pDRIVE5s-SV40-hAlb

A plasmid with a composite promoter based on the human Albumin promoter and the SV40 enhancer

Catalog # pdrive5s-sv40halb

For research use only

Version # 09I24-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5s plasmid.
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1 Δdcm ΔsbcC-sbcD.*
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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.

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 *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 *BspL*U11 I.

- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (*Nco* I and *Nhe* I) for easy replacement with a different gene of interest.

COMPOSITE PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	Albumin	Human	175
5'UTR	Albumin	Human	39
Enhancer	SV40	Viral	235

Albumin promoter

The albumin gene is transcribed at very high levels in fetal liver and unlike the adjacent AFP gene remains active after birth. A small segment of the albumin 5' flanking region, from -170 to +20, is sufficient for promoter activity and specificity[1]. This segment contains the CCAAT box, TATA box and the binding site for HNF1, a liver specific transcription factor. The albumin promoter is a liver-specific promoter. It can drive the expression of a transgene, such as HSVtk, specifically in hepatocellular carcinoma cells eliminating the risk of sytstemic toxicity of GCV[2].

SV40 enhancer

The simian virus 40 enhancer is comprised of a 72-base-pair repeat. Its efficiency to increase promoter activity has been demonstrated by many groups in the mid-eighties[3,4,5]. The SV40 enhancer exhibits a pronounced host range in its enhancement of gene expression; the enhancement varies from 2-fold in nonpermissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[6].

References

1. Power SC. et al. 1994. Biochem Biophys Res Com 203(3): 1447-1456. **2. Kuriyama S. et al. 1997.** Int. J Cancer. 71(3): 470-5. **3. Byrne BJ et al. 1983.** Proc Natl Acad Sci U S A 80(3):721-5. **4. Wasylyk B et al. 1984.** Nucleic Acids Res. 12(14):5589-608. **5. Ondek B et al. 1987.** EMBO J. 6(4):1017-25. **6. Dean DA et al., 1999.** Exp Cell Res. 253(2):713-22.

PLASMID FEATURES

• **SEAP gene** encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods

• **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

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.*

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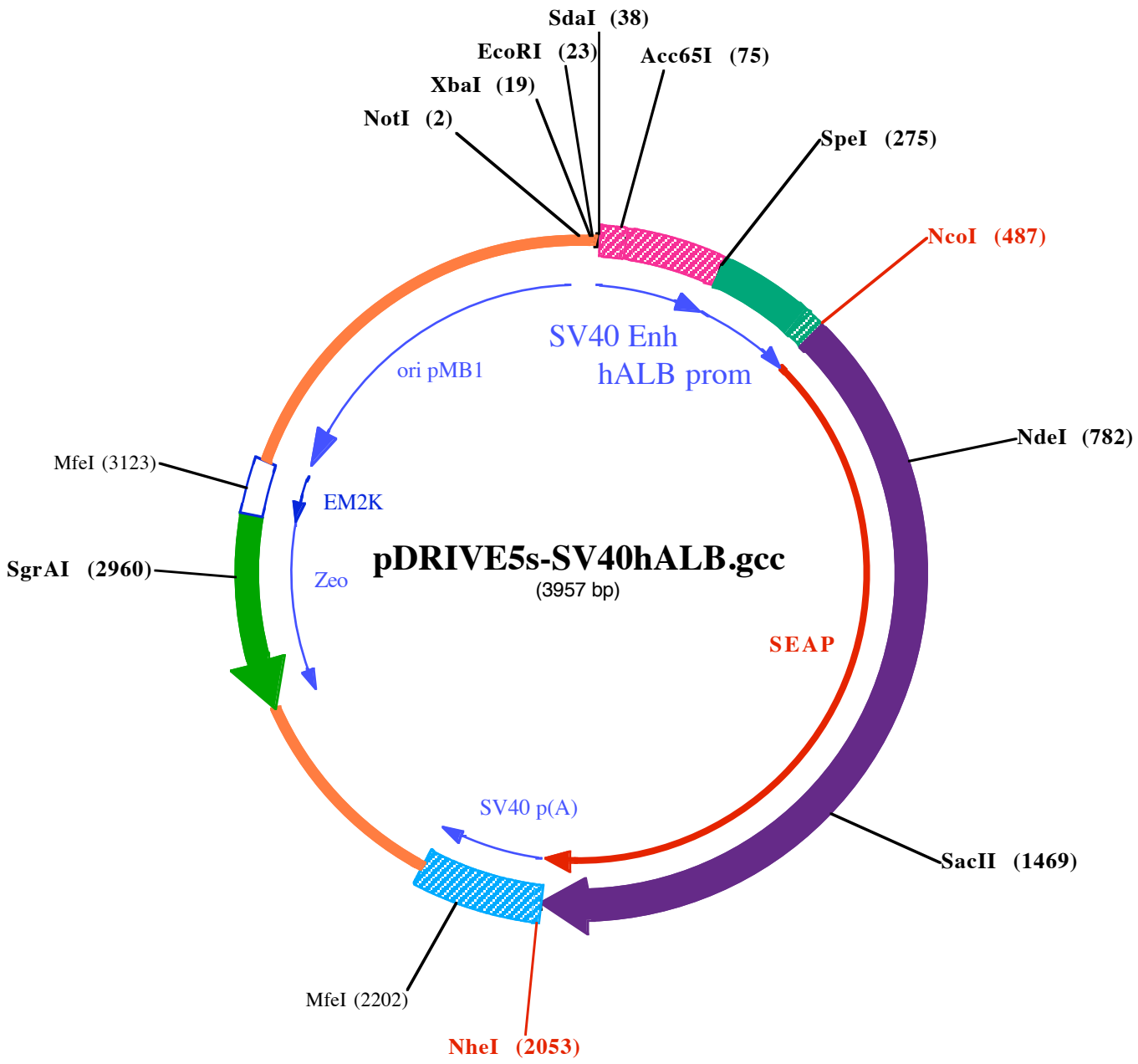
SdaI (38)
EcoRI (23)
Acc65I (75)
XbaI (19)
NotI (2)
SpeI (275)
NcoI (487)
SV40 Enh
hALB prom
ori pMB1
NdeI (782)
MfeI (3123)
EM2K
SgrAI (2960)
Zeo
pDRIVE5s-SV40hALB.gcc
(3957 bp)
SEAP
SV40 p(A)
SacII (1469)
MfeI (2202)
NheI (2053)

InvivoGen

EcoRI (23)
NotI (2) XbaI (19) SdaI (38) Acc65I (75)
1 GCGGCCGCGTCGACGATATCTAGAATTCGGATCCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCA

101 GCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGT

SpeI (275)
201 GGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCAGATGGTAAATATACAC

301 AAGGGATTTAGTCAAACAATTTTTTGGCAAGAATATTATGAATTTTGTAATCGGTTGGCAGCCAATGAAATACAAAGATGAGTCTAGTTAATAATCTACA

NcoI (487)
401 ATTATTGGTTAAAGAAGTATATTAGTGCTAATTTCCCTCCGTTTGTCCTAGCTTTTCTCTTCTGTCAACCCCACACGCCTTTGGCACcATGGTTCTGGGG
1 M V L G
501 CCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCG
5 P C M L L L L L L L G L R L Q L S L G I I P V E E E N P D F W N R
601 AGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTAC
38 E A A E A L G A A K K L Q P A Q T A A K N L I I F L G D G M G V S T

NdeI (782)
701 GGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAG
71 V T A A R I L K G Q K K D K L G P E I P L A M D R F P Y V A L S K
801 ACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAG
105 T Y N V D K H V P D S G A T A T A Y L C G V K G N F Q T I G L S A
901 CCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCAC
138 A A R F N Q C N T T R G N E V I S V M N R A K K A G K S V G V V T T
1001 CACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAG
171 T R V Q H A S P A G T Y A H T V N R N W Y S D A D V P A S A R Q E
1101 GGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACC
205 G C Q D I A T Q L I S N M D I D V I L G G G R K Y M F R M G T P D
1201 CTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTG
238 P E Y P D D Y S Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W
1301 GAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGAC
271 N R T E L M Q A S L D P S V T H L M G L F E P G D M K Y E I H R D

SacII (1469)
1401 TCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCA
305 S T L D P S L M E M T E A A L R L L S R N P R G F F L F V E G G R
1501 TCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGA
338 I D H G H H E S R A Y R A L T E T I M F D D A I E R A G Q L T S E E
1601 GGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAG
371 D T L S L V T A D H S H V F S F G G Y P L R G S S I F G L A P G K
1701 GCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCG
405 A R D R K A Y T V L L Y G N G P G Y V L K D G A R P D V T E S E S
1801 GGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCT
438 G S P E Y R Q Q S A V P L D E E T H A G E D V A V F A R G P Q A H L
1901 GGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGC
471 V H G V Q E Q T F I A H V M A F A A C L E P Y T A C D L A P P A G

NheI (2053)
2001 ACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACC
505 T T D A A H P G R S R S K R L D •
2101 ACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA

MfeI (2202)
2201 ACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAA

2301 ATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTG
2401 CATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGC
2501 ACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATG
2601 CTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTT
2701 ATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCAT
125 • D Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M
2801 GGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTG
93 A P G S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N

SgrAI (2960)
2901 TCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGA
59 D P V V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L
3001 GCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAG
26 R D T W F E V A G A V D R A T L V P V A S T L K A M

MfeI (3123)
3101 AGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTT

3201 AATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCAT

3301 CACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTC

3401 CGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGT

3501 CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACAC

3601 GACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCT

3701 ACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGG

3801 TAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGG

3901 AACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA