

# pDRIVE-chEF1 $\alpha$ /RU5'

A plasmid with a composite promoter consisting of the chimpanzee Elongation Factor-1 alpha and HTLV 5' UTR

Catalog # pdrive-chef1ru5

For research use only

Version # 04L15-SV

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-chEF1 $\alpha$ /RU5'.
- GT100 genotype is: *F*-, *mcrA*,  $\Delta$ (*mrr-hsdRMS-mcrBC*),  $\Delta$ 80*lacZ* $\Delta$ M15,  $\Delta$ *lacX74*, *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® is 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 include *Sda* I, *Pst* I, and *Spe* I. *Sda* I is compatible with *Nsi* I and *Pst* I. *Spe* I is compatible with *Avr* II, *Nhe* I and *Xba* I. The 3' restriction site is *Nco* I which includes the ATG start codon, and is compatible with *BspH* I and *BspLU11* 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 $\alpha$	Chimpanzee	672
5'UTR	HTLV	Viral	267
Intron	-	-	-
Enhancer	-	-	-

### **EF-1 $\alpha$ 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 promoters<sup>1</sup>, and on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo<sup>2</sup>. The chimpanzee EF-1 $\alpha$  promoter shares a 98.46% homology to the human EF-1 $\alpha$  promoter.

## PLASMID FEATURES

- **LacZ gene** encodes  $\beta$ -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.

### 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:

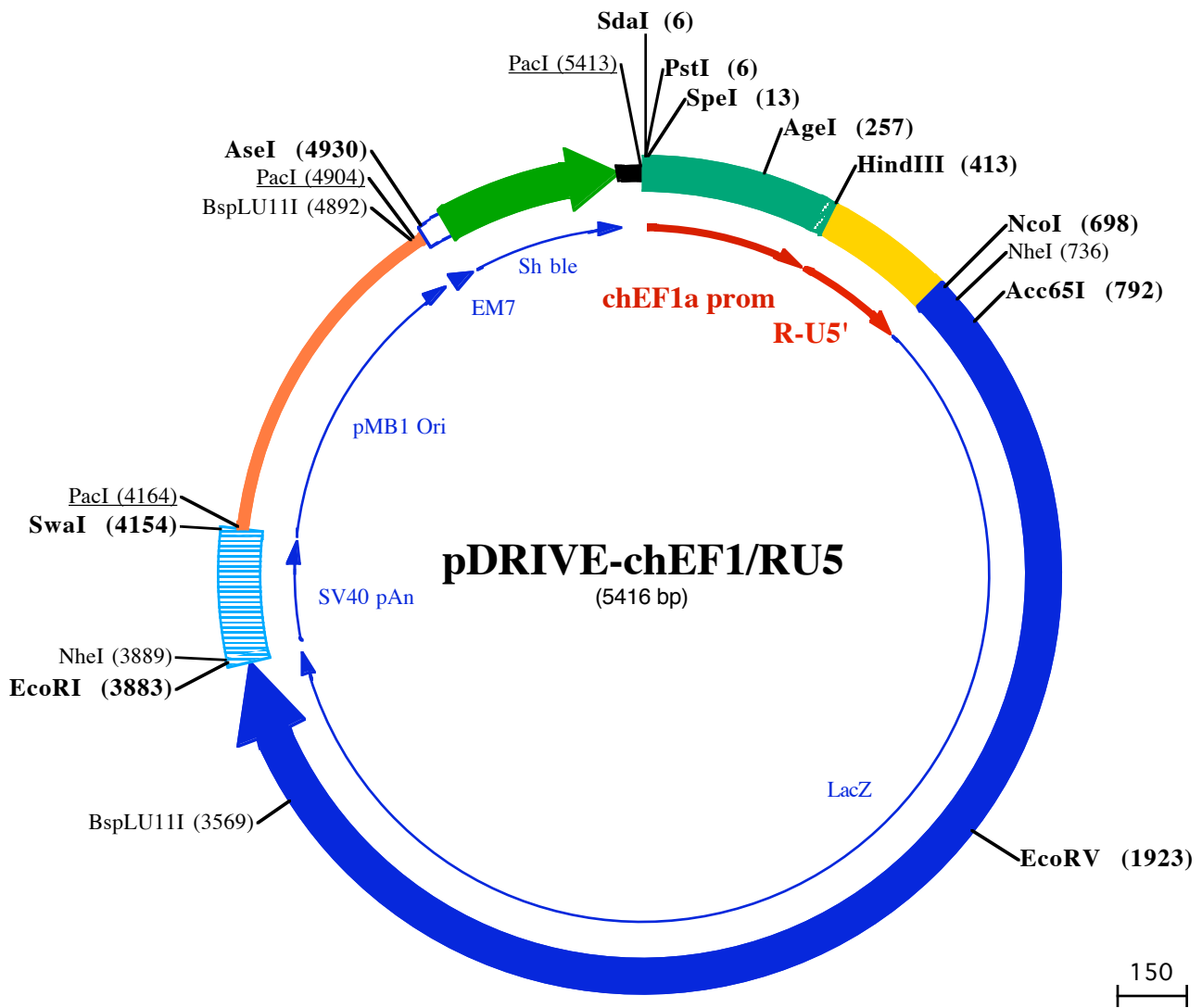
- 1- Kim DW *et al.* (1990). *Gene*. 91(2): 217-23.
2. Guo ZS *et al.* (1996). *Gene Ther.* 3(9):802-10

## TECHNICAL SUPPORT

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BspLU11I (3569)  
3501 GCCGAAGAAAACCTATCCCAGCGCCTTACTGCCGCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGCTCTCCGAGCGAA  
934▶ yProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGlu  
3601 AACGGTCTGCGCTGCGGGACGCGGAATTGAATTATGGCCACACCAGTGGCGCGGACTTCCAGTTCAACATCAGCCGCTACAGTCAACGCAACTGA  
968▶ AsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuM  
3701 TGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTCCATATGGGATTGGTGGCGACGACTCTGGAGCCC  
1001▶ etGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyThrTrpLeuAsnIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerPr

NheI (3889)  
**EcoRI (3883)**  
3801 GTCAGTATCGGCGGAATTACAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAATAATAATCTAGTCGAGAATTCGCTAGCTCGACA  
1034▶ oSerValSerAlaGluLeuGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys•••  
3901 TGATAAGATACATTGATGAGTTTGGACAAACCACAACACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAA  
4001 ATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTACAGGGGAGG

PacI (4164)  
**SwaI (4154)**  
4101 TGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAAGTCCATGACCAAAATCCCTTAACGTGAGTTT  
4201 TCGTTCCTACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGTAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACAC  
4301 CGCTACCAGCGGTGTTTTGTTTCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTCAGCAGAGCGCAGATACCAATACTGTTCTTCT  
4401 AGTGTAGCCGTAGTTAGGCCACCACTCAAGAAGCTCTGTAGCACCAGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGAT  
4501 AAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGAGC  
4601 GAACGACCTACACGAAGTACCTACAGCGTGTAGCTATGAGAAAGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAG  
4701 GGTGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCTGGTATCTTATAGTCTGTCGGGTTTCCGCACCTCTGACTTGAGCGTCGATTT

PacI (4904)  
BspLU11I (4892)  
4801 TTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGCCCTTTTACGGTTCCTGGCCTTTTGTGGCCTTTTGTCCACATGTTCT

**AseI (4930)**  
4901 TAATTAATTTTTCAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTG  
5001 ACCAGTGTGTCCAGTGTCTACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGTTTCTCCAGAGATTTTGTGGAGGATGACT  
5101 TTGACGGTGTGGTCAGAGATGATGTACCCTGTTTACTCTCAGCAGTCCAGGACCAGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACT  
5201 GGATGAGCTGTATGCTGAGTGGAGTGGTGGTCTCCACCACTTCCAGGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGA  
5301 GAGTTTGCCTGAGAGACCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGTAACAAAAACCCCGCCCGGGGG

PacI (5413)  
5401 GTTTTTTGTAAATTA