

# pDUO-mcs

A plasmid containing two multiple cloning sites and the blasticidin resistance gene

Catalog # pduo-mcs

For research use only

Version # 05K15-SV

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT110 *E. coli* bacteria transformed with the pDUO-mcs plasmid.
- GT110 genotype is: *F*-, *mcrA*,  $\Delta(mrr-hsdRMS-mcrBC)$ ,  $\Delta\text{Ø80lacZ}\Delta M15$ ,  $\Delta lacX74$ , *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® Blas

### Storage and Stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Blas 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

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionary conserved receptors, homologues of the *Drosophila* Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharides (LPS), peptidoglycans and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade that involves a number of proteins, such as MyD88 and IRAK. This signaling cascade leads to the activation of the transcription factor NF- $\kappa$ B which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

To date, ten toll-like receptors have been reported in humans (TLR1 to TLR10) and only nine in mice (TLR1 to TLR9). In many instances, TLRs require the presence of a co-receptor to initiate the signaling cascade. One example is TLR4 which interacts with MD2 and CD14 to induce NF- $\kappa$ B in response to LPS stimulation.

pDUO is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other.

The genes cloned into pDUO comprise the coding sequence (without introns) from the ATG to the Stop codon.

## PLASMID FEATURES

• **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein<sup>4</sup>. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

**MCS1** includes the following restriction sites:

*Age* I, *Eco* RV, *Bam* HI, *Sal* I and *Avr* II

- *Age* I is compatible with *Bsp* EI and *Sgr* AI.
- *Eco* RV is compatible with any blunt-end restriction enzymes.
- *Bam* HI is compatible with *Bgl* II, *Bst* YI and *Bcl* I.
- *Sal* I is compatible with *Ava* I and *Xho* I.
- *Avr* II is compatible with *Xba* I, *Spe* I and *Nhe* I.

**MCS2** includes the following restriction sites:

*Sgr* AI, *Bgl* II, *Xho* I and *Nhe* I

- *Sgr* AI is compatible with *Bsp* EI and *Age* I.
- *Bgl* II is compatible with *Bam* HI, *Bst* YI and *Bcl* I.
- *Xho* I is compatible with *Ava* I and *Sal* I.
- *Nhe* I is compatible with *Xba* I, *Spe* I and *Avr* II.

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. 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 plasmids<sup>5</sup>.

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is several-fold more active than the SV40 enhancer<sup>6</sup>.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*<sup>7</sup>

• **pMB1 Ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression<sup>8</sup>.

## TECHNICAL SUPPORT

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- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.
- **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

## METHODS

### Growth of pDUO-transformed bacteria:

**Use sterile conditions** to do the following:

- 1- Resuspend the lyophilized *E. coli* strain 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 an blasticidin LB agar plate prepared with the *E. coli* Fast-Media® Blas agar provided.
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with blasticidin using the Fast-Media® Blas liquid provided.
- 5- Extract the pDUO plasmid DNA using the method of your choice.

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

### References:

1. Miyake K. *et al.*, 2000. Innate recognition of lipopolysaccharide by Toll-like receptor 4/MD-2 and RP105/MD-1. *J Endotoxin Res*, 6(5):389-91.
2. Miyake K. *et al.*, 1998. Mouse MD-1, a molecule that is physically associated with RP105 and positively regulates its expression. *J Immunol*, 161(3):1348-53.
3. Nagai Y. *et al.*, 2002. Requirement for MD-1 in cell surface expression of RP105/CD180 and B-cell responsiveness to lipopolysaccharide. *Blood*, 99(5):1699-705.
4. Eisenstein RS. and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. *Enzyme* 44(1-4):42-58
5. Dean DA. *et al.* 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22
6. Boshart M. *et al.* 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 141(2):521-30
7. Carswell S., and Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258
8. Ramesh N *et al.* 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. *Nucleic Acids Res.* 24(14):2697-700

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

*E. coli* Fast-Media® Blas is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave.

- 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.

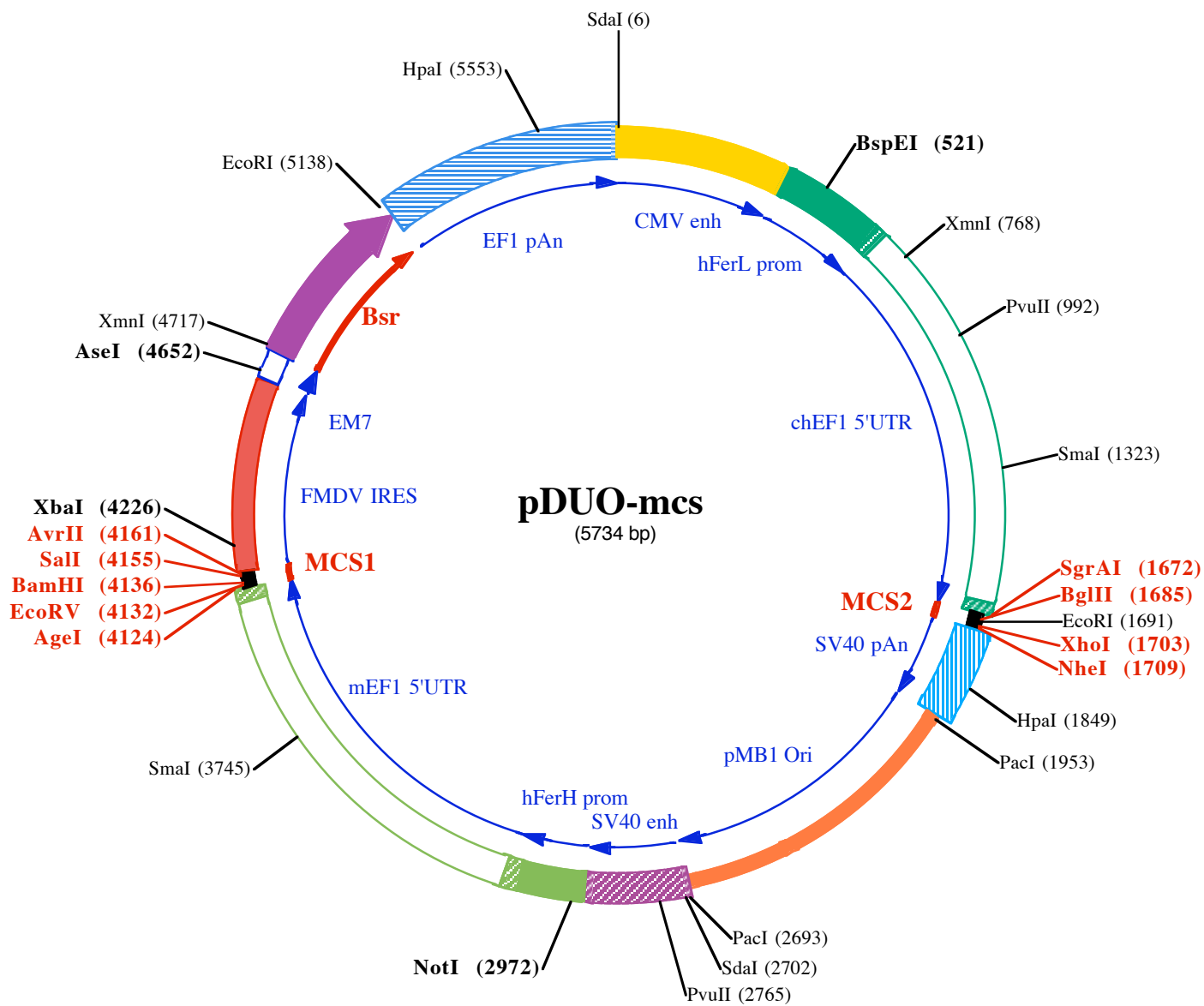
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### TECHNICAL SUPPORT

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3601 caggctggggtagcgtgccgagccatgtggccccagcaccggcagcatctggcctggcggcggcggttgcctgcctccctaactagggtgagggc

SmaI (3745)

3701 atcccgctccggcaccagttgctgctggaagatggccgctcccgggcccctgttgcaaggagctcaaaatggaggacgcggcagcccgggtggagcgggc

3801 gggtgagtcacccacacaaaggaagagggcctggctccctcaccggctgctgcttctgtgaccccgtggctctatcggcgcgaatagtcacctcgggctt

3901 ttgagcacggctagtcgcgccggggggaggggatgtaatggcctggagttgttccacatttgggtgggtggagactagtcaggccagcctggcgtggaa

4001 gtcatttttggaaatttgtccccttgagttttgagcggagctaattctcgggcttcttagcggttcaaaggtatcttttaaaccttttttagGTGTTGTG

EcoRV (4132)                      AvrII (4161)

AgeI (4124)   BamHI (4136)                      SalI (4155)

4101 AAAACCACCGCTAATTCAAAGCAACCGGTGATATCGGATCCACGCGTATCGATTGTGACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT

XbaI (4226)

4201 TGAAACTCCGCTGGTCTTCCAGGCTAGAGGGTAACACTTTGACTGCGTTTGGCTCCACGCTCGATCCACTGGCAGTGTTAGTAACAGCACTGTT

4301 GCTTCGTAGCGGAGCATGACGGCCGTGGAACTCCTCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACACGGGCCGTCATGTGTGCAACC

4401 CCAGCACGGCGACTTTACTGCGAAACCCACTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAG

4501 GTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTACCGGAGGTCGGC

AseI (4652)

4601 ACCTTTCCTTTGCAATTAAGTACCTATGAATACAAGTACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAG

XmnI (4717)

4701 GAGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCAC

4801 CATGTCGGGGCGGCATCAGGACCAAGACTGGGAGATCATCTCTGCTGCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCATTG

4901 CCATTGGGTCTGCTGTGAGCAACGGGAGGACTTTGACACCATTGGCTGTGAGGACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGT

5001 CAGCCCCGTGGCATGTGCAGAGAGCTCATCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAACCCACCTTGAG

5101 GAACTCATCCCCCTCAAGTACACCAGGAACCTAAACCTGAATTCGCTAGGATATTAGCTAGATTATCCCTAATACCTGCCACCCACTCTTAATCAGTGGT

EcoRI (5138)

5201 GGAAGAACGGTCTCAGAAGCTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAACCTTCAGAAGGA

5301 AAGGAGAATGTTTTGTGGACCATTGGTTTTCTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTTAAAGAAACAATTG

5401 ACCAAAAATTTGTACAGAAATTTGAGACCATTAAAAAGTTAAATGAGAAACCTGTGTGTTCCCTTGGTCAACCCGAGACATTTAGTGAAAGACAT

HpaI (5553)

5501 CTAATTCGGTTTTACGAATCTGAAACTTCTTGAAAATGTAATTCCTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCACATAATTGG

5601 AAGGGGAAGGAATATCATTTAAAGCTATGGGAGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGCTACTAAAACAGGC

5701 CAAAAACCTGAGTCCTTGGTTGCATAGAAAGCTG