

pDRIVE5s-hB29

A plasmid with a native tissue specific human B29 (immunoglobulin beta) promoter

Catalog # pdrive5s-hb29

For research use only

Version # 09F17-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5s plasmid.
- GT116 genotype is: *F*, *mcrA*, $\Delta(mrr-hsdRMS-mcrBC)$, $\emptyset 80lacZ\Delta M15$, $\Delta lacX74$, *recA1*, *endA1* Δdcm $\Delta sbaC-sbcD$.
- 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

pDRIVE5s is an expression plasmid containing a native or composite promoter of interest. pDRIVE5s 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 *Neo* 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 pDRIVE5s 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.

PROMOTER CHARACTERISTICS

Human B29 promoter

Complete Promoter size: 1219 bp

Specificity: B cells

B29 (Igbeta) is a B-cell-specific member of the immunoglobulin gene superfamily that is expressed throughout B-cell development. The product of the B29 gene is an essential component of the B cell receptor and plays a critical role in B cell development. The B29 gene lacks either a TATA or a CAAT box and transcription is initiated at multiple sites¹. The minimal promoter of the human B29 gene is contained within a <200-bp region 5' of these multiple start sites². This minimal promoter exhibits B-cell-specific activity and contains SP1, ETS, OCT, and IKAROS/LYF-1 transcription factor motifs³.

- 1. Hermanson GG, et al. 1989.** Immunoglobulin enhancer and promoter motifs 5' of the B29 B-cell-specific gene. Proc Natl Acad Sci U S A 86(19):7341-5.
- 2. Omori SA, & Wall R. 1993.** Multiple motifs regulate the B-cell-specific promoter of the B29 gene. Proc Natl Acad Sci USA 90(24):11723-7.
- 3. Thompson AA, et al., 1996.** The promoter and 5' flanking sequences controlling human B29 gene expression. Blood. 87(2):666-73.

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 pDRIVE5s 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 pDRIVE5s-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 pDRIVE5s 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.

TECHNICAL SUPPORT

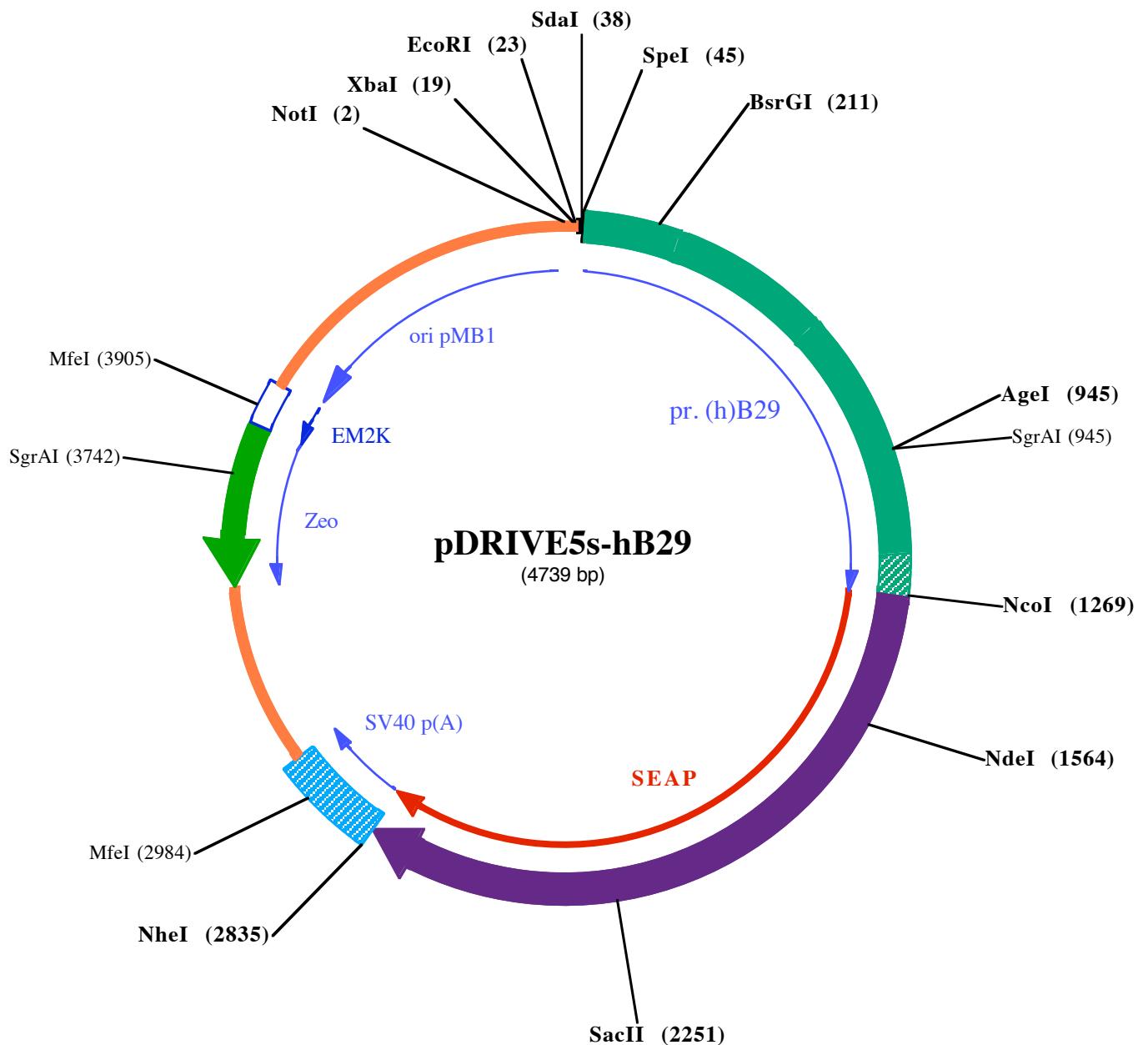
Toll free (US): 888-457-5873

Outside US: +(1) 858-457-5873

Europe: +33 562-71-69-39

E-mail: info@invivogen.com

Website: www.invivogen.com



EcoRI (23)

NotI (2)

XbaI (19)

SdAI (38) SpeI (45)

1 GCGGCCGCGTCGACGATATCTAGAATT CGGAT CCTGCAGGGCCACTAGTaaACGGAGGGTTGTGAGGAGAGT GAGAGGTGGACAGAGGCACCGACGAT

101 TTAGCATCTCCTCTCTGGGGTCGAGGATGAGAGACAAAAAAAGAAGCTGCCAGAACATAAATTCAAGGGCTCAGCTGCCAGGGCTGAGGTCTG

BsrGI (211)

201 CAAGCATGCTGTGACACTTGTGCATGTTGTGCCCTGACAAGGGCATCTGAAGGGCTGACTGGACCCAGGGCAGGGCGCAAAGGTGAGTTAT

301 ATCAGTTCTGAGCACGTGGCTCCATCCAGCACTCTGAGGACAGGAGATACTGGAGGACCTGAGGGCTCCCCACACCAGCTCTGTTCCCTGC

401 CCAAGACCCCCTGGACCTGCAGACAACAATTCAACGCACTCAGAGTCCCACAGTTAAGAACTCCCTGAAGAAGCCCCCAGTGGCTGCGTGGATTTC

501 GCAAAGCTGTCTCCACCTACATCCACCCCTGTTGGCAGCCCACATACTCTTACAGCATGAGGAAGGGAGGCCTCACCAAGACCTGGACTGAATC

601 TTCTCCCAGTGGCTGCCACACCTGACCTGCTCTGCTCCAGAACCTCTGAGGCTCCATCTCCACAGGGTCAACTTCAACATGGCTGCCACTCCA

701 GCCAAGAGGCTCTGCTCTGGGCCCTCCAGATGCCACCTGGCTCTGAGGCTCCATCTCCAGTGTCCCTTCCGCTGGGTGAGGAATAGTAGT

801 TCAGGACAGAGGAGCTAAGTTCAAGGTTCAATTCAAGGACAGGTGCCTATTCGCTCACGGCCAGGAATAGAGACTTGGCGGCTCGGCCCTCGGGAG

AgeI (945)

SgrAI (945)

901 TTGGCAGACGGCAGAGGGAGGCTGGCTGGCCAGGGATGACCACCGGTGGGTAAGCACAGACAGAGGGAGCACAGCTCCCCAGAACAGACTGAGA

1001 GGCCCCCCCAGAGGCATCCACAGAGGACCCCAAGCTGTGCTGCCAAGCTGGGGACCCCAAACCTTACGGGCCCCAGCTGACAAAAGCTGCCCTCCCCA

1101 GGGTCCCCGGAGAGCTGGTGCTCCCTGGTCCCAATTGATGGCAGGAAGGGGCTGGTGAGGAAGAGGCGGGAGGGACAGGCTGCAGCCGTGC

NcoI (1269)

1201 AGTTACACGTTTCTCCAAGGAGCCTCGACGTTGACGGTTTGGGGTCGGGAAGAGCGGTGACATGGTTCTGGGCCCTGCATGCTGCTGCTG

1301 CTGCTGCTGCTGGGCCCTGAGGCTACAGCTCTCCCTGGCATCATCCAGTTGAGGAGAGAACCGGACTCTGGAACCGCGAGGCAGGGCCCTGG

11▶ L L L L G L R L Q L S L G I I P V E E N P D F W N R E A A E A L

1401 GTGCCGCCAAGAACGCTGAGCCTGCACAGACAGCCCAAGAACCTCATCATCTCCCTGGCGATGGATGGGGTGTCTACGGTACAGCTGCCAGGAT

44▶ 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 V T A A R I

NdeI (1564)

1501 CCTAAAAGGGCAGAAGAACAAACTGGGCCCTGAGATAACCCCTGGCTATGGACCGCTTCCATATGGCTCTGTCAGAACACATAATGTAGACAAA

77▶ 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 T Y N V D K

1601 CATGTGCCAGACAGTGGAGGCCACAGGCCACGGCTACCTGTGCGGGTCAAGGGCAACTTCCAGACCATTGGCTGAGTCAGCCGCCCTTAAACAGT

111▶ 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 A A R F N Q

1701 GCAACACGACACGGCAACGAGGTCACTCCGTGATGAATCGGGCAAGAACAGCAGGAAGTCAGTGGAGTGGTAACCACACAGAGTGCAGCACGC

144▶ 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 R V Q H A

1801 CTCGCCAGCCGGCACCTACGCCACACGGTAACCGCAACTGGTACTCGGACGCCACGTGCCCTGCCCTGGCCGCCAGGAGGGTGCAGGACATCGCT

177▶ S P A G T Y A H T V N R W Y S D A D V P A S A R Q E G C Q D I A

1901 ACGGAGCTCATCTCCAAACATGGACATTGATGTGATCTGGTGGAGGCCAAAGTACATGTTGCGATGGAAACCCCGACACCTGAGTACCCAGATGACT

211▶ 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 P E Y P D D

2001 ACAGCCAAGGTGGGCCAGGCTGGACGGGAAGAATCTGGTGAGGAATGGCTGGCAAGGCCAGGGTGCCTGATGGAAACCGCACTGAGCTCAT

244▶ Y S Q G 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 N R T E L M

2101 GCAGGCTCCCTGGACCCGCTGTGACCCATCTCATGGTCTCTTGGAGCTGGAGACATGAAATACCGAGATCCACCGAGACTTACACTGGACCCCTCC

277▶ 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 S T L D P S

SacII (2251)

2201 CTGATGGAGATGACAGAGGCTGCCCTGCGCTGAGCAGGAACCCCGCGCTTCTCTCTTCTGGAGGGTGGTCGCATCGACCAAGGTCACTACAG

311▶ 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 I D H G H

2301 AAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTCAGCACGCCATTGAGAGGGCGGCCAGCTCACAGCAGGAGGACACGCTGAGCCTCGT

344▶ 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 D T L S L V

2401 CACTGGCACCACCTCCACGCTCTCTCCCTGGAGGCTACCCCTGCGAGGGAGCTCATCTCGGCCCTGGCAAGGCCGGACAGGAAGGCC

377▶ 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 A R D R K A

2501 TACACGGCTCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCCCGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTACCGC

411▶ 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 G S P E Y R

2601 AGCAGTCAGCAGTGGCCCTGGACGAAGAGACCCACGGCAGGGAGCTGGCGCGCCGGCAGGCGCACCTGGTACCGCGTGCAGGA

444▶ 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 V H G V Q E

2701 GCAGACCTTCATAGGCCACGTATGCCCTCGCCCTGGACCTGCGACCTGGCCGGCCCCGGCAGGCCACCGAGCCGACCGC

477▶ 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 T T D A A H

NheI (2835)

2801 CCGGGCGGTCCGGTCCAAGCGTCTGGATTGAGCTAGCTGGCCAGACATGATAAGATACTTGTGAGTTGGACAAACACAAACTAGAATGCCAGTGA

511▶ P G R S R S K R L D •

MfeI (2984)

2901 AAAAATGCTTATTGTGAAATTGTGATGCTATTGCTTATTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACAAATTGCAATTGCTATTG

3001 TGTTTCAGGTTCAAGGGGGAGGTGTGGGAGGTTTTAAAGCAAGTAAACCTCTACAAATGTGATGGTGAATTAACTAAACAGCATAGCAAAACT

3101 TTAACCTCAAATCAACGCTCTACTTGAATCCCTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTTGTGCCATTGCAATTAGCTGTTGCA

3201 TCACCTCTTCTCATGGAGTTAAAGATATAGTGTATTCTCCAAAGGTTGAACTAGCTCTTCATTTCTTATGTTAAATGCACTGACCTCCACATTCC

3301 CTTTTAGAAAAATTTCAGAAATAATTAAACATCATGGCAATGAAAATAATGTTTTATTAGGCAGAACCTAGCTCAAGGCCCTTCATAAT

3401 ATCCCCCAGTTAGTAGTGGACTTGGAAACAAAGGAACCTTAAAGAAATTGGACAGCAAGAAGCGAGCTTGTGCTTACAGTCCTGCTCC

3501 CTGCCACAAAGTGCACCGAGTTGCCGGCCGGTCGCCAGGGCGAACTCCCCCCCCACGGCTGCTCCCGATCTCGGTATGGCGGCCGGAGGCCTC
120◀ 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 A P G S A D
3601 CCGGAAGTTCGTGGACACGACCTCCGACCCTCGGCTACAGCTCGTCCAGGCCGCCACCCACCCCAGGCCAGGGTGTGTCCGGCACCACTGGTCC
87◀ 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 D P V V Q D
SgrAI (3742)
3701 TCGACCCGCTGATGAACAGGGTCACGTGGTCCCGAACACCCGGAAACTCGTCCTCCACGAAGTCCGGAGAACCCGAGCCGTCGGTCCAGAACT
53◀ 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 R D T W F E
3801 CGACCGCTCCGGCGACGTCGCGCGGTGAGCAGCGACTGGTCAACTGGCATGATGGCTCCTCTGTCAAGGAGAGGAAAGAGAAGAAGGTT
20◀ V A G A V D R A T L V P V A S T L K A M ←
MfeI (3905)
3901 AGTACAATTGCTATAGTGAGTTGTATTATACTATGCAAGATATACTATGCCAATGATTAATTGTCAA ACTAGGGCTGCAGGTTAATTAGAACATGTGAGC
4001 AAAAGGCCAGAAAAGGCCAGGAACCGTAAAAGGCCGTTGCTGGCTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCA
4101 AGTCAGAGGTGGCAAACCCGACAGGACTATAAGATACCAAGCGTTCCCTCTGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGG
4201 GATACTGTCCGCTTCTCCCTCGGAAGCGTGGCGTTCTCATAGCTCACGCTGTAGGTATCTCAGTTGGTAGGTCTCGCTCCAAGCTGG
4301 CTGTGTGCACGAACCCCCGTTCAAGCCGACCGCTGCCCTATCCGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGG
4401 GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTAT
4501 TTGGTATCGCGCTCTGTAAGCCAGTTACCTTGGAAAAAGAGTTGGTAGCTCTGATCCGCAAACAAACCAACCGCTGGTAGGGTGGTTTTTGT
4601 TTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAA
4701 GGGATTGGTCATGGCTAGTTAATTAAACATTAAATCA