

pDRIVE-SV40/hAlb

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

Catalog # pdrive-sv40-halb

For research use only

Version # 05L13-MT

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-SV40/hAlb.
- 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 Hep-G2, 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.

COMPOSITE PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Core Promoter	Albumin	Human	175
S'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 specificity¹. 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 systemic toxicity of GCV².

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 non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids⁶.

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 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:

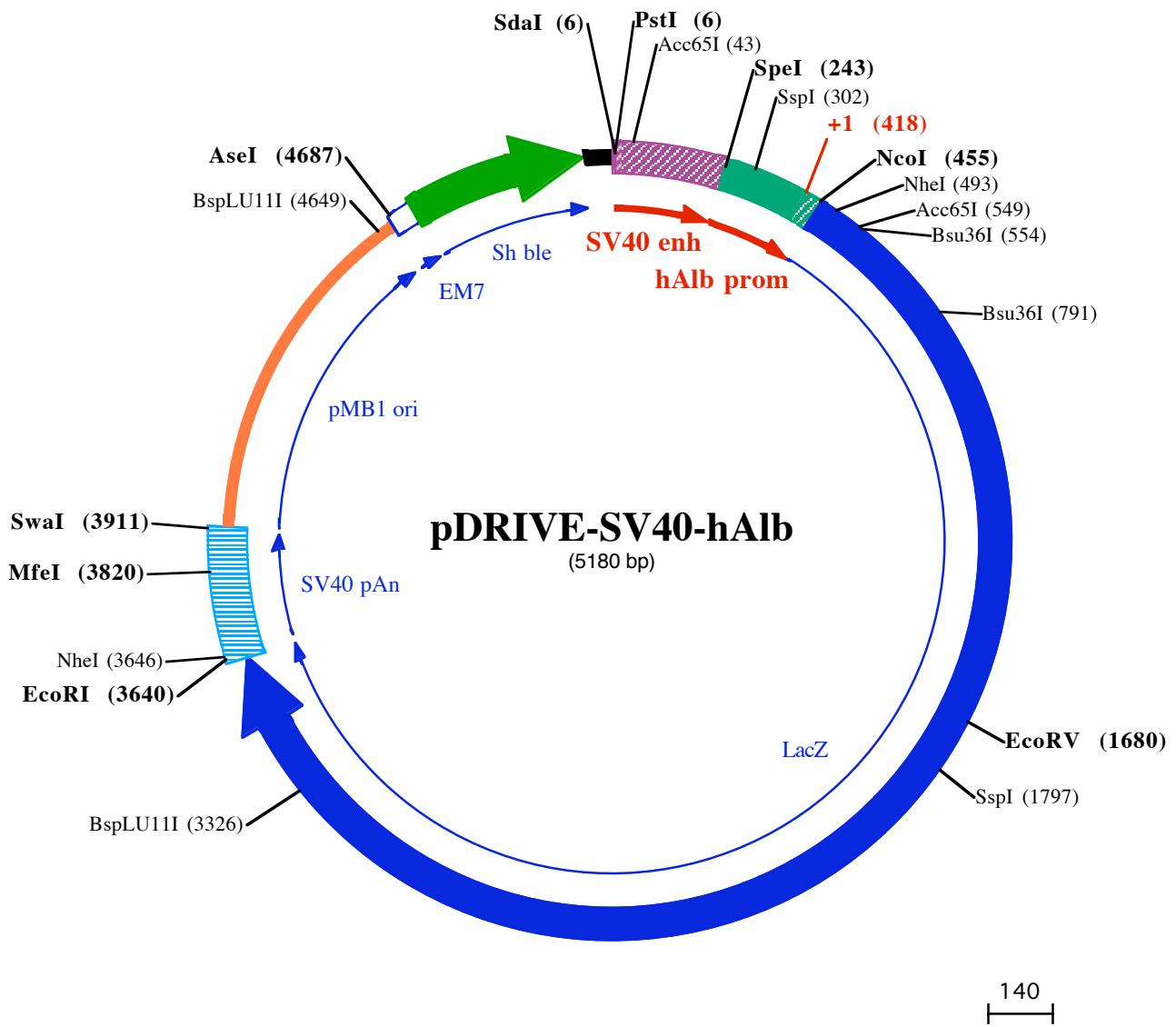
- 1- Power SC. et al. 1994. Biochem Biophys Res Com 203(3): 1447-1456.
- 2- Kuriyama S. et al. 1997. Int. J Cancer. 71(3): 470-5.
- 3- Byrne BJ et al. 1983. Proc Natl Acad Sci U S A 80(3):721-5
- 4- Wasylkyk B et al. 1984 Nucleic Acids Res. 12(14):5589-608
- 5- Ondek B et al. 1987. EMBO J. 6(4):1017-25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

TECHNICAL SUPPORT

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NheI (3646)
EcoRI (3640)

3601 TACCAAGTTGGTCTGGTGTCAAAAATAATACTAGTCGAGAATTGCTAGCTGCACATGATAAGATACATTGATGAGTTGGACAACCACAACTAGAAC
1049 Tyr Gl nLeu Val Trp Cys Gl nLys ***
3701 GCAGTGAAAAAAATGCTTATTTGAAATTGTGATGCTATTGCTTATTGAAATTGTGATGCTATTGCTTATTGTAACCATTATAAGCTGA

MfeI (3820)

3801 ATAAACAAGTTAACACAACAATTGATTTCAGGTTAGGGGAGGTGTGGAGGTTTAAAGCAAGTAAACCTCTACAAATGTGG

SwaI (3911)

3901 TAGATCCATTAAATGTTAATTAACTAGCCATGACCAAAATCCCTTAACGTGAGTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGAT
→
4001 CTTCTTGAGATCCTTTCTGCGCTAATCTGCTGCTGCAAACAAAAACCCACCGCTACCAGCGTGGTTGTTGCCGATCAAGAGCTACCAAC
4101 TCTTTTCCGAAGGTAACTGGCTCAGCAGAGCGCAGATAACAAACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACACTCAAGAACTCTGTAGCA
4201 CCGCCTACATACCTCGCTGCTAATCTGTTACAGTGGCTGCTGCCAGTGGAGCGAACCTACACCGAACTGAGATAACCTACAGCGTAGCTATG
4301 ATAAGGCGCAGCGGTCGGCTGAACGGGGGTTCTGCACACAGCCAGCTGGAGCGAACGACCTACACCGAACTGAGATAACCTACAGCGTAGCTATG
4401 AGAAAGCGCACGCTCCCGAAGGGAGAAAGGCGACAGGTATCGGTAAAGCGCAGGGTGGAAACAGGAGAGCGCAGGAGGGCTCCAGGGGGAAAC
4501 GCCTGGTATCTTATAGTCTGCGGTTGCCACCTCTGACTTGAGCGTCATTGATGCTCGTCAAGGGGGCGGAGCCTATGGAAAACGCCA

BspLU1II (4649)
AseI (4687)

4601 GCAACCGGCCCTTTTACGGTCTGGCTTTGCTGGCTTTGTCACATGTTCTTAATTAAATTTCAAAAGTAGTTGACAATTAAATCATCGGCAT
4701 AGTATATCGCATAGTATAATCGACTCACTATAGGGGCCATCATGGCCAAGTGACAGTGCTGCTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAG
→ 1► Met tAl aLys Leu Thr Ser Al aVal Pro Val Leu Thr Al aArg Asp Val Al aGl yA
4801 CTGTTGAGTTCTGGACTGACAGTTGGGTTCTCAGAGATTGAGGATGACTTGAGGTGGTCAAGAGATGATGTCACCCCTGTCATCTCAGC
19► l aVal Gl uPhe Trp Thr Asp Arg Leu Gl yPhe Ser Arg Asp Phe Val Gl uAsp Asp Phe Al aGl yVal Val Arg Asp Asp Val Thr Leu Phe l eSer Al
4901 AGTCCAGGACCAGGTGGCTGACACACCCCTGCTGGGTGAGAGACTGGATGAGCTGTATGCTGAGTGGAGTGGAGTGGCTCCACCAAC
52► aVal Gl nAsp Gl nVal Val Pro Asp Asn Thr Leu Al aTrp Val Trp Val Arg Gl yLeu Asp Gl uLeu Tyr Al aGl uTrp Ser Gl uVal Val Ser Thr Asn
5001 TTCAAGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGAGAGTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTG
86► Phe Arg Asp Al aSer Gl yPro Al aMet Thr Gl uL i eGl yGl uGl nPro Trp Gl yArg Gl uPhe Al aLeu Arg Asp Pro Al aGl yAsn Cys Val His Phe V
5101 TGGCAGAGGAGCAGGACTGAGGATAAGAATTGAGTTCAAGAAAAGGGGCTGAGTGGCCCTTTTCAACTTAATTAA
119► aAl aGl uGl uGl nAsp ***