pDRIVE-hGPIIb

A plasmid with the tissue specific human GPIIb promoter

Catalog # pdrive-hgp2b

For research use only

Version # 12H16-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by pDRIVE-hGPIIb
GT116 genotype is: *F- mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZM15 ΔlacX74 recA1 rpsL (StrA) endA1 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® is 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 *Bsp*H I and *Pag* 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

Human integrin alpha IIb promoter

Complete Promoter size: 932 bp

Specificity: Megakaryocytes

Integrin αIIb is the alpha-subunit of the alpha IIb/beta 3 complex found on the surface of platelets. The GPIIb gene encoding integrin alpha IIb is only expressed on maturing megakaryocyes and the platelets derived from them. An 800 bp fragment located upstream of the initiation start site of the αIIb gene contains cis-acting elements necessary for lineage specific expression[1]. This promoter fragment, which lacks a TATA box, binds GATA and Ets factors to induce high level, megakaryocyte-targeted gene transcription in human cells, rat cells and transgenic mice[2].

1. Uzan G. *et al.*, 1991. Tissue-specific expression of the platelet GPIIb gene. J Biol Chem. 266(14):8932-9.
2. Wilcox DA. *et al.*, 1999. Integrin alphaIIb promoter-targeted expression of gene products in megakaryocytes derived from retrovirus-transduced human hematopoietic cells. Proc Natl Acad Sci U S A. 96(17):9654-9.

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.
Note: *For long-term storage of the pDRIVE-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.*

Selection of bacteria with *E. coli* Fast-Media Zeo:

E. coli Fast-Media® Zeo is a **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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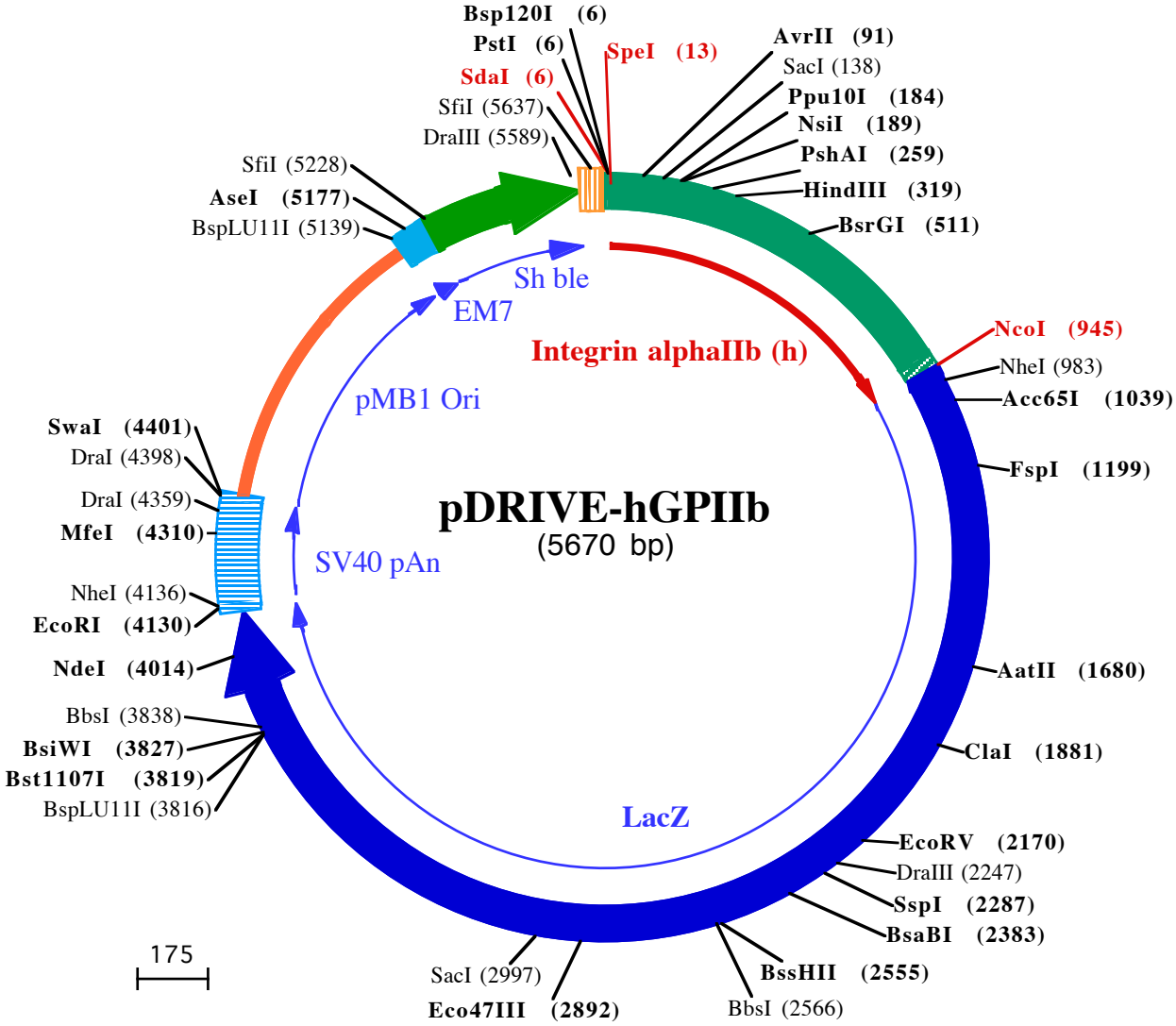
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Integrin alphaIIb (h)
Sh ble
EM7
pMB1 Ori
SV40 pAn
LacZ
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PstI (6)
SdaI (6)
SfiI (5637)
DraIII (5589)
SpeI (13)
AvrII (91)
SacI (138)
Ppu10I (184)
NsiI (189)
PshAI (259)
HindIII (319)
BsrGI (511)
SfiI (5228)
AseI (5177)
BspLU11I (5139)
NcoI (945)
NheI (983)
Acc65I (1039)
FspI (1199)
AatII (1680)
ClaI (1881)
EcoRV (2170)
DraIII (2247)
SspI (2287)
BsaBI (2383)
BssHII (2555)
BbsI (2566)
SacI (2997)
Eco47III (2892)
BbsI (3838)
BsiWI (3827)
Bst1107I (3819)
BspLU11I (3816)
NdeI (4014)
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DraI (4359)
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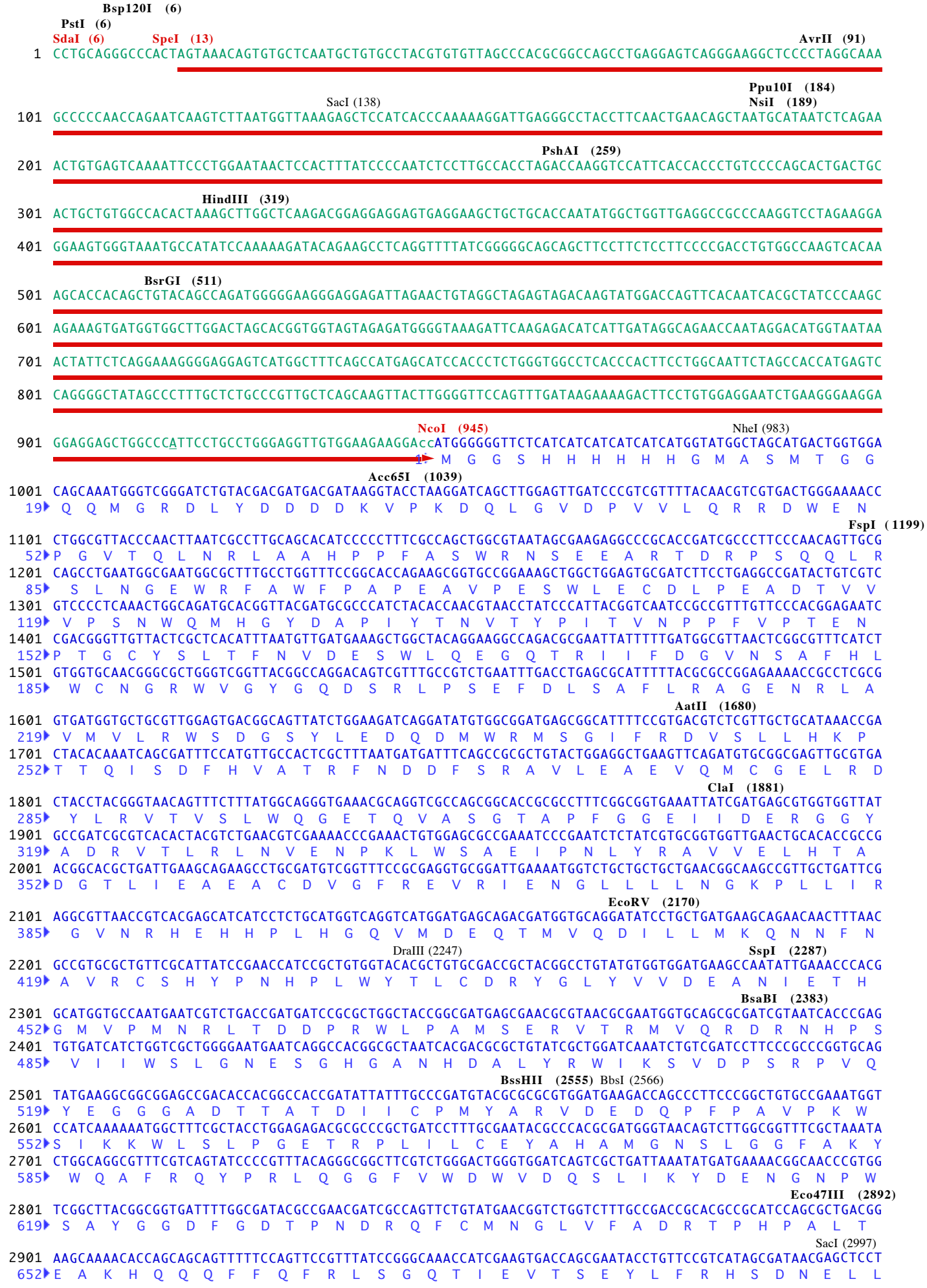
175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FspI (1199)
AatII (1680)
ClaI (1881)
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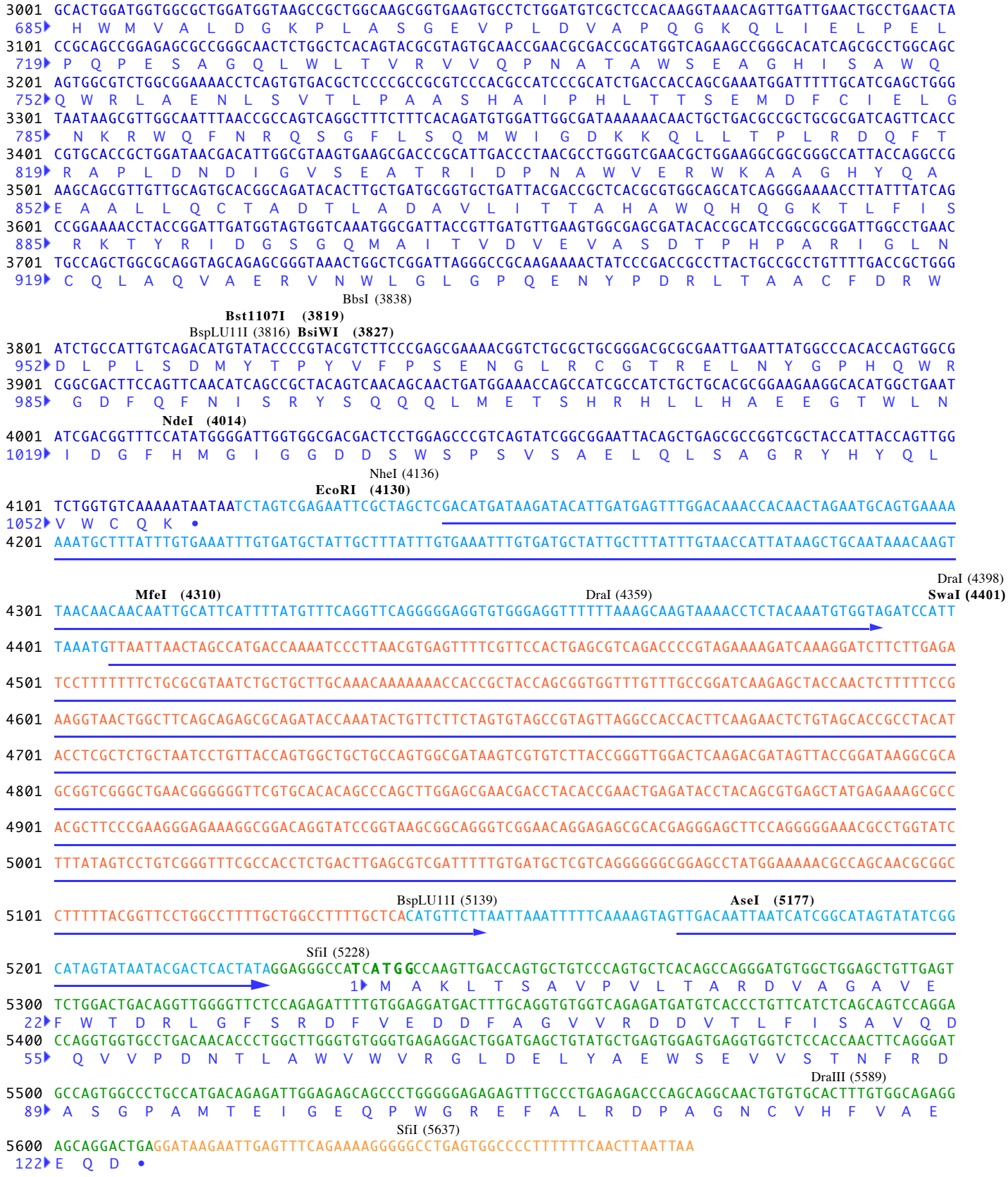
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685 H W M V A L D G K P L A S G E V P L D V A P Q G K Q L I E L P E L
3101 CCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGC
719 P Q P E S A G Q L W L T V R V V Q P N A T A W S E A G H I S A W Q
3201 AGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGG
752 Q W R L A E N L S V T L P A A S H A I P H L T T S E M D F C I E L G
3301 TAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACC
785 N K R W Q F N R Q S G F L S Q M W I G D K K Q L L T P L R D Q F T
3401 CGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCG
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3501 AAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAG
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3601 CCGGAAAACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAAC
885 R K T Y R I D G S G Q M A I T V D V E V A S D T P H P A R I G L N
3701 TGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGG
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BbsI (3838)
Bst1107I (3819)
BspLU11I (3816) BsiWI (3827)
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985 G D F Q F N I S R Y S Q Q Q L M E T S H R H L L H A E E G T W L N
NdeI (4014)
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NheI (4136)
EcoRI (4130)
4101 TCTGGTGTCAAAAATAATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAA
1052 V W C Q K •
4201 AAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGT
DraI (4398)
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SwaI (4401)
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BspLU11I (5139)
AseI (5177)
5101 CTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTAATTAAATTTTTCAAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGG
SfiI (5228)
5201 CATAGTATAATACGACTCACTATAGGAGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGT
1 M A K L T S A V P V L T A R D V A G A V E
5300 TCTGGACTGACAGGTTGGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCCTGTTCATCTCAGCAGTCCAGGA
22 F W T D R L G F S R D F V E D D F A G V V R D D V T L F I S A V Q D
5400 CCAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGAT
55 Q V V P D N T L A W V W V R G L D E L Y A E W S E V V S T N F R D
DraIII (5589)
5500 GCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGG
89 A S G P A M T E I G E Q P W G R E F A L R D P A G N C V H F V A E
SfiI (5637)
5600 AGCAGGACTGAGGATAAGAATTGAGTTTCAGAAAAGGGGGCCTGAGTGGCCCCTTTTTTCAACTTAATTAA
122 E Q D •

Fast-Media® Zeo Agar

Microwaveable media for selection and propagation of Zeocin™ resistant *E. coli*

Catalog # fas-zn-s

For research use only

Version # 12B10-MM

PRODUCT INFORMATION

Contents:

- 20 individual sealed pouches of Fast-Media® Zeo Agar. Each pouch contains the necessary amount of powder for the preparation of **200 ml** of **Lysogency Broth (LB) based solid medium** supplemented with **Zeocin™**. Lysogeny Broth is also known as Luria Broth.

Effective concentration: Zeocin™ 25 µg/ml

Storage and stability:

- Fast-Media® Zeo Agar 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® Zeo Agar broths are stable for 4 weeks at 4°C, retaining sterility and selective properties.

Quality control:

The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the Zeocin™ resistance gene are used as positive controls for Fast-Media® Zeo Agar.

METHOD

For customer convenience, the following 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 before use.

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.*

SPECIAL HANDLING

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

FAST-MEDIA® FEATURES

Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of sterile *E.coli* growth medium in about five minutes using a **microwave** instead of an autoclave.

Fast-Media® is supplied with a choice of antibiotics for selection (see table below), and chromogenic substrates, for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies. Fast-Media® Base is supplied without selective antibiotics.

Several Fast-Media® are available:

- **Fast-Media® TB,** Terrific Broth based liquid medium
- **Fast-Media® LB,** Lysogency Broth (LB) based liquid medium
- **Fast-Media® Agar,** LB based solid medium
- **Fast-Media® Agar X-Gal,** LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc** LB based solid medium containing X-Gluc.

Fast-Media®	Agar	Agar X-Gal	Agar X-Gluc	LB	TB
Base	X				X
Ampicillin	X	X		X	X
Blasticidin	X	X			X
Hygromycin	X	X			X
Kanamycin	X	X		X	X
Puromycin	X				X
Zeocin™	X	X	X		X

RELATED PRODUCTS

Product	Catalog Code
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar X-Gal	fas-zn-x
Fast-Media® Zeo Agar X-Gluc	fas-zn-g

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InvivoGen
3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA

Fast-Media® Zeo TB

Microwaveable media for selection and propagation of Zeocin™ resistant *E. coli*

Catalog # fas-zn-l

For research use only

Version # 12B09-MM

PRODUCT INFORMATION

Contents:

- 20 individual sealed pouches of Fast-Media® Zeo TB. Each pouch contains the necessary amount of powder for the preparation of **200 ml** of **Terrific Broth (TB) based liquid medium** supplemented with **Zeocin™**.

Effective concentration: Zeocin™ 25 µg/ml

Storage and stability:

- Fast-Media® Zeo TB 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® Zeo TB broths are stable for 4 weeks at 4°C, retaining sterility and selective properties.

Quality control:

The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the Zeocin™ resistance gene are used as positive controls for Fast-Media® Zeo TB.

METHOD

For customer convenience, the following 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 before use.

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.*

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- **Fast-Media® Agar,** LB based solid medium
- **Fast-Media® Agar X-Gal,** LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc** LB based solid medium containing X-Gluc.

Fast-Media®	Agar	Agar X-Gal	Agar X-Gluc	LB	TB
Base	X				X
Ampicillin	X	X		X	X
Blasticidin	X	X			X
Hygromycin	X	X			X
Kanamycin	X	X		X	X
Puromycin	X				X
Zeocin™	X	X	X		X

RELATED PRODUCTS

Product	Catalog Code
Fast-Media® Zeo Agar	fas-zn-s
Fast-Media® Zeo Agar X-Gal	fas-zn-x
Fast-Media® Zeo Agar X-Gluc	fas-zn-g

TECHNICAL SUPPORT
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