pDUO-mcs

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

Catalog # pduo-mcs

For research use only

Version # 14C19-MM

PRODUCT INFORMATION

Content:

- 20 µg of pDUO-mcs provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20˚C.
- Resuspended DNA should be stored at -20˚C and is stable up to 1 year.
- Store *E. coli* Fast-Media® at room temperature in a dry and cool place. Fast-Media® pouches are stable 2 years when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.

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-κB which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

Ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. 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-κB 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.

pDUO-mcs does not contain a TLR gene and can be used in conjunction with other vectors of the pDUO family to serve as experimental controls.

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[4]. 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[5].

• **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 severalfold more active than the SV40 enhancer[6].

• **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.*[7]

• **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[8].

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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

Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water. Store resuspended plasmid at -20°C.
Plasmid amplification and cloning:
Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.
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.*

References:

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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

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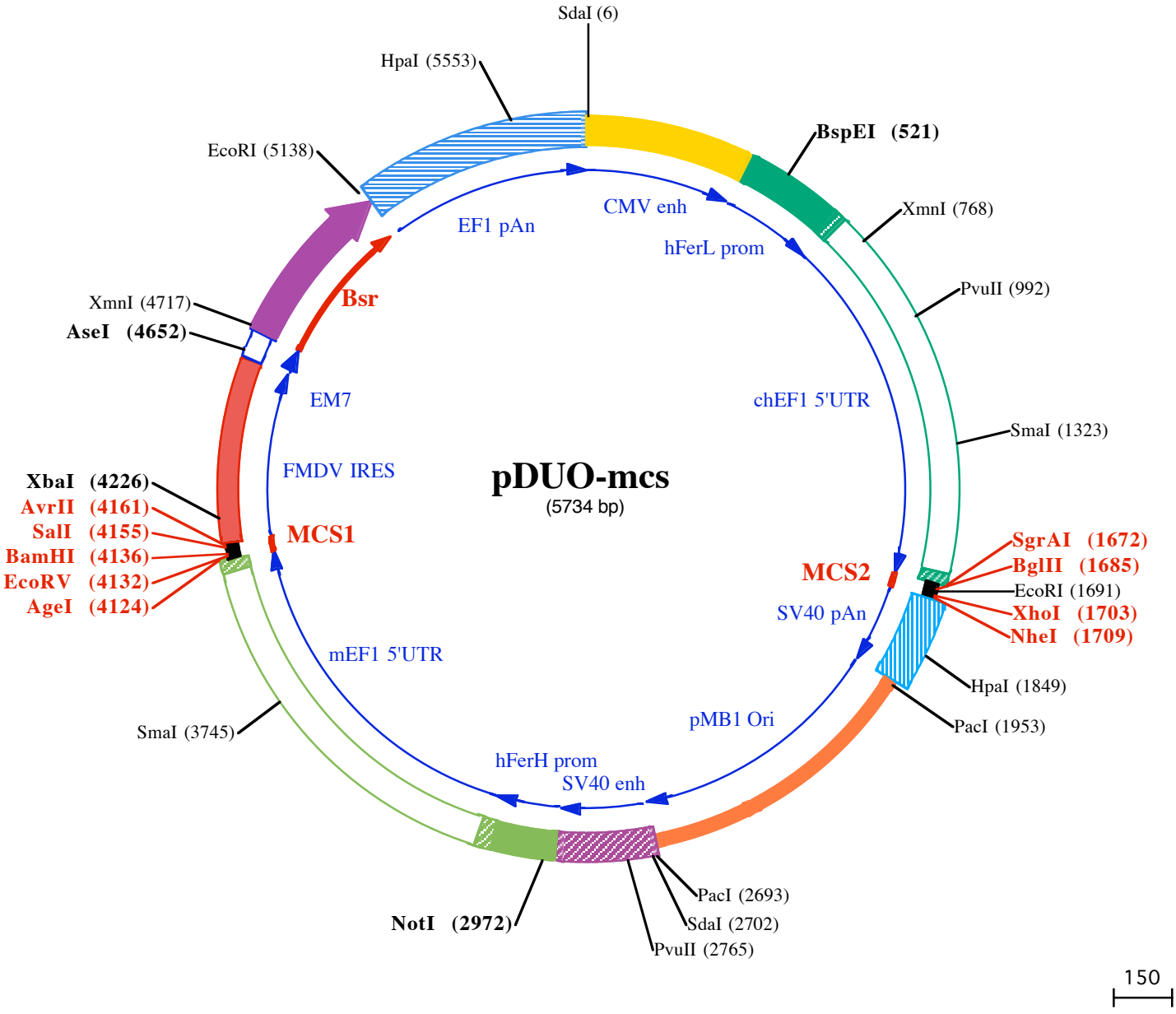
pDUO-mcs
(5734 bp)
SdaI (6)
HpaI (5553)
EcoRI (5138)
BspEI (521)
XmnI (768)
PvuII (992)
SmaI (1323)
SgrAI (1672)
BglII (1685)
EcoRI (1691)
XhoI (1703)
NheI (1709)
HpaI (1849)
PacI (1953)
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SdaI (2702)
PvuII (2765)
NotI (2972)
SmaI (3745)
XbaI (4226)
AvrII (4161)
SalI (4155)
BamHI (4136)
EcoRV (4132)
AgeI (4124)
XmnI (4717)
AseI (4652)
Bsr
MCS1
MCS2
EF1 pAn
CMV enh
hFerL prom
chEF1 5'UTR
SV40 pAn
pMB1 Ori
hFerH prom
SV40 enh
mEF1 5'UTR
FMDV IRES
EM7
150


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