

pDRIVE-heIF4A1

A plasmid with the native human Eukaryotic Initiation Factor 4A1 promoter

Catalog # pdrive-heif4a1

For research use only

Version # 04L15-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by a pDRIVE plasmid.
- GT100 genotype is: *F*-, *mcrA*, Δ (*mrr-hsdRMS-mcrBC*), Δ 80*lacZ* Δ M15, Δ *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® 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	eIF4A1	Human	523
5'UTR	eIF4A1	Human	18
Enhancer	-	-	-

eIF4A1 promoter

The eIF4A1 gene encoding eukaryotic initiation factor 4A is ubiquitously expressed in eukaryotic cells. The minimal promoter of the eIF4A1 gene consists of approximately 300 base pairs, 80% of which were identical with those in the corresponding mouse promoter. The minimal promoter contains TATA and CAAT motifs and consensus sequences binding to SP1 and AP2'. The eIF4A1 promoter is a versatile promoter giving high-levels of expression. In some cell lines, such as macrophages, levels of expression are higher than that obtained with the CMV promoter².

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:

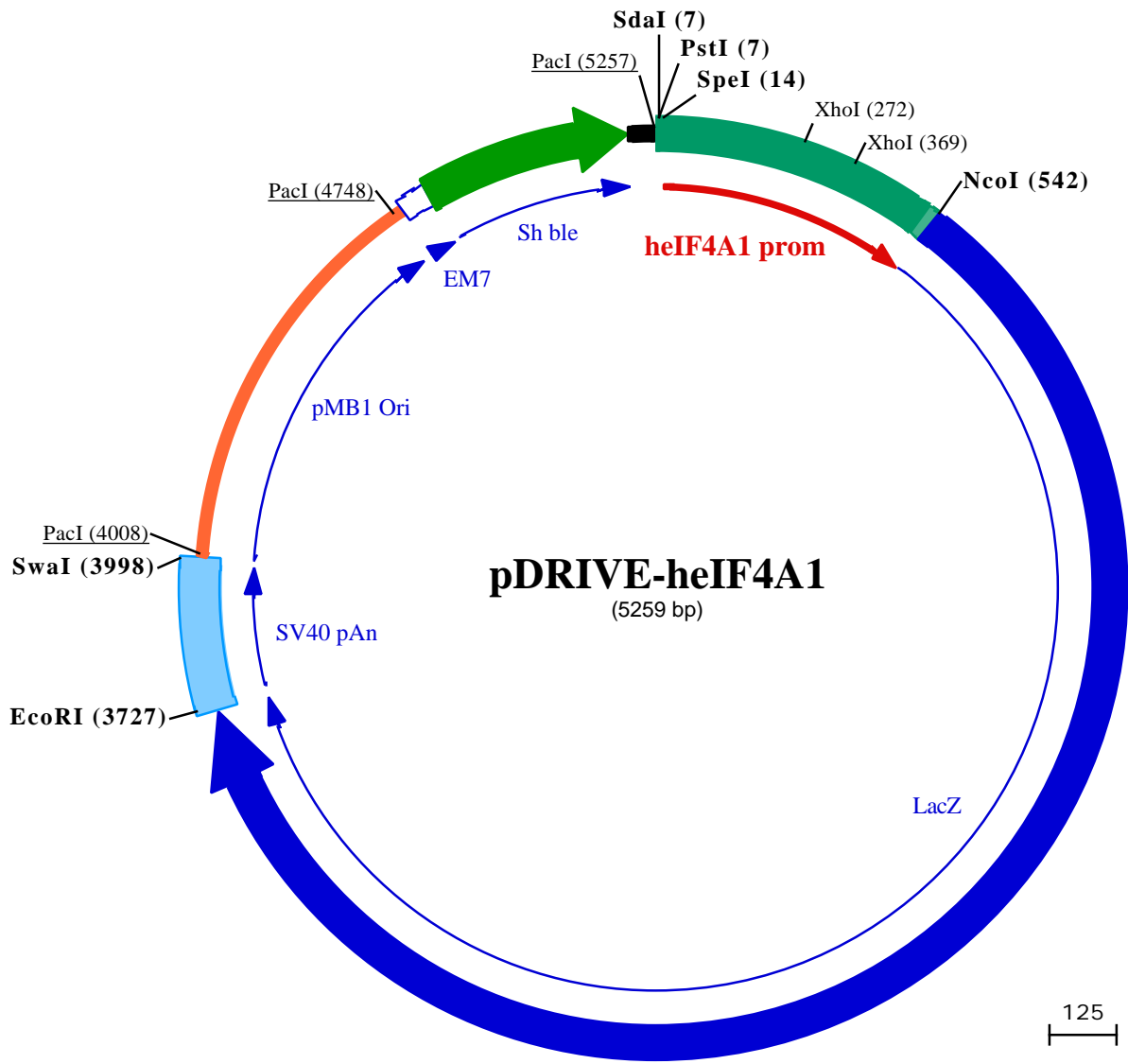
- 1- Kukimoto I et al. (1997). Biochem Biophys Res Commun 233(3):844-7
2. Quinn CM et al. (1999). Genomics 62(3):468-76

TECHNICAL SUPPORT

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PstI (7)
SdaI (7) SpeI (14)

1 CCTGCAGGGCCCACTAGTTGATTTCCTTCATCCCTGGCACACGTCACAGGCAGTGTGCAATCCATCTCTGCTACAGGGGAAAAACAATAACATTTGAGTCC
101 AGTGGAGACCGGGAGCAGAAGTAAAGGGAAGTGATAACCCACAGAGCCCGGAAGCCTCTGGAGGCTGAGACCTCGCCCCCTTGGTGATAGGGCTACG
XhoI (272)
201 GAGCCACATGACCAAGGCACTGTCCCTCCGCACGTGTGAGAGTGCAGGGCCCAAGATGGCTGCCAGGCTCGAGGCGCTACTTCTATGTCACTTCC
XhoI (369)
301 GTACCGCGGAGAAAGCGGGCCCTCCAGCCAATGAGGCTGCGGGCGGGCCCTTACCTTGATAGGCACTCGAGTTATCCAATGGTGCCTGCGGGCCGGAG
401 CGACTAGGAACAACTCATGCCAGATTGCTGAGCGCCGGCAGGCGGGCGGGCGGCAACCAATGCGATGGCCGGGGCGGAGTCGGGCGCTCTATA

NeoI (542)

501 AGTTGTGCGATAGCGGGGCACTCCGCCCTAGTTTCTAAGGACCATGGGGGTTCTCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGC
1 MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnG
601 AAATGGGTCGGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGCTGTTTACACCGCTGCTGGGAAAAACCCTGG
20 InMetGlyArgAspLeuTyrAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProG
701 CGTTACCAACTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCAACAGTTGCGCAGC
53 yValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSer
801 CTGAATGGCGAATGGCGTTTCCGCTGGTTCCGGCACCAGAAGCGGTGCCGAAAGCTGGCTGGAGTGCATCTTCCCTGAGGCGGATACTGTCGCTGCC
87 LeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValP
901 CCTCAAATGGCAGATGCAGGTTACGATGCGCCACTACACCAACGTAACCTATCCCATACGGTCAATCGCCGTTTGTCCACGGAGAATCCGAC
120 roSerAsnTrpGlnMetHisGlyTyrAspAlaProI leTyrThrAsnValThrTyrProI leThrValAsnProProPheValProThrGluAsnProTh
1001 GGGTTGTACTCGCTCACATTAATGTTGATGAAAGCTGGCTACAGGAAGCCAGCAGCGAATTATTTTGTGAGCGTAACTCGGCGTTTCACTCTGTGG
153 rGlyCysTrpSerLeuThrPheAsnValAspGluSerTrpLeuGlnGlyGlnThrArgI leI lePheAspGlyValAsnSerAlaPheHisLeuTrp
1101 TGCAACGGGCGTGGTGGTTACGGCCAGGACAGTCTGTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGAGAAAAACCCCTCGCGTGA
187 CysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValM
1201 TGGTGTGCTGGTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGCTCGTTGCTGCATAAACCGACTAC
220 etValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyI lePheArgAspValSerLeuHisLysProThrTh
1301 ACAATACAGGATTTCCATGTTGCCACTCGCTTAAATGATGATTTACGCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGCGGAGTTGCGTGACTAC
253 rGlnI leSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyr
1401 CTACGGGTAACAGTTTCTTTATGGCAGGGTGAACCGCAGGTCGCCAGCGGCCACCGCCCTTTCGGCGGTGAATATCGATGAGCGTGGTGGTTATGCC
287 LeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyTrpAlaProPheGlyGlyGluI leI leAspGluArgGlyGlyTrpAlaA
1501 ATCCGCTCACACTCTGAAACGTCGAAAACCCGAACTGTGGAGCGCGAAATCCCGAATCTCTATCGTGGCTGGTTGAACCTGACACCCCGCAGC
320 spArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluI leProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspG
1601 CACGCTGATTGAAGCAGAAGCCTGCGATGTGCGTTTCCGCGAGGTGCGGATGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTGAGGC
353 yThrLeuI leGluAlaGluAlaCysAspValGlyPheArgGluValArgI leGluAsnGlyLeuLeuLeuAsnGlyLysProLeuLeuI leArgGly
1701 GTTAACCGTCACGAGCATCCTCTGCATGGTCAAGTATGAGCAGAGATGAGTGCAGGATATCCTGCTGATGAAGCAACAACCTTAAACCGCG
387 ValAsnArgHisGlnHisHisProLeuHisGlyGlnValMetAspGluValTrpMetValGlnAspI leLeuLeuMetGlnAsnGlnAsnProAlaV
1801 TGGCTGTTCGATTATCCGAACCATCCGCTGTGGTACAGCTGTGGCAGCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAACCCACGGCAT
420 alArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnI leGluThrHisGlyMe
1901 GGTCCCAATGAATCGTCTGACCGATGATCCGCGTGGCTACCGCGATGAGCGAACCCTAACCGCAATGGTGCAGCGGATCGTAATCACCCGAGTGTG
453 tValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValTrpValAspGlnTrpArgMetValGlnArgAspArgSerVal
2001 ATCATCTGGTCCGCTGGGAATGAATCAGGCCACGGCCTAATCAGCAGCGCTGTATCGCTGGATCAAACTGTGCTGATCCTTCCCGCCGGTGCAGTATG
487 I leI leTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpI leLysSerValAspProSerArgProValGlnTyrG
2101 AAGCGCGGGAGCCGACACCAGCCACCGATATATTGCCCCGATGACGCGCGGTGGATGAAGACCAGCCCTTCCCGCTGTGCCGAAATGGTCCAT
520 luGlyGlyGlyAlaSerThrThrAlaThrAspI leI leCysProMetTyrAlaArgValAspGluAsnProPheProAlaValProLysTrpSerI
2201 CAAAAATGGCTTTCGCTACCTGAGAGACGCGCCGCTGATCCTTTCGAAATACGCGACCGATGGGTAACAGTTCGCGGTTTCGCTAAATACTGG
553 eLysLysTrpLeuSerLeuProGlyGluThrArgProLeuI leLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrp
2301 CAGCGTTTCGTGATATCCCGTTCACAGGGCGCTTCTGCTGGGACTGGGTGGATAGTCCGCTGATTAATATGATGAAAACCGCAACCCGTTGGTCCG
587 GlnAlaPheArgGlnTrpArgLeuGlnGlyPheValTrpAspTrpValAspGlnTrpSerLeuI leLysTyrAspGlyAsnGlyAsnProTrpSerA
2401 CTTACCGCGGATTTTGGCGATACGCCAAGCATGCCAGTCTGTATPAAACGGTCTGGTCTTTCGCGACCGCAGCAGTCCAGCGCTGACGGAAGC
620 laTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAl
2501 AAAACACCAGCAGCAGTTTTTCCAGTTCGTTTATCCGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCTGCAC
653 aLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrI leGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeuLeuHis
2601 TGGATGGTGGCGTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCCTGGATGTCGCTCCACAAGGTAACAGTTGATTGAAGTGCCTGAAGTACCCG
687 TrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGlnGlyLysGlnLeuI leGluLeuProGluLeuProG
2701 AGCCGGAGAGCGCGGCAACTCTGGCTCACAGTACGCTAGTGAACCGAACCAGCGACCGCATGGTCAAGAGCCGGGCACATCAGCGCTGCCAGCAGT
720 InProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisI leSerAlaTrpGlnGlnTr
2801 CGCTGCGCGGAAAAACCTCAGTGTGACGCTCCCGCCGCTCCACGCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGTAAT
753 pTrpLeuAlaGluAsnSerValThrLeuProAlaAlaSerHisAlaI leProHisLeuThrThrSerGluMetAspPheCysI leGluLeuProGlyAsn
2901 AAGCGTTGGCAATTTAACCGCCAGTCAAGCTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACCTGCTGACGCGCTGCGGATCAGTTCACCCG
787 LysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpI leGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgA
3001 CACCGCTGGATAACGACATTGGCGTAAGTGAAGCCACCCGATGACCTAACCGCTGGTTCGAACGCTGGAAGCGCGGGCCATTACCAGGCCGAAGC
820 laProLeuAspAsnAspI leGlyValSerGluAlaThrArgI leAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisTyrGlnAlaGluAl
3101 AGCGTTGTGAGTGCAGCGCAGATACACTTGCCTGATCGGCTGATTACGACCGCTACCGCTGGCAGCATCAGGGGAAAAACCTTATTATCAGCCGG
853 aAlaLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeuI leThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheI leSerArg
3201 AAAACCTACCGGATTGATGGTGTGATGTTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACCCGCATCCGGCCGGATTGGCTGAACTGCC
887 LysThrTyrArgI leAspGlySerGlyGlnMetAlaI leThrValAspValGluValAlaSerAspThrProHisProAlaArgI leGlyLeuAsnCysG
3301 AGCTGGCGAGGTAGCAGGGGTAACCTGGCTGGATTAGGCGCGCAAGAAACTATCCGACCGCTTACTGCGCGTGTGTTGACCGCTGGGATCT
920 InLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLe
3401 GCCATTGTCAGACATGTATACCCGTCAGTCTTCCGAGCGAAAAACGGTCTGCGCTGCGGGACGCGGAATTGAATTATGGCCACACCGTGGCGGGC
953 uProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGly
3501 GACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCACTGATGGAACAGCCATCGCCATCTGCTGCACGGGAGAAAGGCATGGCTGAATATCG
987 AspPheHisAsnI leSerArgTyrSerGlnGlnGlnMetGluThrSerHisArgHisLeuLeuHisAlaGluGlyThrTrpLeuAsnI leA

