pDRIVE5s-herbB2

A plasmid with a native tumor-specific human c-erbB2 promoter

Catalog # pdrive5s-herbb2

For research use only

Version # 11G05-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' restriction site is Sda I is compatible with Nsi I and Pst 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 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 c-erbB2/neu oncogene

Complete Promoter size: 891 bp

Specificity: Tumor

c-erbB2/neu oncogene (neu+) encodes a 185 kDa transmembrane protein with intrinsic tyrosine kinase activity, highly homologous with the epidermal growth factor receptor. The oncogene c-erbB2 is overexpressed in approximately one-third of breast and pancreatic tumors (and in a smaller proportion of other tumors). This overexpression involves transcriptional up-regulation of the c-erbB2 gene. The c-erbB2 promoter was shown to drive specific expression in cells overexpressing the oncogene[1]. Basal activity of the c-erbB2 promoter is equivalent to one third of the herpes simplex thymidine kinase promoter but can be significantly induced by several agents such as epidermal growth factor, dibutyryl cAMP and retinoic acid[2].

1. Harris JD. *et al*. 1994. Gene therapy for cancer using tumor-specific prodrug activation. Gene Ther. 1(3):170-5.
2. Hudson LG. *et al*. 1990. Structure and inducible regulation of the human c-erb B2/neu promoter. J Biol Chem. 265(8):4389-93.

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.*

TECHNICAL SUPPORT
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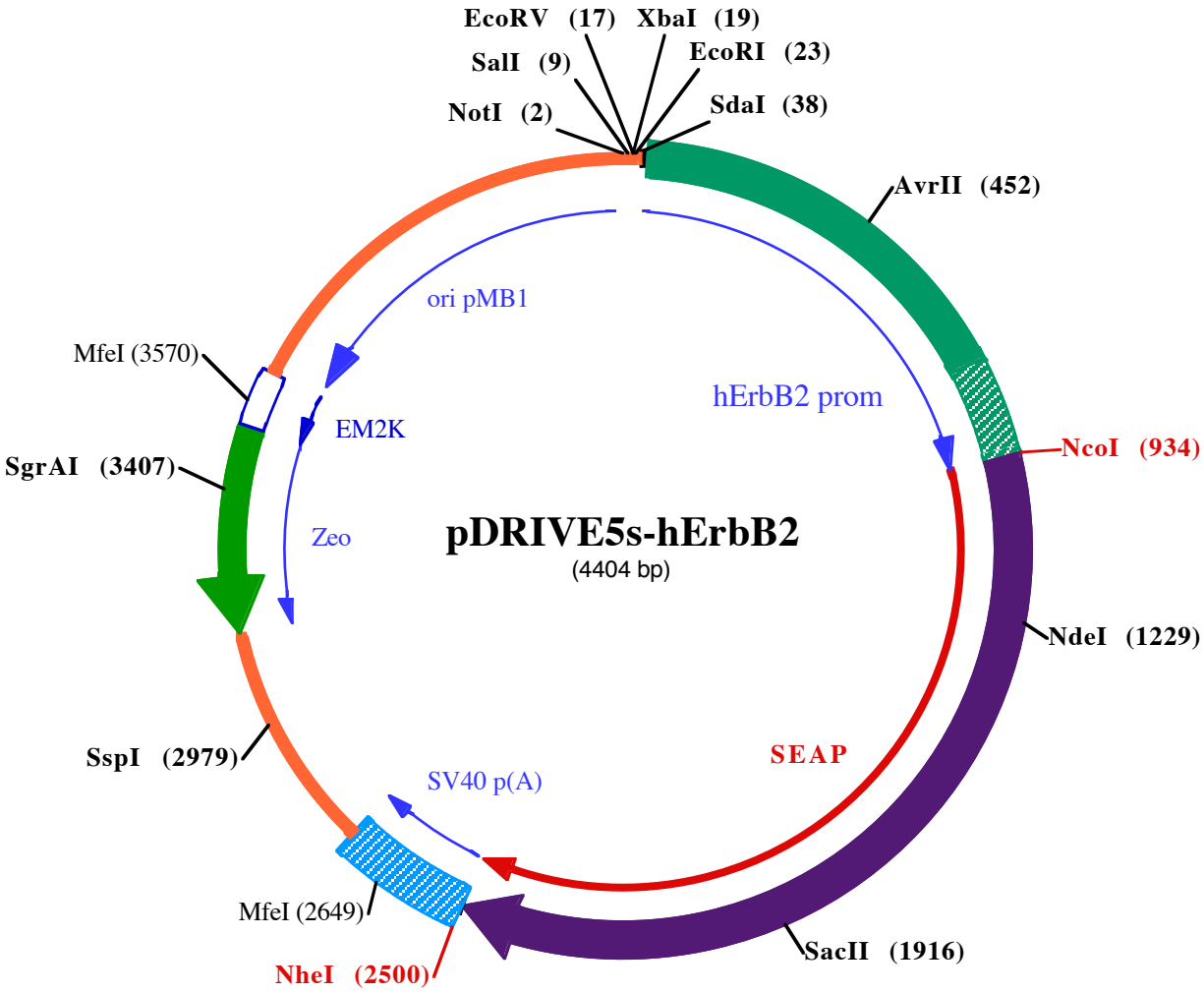
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XbaI (19)
SalI (9)
EcoRI (23)
NotI (2)
SdaI (38)
AvrII (452)
ori pMB1
MfeI (3570)
hErbB2 prom
EM2K
NcoI (934)
SgrAI (3407)
Zeo
pDRIVE5s-hErbB2
(4404 bp)
NdeI (1229)
SEAP
SspI (2979)
SV40 p(A)
MfeI (2649)
NheI (2500)
SacII (1916)


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EcoRV (17)
NotI (2) SalI (9) XbaI (19) SdaI (38)
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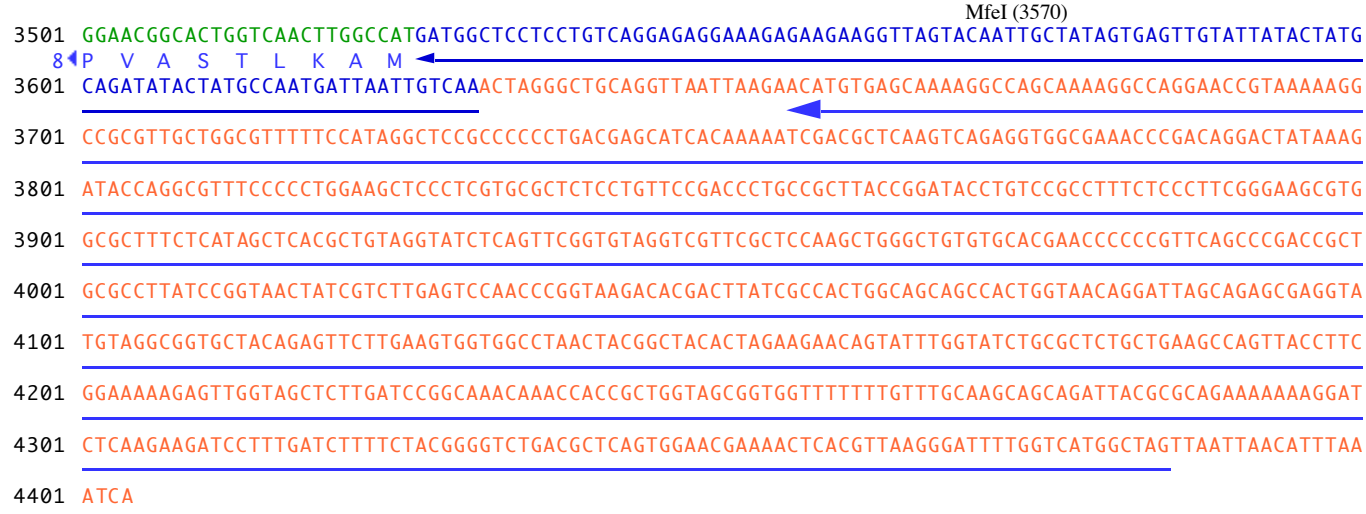
MfeI (3570)
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8 P V A S T L K A M
3601 CAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
3701 CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG
3801 ATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTG
3901 GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT
4001 GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
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4201 GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGAT
4301 CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAA
4401 ATCA

Fast-Media®

Microwaveable media for selection and propagation of *E. coli* transformants

Catalog # fas-xx-l, fas-xx-s, fas-xx-xgal

For research use only

Version # 10G07-MM

PRODUCT INFORMATION

Contents:

E. coli **Fast-Media®** are prepared as individual sealed pouches containing the necessary amount of powder for preparation of 200 ml of selective liquid or agar medium.

30 pouches are supplied for each order of TB or Agar and 20 pouches are supplied for each order of XGal Agar.

Storage and stability:

Fast-Media® are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.

When properly prepared, **Fast-Media®** plates or broths are stable for 4 weeks at 4˚C, and remain sterile and selective**.**

Quality control:

The high quality and performance of each formulation has been tested with some widely used and proprietary *E. coli* K12 derived strains*. These include DH5α, Top10, MC1061, XL1 blue, JM 109, TB1, GT100, GT110, GT115, GT116.

The adequate plasmids carrying the appropriate *E. coli* resistance genes are used as positive control.

**E. coli* recipient strains carrying the Tn5 transposon are resistant to Kanamycin and Zeocin™.

GENERAL PRODUCT USE

E. coli **Fast-Media®** are microwaveable ready-to-use solid or liquid media, supplied with a selective antibiotic, and chromogenic substrates (for five references), therefore designed for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies.

- Fast-Media® Agar formulation is LB based agar medium supplemented with selective antibiotic, it is used for selection of resistant *E. coli* colonies after transformation by vectors carrying a selection resistance gene.

- Fast-Media® X-Gal formulation is a LB based agar medium supplemented with selective antibiotic, X-Gal and IPTG. It is used for detection of blue/white resistant colonies after transformation by a vector carrying *LacZ* gene.

- Fast-Media® TB formulation is a Terrific Broth based liquid medium supplemented with selective antibiotic. It's used for high cell density culture of transformed bacteria, and extraction of high quantity and quality of required plasmid.

FAST-MEDIA® FEATURES

E. coli **Fast-Media®** offer researchers a quick and convenient way to prepare 200 ml of liquid culture medium, or 8-10 agar plates in about five minutes USING A MICROWAVE INSTEAD OF AN AUTOCLAVE.

E. coli **Fast-Media®** are available with a large variety of prokaryotic selective agents including Ampicillin, Blasticidin S, Hygromycin B, Kanamycin, Puromycin and Zeocin™ (see table below). **Fast-Media®** is also available with no selective agent (Base) that can be prepared with or without antibiotics.

	Agar	X-Gal	TB
Base	√		√
Ampicillin	√	√	√
Blasticidin	√	√	√
Hygromycin	√	√	√
Kanamycin	√	√	√
Puromycin	√		√
Zeocin™	√	√	√

SPECIAL HANDLING

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breath the dust**.**

METHOD

For customer convenience, procedure is directly printed on each pouch.

1- Pour the pouch contents into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled or deionized water.
3- Mix thoroughly by swirling the glass bottle or flask.
4- Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).
Do not heat in a closed container.
5- Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6- Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7- Allow the medium to cool to 50-55 °C, use directly for liquid medium, or pour plates for solid medium.
Caution: *Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.*
Note*: Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.*

For preparation of supplemented Fast-Media® Base.

- Follow the instructions above and when media has cooled to 50-55 ˚C add the antibiotic at the appropriate concentration for selection of *E. coli*.

TECHNICAL SUPPORT
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