pDRIVE-hFerL/RU5’

A plasmid with a composite promoter comprised of the human Ferritin Light and HTLV 5’ UTR

Catalog # pdrive-hferlru5

For research use only

Version # 04L15-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-hFerL/RU5’.
- GT100 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, 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 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 *EcoR* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	FerL	Human	537
5’UTR	HTLV	Viral	267
Intron	-	-	-

hFerL/HTLV promoter

Ferritin is a ubiquitous iron storage protein. Ferritin is a 24 subunit protein composed of two subunit types termed H (heavy) and L (light) which perform complementary functions in the protein. The synthesis of ferritin is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through interaction between a 28-nucleotide iron-responsive element (IRE) located in the 5’ UTR of ferritin mRNAs and a cytosolic protein, the iron regulatory protein[1].To eliminate the iron regulation of the ferritin promoter, the 5’ UTR of FerL has been replaced by the 5’ UTR of the HTLV. This modification makes the FerL promoter ubiquitous, strong and constitutive.

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.

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-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.*

References:

1- Eisenstein RS & Munro HN. (1990). Enzyme 44(1-4): 42-58.

TECHNICAL SUPPORT
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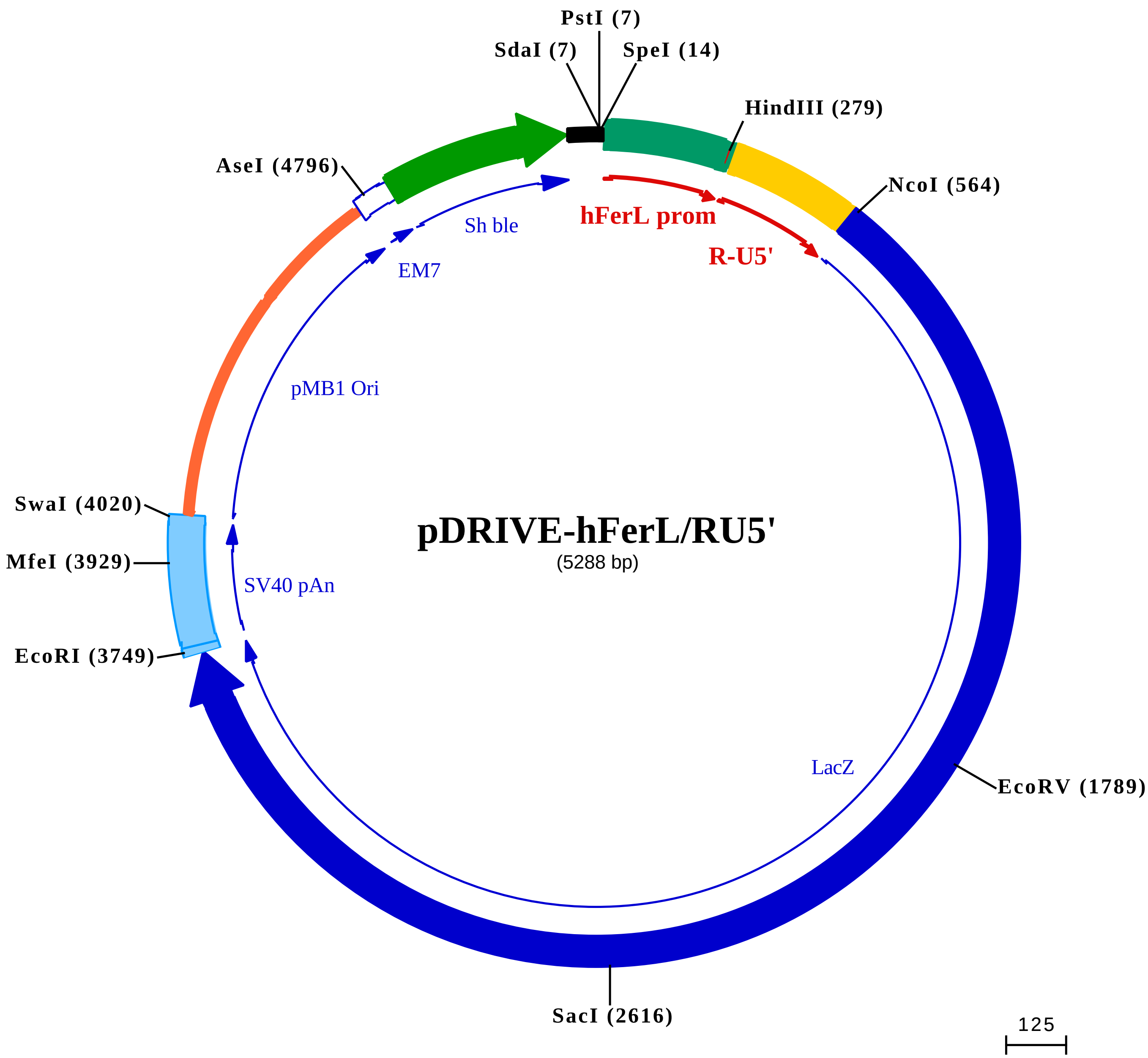
PstI (7)
SdaI (7)
SpeI (14)
HindIII (279)
NcoI (564)
AseI (4796)
Sh ble
hFerL prom
R-U5'
EM7
pMB1 Ori
SwaI (4020)
MfeI (3929)
EcoRI (3749)
SV40 pAn
pDRIVE-hFerL/RU5'
(5288 bp)
LacZ
EcoRV (1789)
SacI (2616)
125

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   PstI (7)
  SdaI (7)          SpeI (14)
   1 CCTGCAGGGCCCACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGGG

 101 GGCTGAGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGCCCTCCCCGAGCGCCCTGCCT

                                                                                  HindIII (279)
 201 CCGAGGGCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTCGAGGGGCTCGCATCTC

 301 TCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCC

 401 GTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCC

                                                                        NcoI (564)
 501 TGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCCACCATGGGGGGTTCTCATCATCATCATCATCATGGTATG
                                                                        1 MetGlyGlySerHisHisHisHisHisHisGlyMet
 601 GCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAAC
  13 AlaSerMetThrGlyGlyGlnGlnMetGlyArgAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnA
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 801 CCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCT
  79 gProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuPro
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1001 TTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGT
 146 heValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyVa
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1201 GGAGAAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCT
 213 GlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValS
1301 CGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGAT
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 313 AspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValV
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1901 CCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCG
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             SacI (2616)
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 679 sSerAspAsnGluLeuLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGlnGlyLysGlnLeu
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                                   MfeI (3929)
3901 AAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTAC

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4201 CTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACT

4301 CTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA

4401 GTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGT

4501 GAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAG

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                                                                                                   AseI (4796)
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116  lHisPheValAlaGluGluGlnAsp•••
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