Before using this product, please read the Limited Use License statement below:

**Important Limited Use License information for pDRIVE5Lucia-chEF1**

The purchase of the pDRIVE5Lucia-chEF1 vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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pDRIVE5Lucia-chEF1
A pDRIVE plasmid encoding chimpanzee EF1 promoter
Catalog code: pdrive5lc-chEF1
https://www.invivogen.com/pdrive-ef1
For research use only
Version 19E06-MM

PRODUCT INFORMATION
Contents:
• 20 µg of pDRIVE5Lucia-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 for up to 1 year.
• Store E. coli Fast-Media® at room temperature in a dry and cool place.
Fast-Media® pouches are stable for 2 years when stored properly.
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
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 SdaI 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 BspLu11I.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5Lucia 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 unique restriction sites (NcoI and NheI) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS
Gene promoter: Elongation Factor 1 (EF1)
Specificity: Ubiquitous
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 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.

- 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 DH5a.
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 400 Watts), 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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InvivoGen
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pDRIVE5Lucia-chEF1
(3953 bp)

EF1alpha(ch) prom

SdaI (38)
SpeI (45)
AgeI (289)
BstXI (522)
PvuII (734)
BglII (781)
SacI (1043)
XhoI (1180)
NcoI (1415)
BglII (1530)
NheI (2049)
SspI (2528)
SgrAI (2956)

100

Zeo

Lucia

SV40 p(A)

EM2K

pMB1 ori
GTGGTCCAGAATCTGACCACCGCTCAGGCAGCTGCGCGCGGTGACCGAAGACGGCAGCTGCAACTTGCTGGCCATTGATGGCTCTCCCTGTCAGGAGAGGA


AAGAAGAAAGGTTAGTCAATTGCTATAAGTAGGTTGTTATATACCTATGCAAGATATACAGAATAGTTAGTCAAACTAGGGCTTCGGTATT

AAGAACATGTGAGCAGAGAACAGGACAGCAAAGGCGGACGTCTGGCTGCGTGGCTTTCTCATAAGGCTTCCGCCCCTGACGAGCATCAC

AAAATCGAGCTCAAGTCAGGCTGCGGAAACCGAAGGACTTTAAGATAACAGGGGTGGTTCTCCCTGCTCTGGCTCCTGCGGTGGTTCTGG

CGTCACAGCTGGGCTGTGCTCAGCAAGACCCCCGCTCGACCGACCGCTGCGCCTTTATCAGGTAACCTACGTCTTGAGTCCAAACCCGGTAGA

TCGCCACTGGCAGCAGCAGCTGTAACAGATTAGCAGACGGAGGATGTAGCGGCGTCTACAGAGTTGATGTGGCTAAGCTACGGCTACAC

TAGAAGAACAGTATTGGTATCTGCCTGCTGCTGAAGCAGGTACTTCCTGGAGAAAAAGGTTGTAGCTCTTTGATCAGGCAAAAACACCGCGT

GGTGTTTTTTTTGGTTGCAAAGCAGCAGATTAGCAGCGACAGAAAAAGGATCTCAAGAAGATCCTTTTGATCCTTTTCTACGGGCTGCTAGTG

AAAACTCAGTTAAGGATTGGTGCTAGTTAAATTAAATCA