCTGAT
3701  ATTACGAGGCGAGTAGTGAGGCCTGTACAGGTTGACTACGGCTACTACAGACAGATATTTGGTATCTGCCTGCTGC
3801  TGAAGCCAGTTACCTTCCGAAAAGAGTTTGAGATCTTTGATCCCGCAAAACACCCGCCTGTAGCGGTTGGTTTTTTGTTTGCAAAGCAGAGATTAC
3901  GGGCAGAAAAAGAGATCTCAAGAGAGTCTCTTTTCTACGGGGGTCTGACGTCAGTGAACGAAACACTCAGTTAAGGGATTGGGTACATGGC
4001  AGTAAATTACATTAAATCA