pDRIVE5SEAP-chEF1
A plasmid with a native ubiquitous chimpanzee elongation factor 1 promoter
Catalog # pdrive5s-chef1

For research use only
Version # 15K12-MM

PRODUCT INFORMATION

Contents:
• 20 µg of pDRIVE5SEAP-chEF1 provided as lyophilized DNA
• 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)

Storage and Stability:
• Product is shipped at room temperature.
• Lyophilized DNA should be stored at -20°C.
• Resuspended DNA should be stored at -20°C and is stable up to 1 year.
• Store E. coli Fast-Media® at room temperature in a dry and cool place.

Quality Control:
• Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
• Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE
pDRIVE5SEAP is an expression plasmid containing a native or composite promoter of interest. pDRIVE5SEAP 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 Sdal which is compatible with NsiI and PstI. The 3’ restriction site is NcoI which includes the ATG start codon. NcoI is compatible with BspHI and BspLU11.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5SEAP 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 restriction sites (NcoI and NheI) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS
Chimpanzee elongation factor 1 promoter
Complete Promoter size: 1365 bp

The EF-1 alpha gene encoding elongation factor-1 alpha is an enzyme which catalyzes the GTP-dependent binding of aminoacyl-tRNA to ribosomes. EF-1α is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The promoter of this housekeeping gene exhibits a strong activity, higher than viral promoters such as SV40 and RSV promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo. InvivoGen has cloned the EF-1α promoter region of different species, among them the chimpanzee, mouse and rat. These sequences have not been described yet. They share respectively 98.46, 47.73 and 45.05% homology with the sequence of the human EF-1α promoter.


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 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 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 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.
pDRIVE5s-chEF1
(4885 bp)