

pDRIVE-rEF1 α

A plasmid with the native rat Elongation Factor-1 alpha promoter

Catalog # pdrive-ref1

For research use only

Version # 05B15-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-rEF1 α .
- GT100 genotype is: *F*-, *mcrA*, Δ (*mrr-hsdRMS-mcrBC*), Φ 80*lacZ* Δ M15, *ΔlacX74*, *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® Zeo

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.
- Promoter activity has been confirmed by transient transfection of 293 cells as well as other selected cell lines.

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' sites are *Sda* I and *Pst* I. *Sda* I is compatible with *Nsi* I and *Pst* I. The 3' restriction site is *Nco* I which includes the ATG start codon, and is compatible with *Bsp*H I, *Afl* III and *Sty* I.
- **Compare the activity of different promoters** in transient transfection experiments. Each pDRIVE promoter drives the expression of the *LacZ* reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the *LacZ* gene is flanked by unique restriction sites (*Nco* I and *Eco*R I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	EF-1 α	Rat	1314
5' UTR	EF-1 α	Rat	998*
Intron*	EF-1 α	Rat	931

*Intron is contained within the 5' UTR. ^Size of 5' UTR without intron is 67 bp.

EF-1 α promoter

The EF-1 alpha gene encodes for elongation factor-1 alpha which 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 promoters¹, and on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo². The rat EF-1 α promoter shares a 45.05% homology to the human EF-1 α promoter.

PLASMID FEATURES

- **LacZ gene** encodes β -galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope.
 - **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.
 - **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
 - **Sh ble** 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

Growth of pDRIVE-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 pDRIVE plasmid DNA using the method of your choice.
- Note:** For long-term storage of the pDRIVE-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

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-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. **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.

References:

- 1- Kim DW *et al.* (1990). *Gene*. 91(2): 217-23.
2. Guo ZS *et al.* (1996). *Gene Ther.* 3(9):802-10

TECHNICAL SUPPORT

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