pMOD2-Blast

A plasmid containing a synthetic blasticidin resistance gene

Catalog # pmod2-blast

For research use only

Version # 17C16-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pMOD2-Blast plasmid DNA.
- 4 pouches of *E. coli* Fast-Media® Amp (2 LB and 2 Agar)

Shipping and storage:

- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Amp at room temperature. Fast-Media® pouches are stable for 18 months when stored properly.

Quality control:

- Plasmid DNA was purified by ion exchange chromatography and lyophilized.
- Plasmid construct has been confirmed by restriction analysis sequencing.
- Sequence integrity of the gene has been verified by double-stranded sequencing.

GENERAL PRODUCT USE

pMOD2 plasmids contain genes that have been chemically synthesized. The DNA sequence of these genes were modified by optimizing the codon usage, reducing or eliminating the CpG motifs and avoiding secondary DNA structures without changing the amino acid sequence of the wild type proteins.

pMOD2 may be used to **subclone the synthetic gene** into another vector. Each synthetic gene is flanked by unique restriction sites allowing convenient excision. Furthermore, two multiple cloning sites (MCS) have been added on both ends of the synthetic gene. They contain several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

PLASMID FEATURES

• Multiple cloning sites

MCS1, located upstream of the synthetic gene, contains the following restriction sites:

Nde I, Bst EII, Avr II, Mfe I, Bgl II, Afl II, Hind III, Pme I

MCS2, located downstream of the synthetic gene, contains the following restriction sites:

Nhe I, Bam HI, Eco RV, Pac I

• Synthetic Bsr gene (bsr-lowCpG): The bsr gene from *Bacillus Cereus* is a small gene (420 bp) that encodes a deaminase and confers resistance to the antibiotic Blasticidin S. The bsr gene was modified by optimizing the codon usage and reducing the number of CpG motifs from 14 to 4.

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

• Amp: The ampicillin resistance gene allows the selection of transformed *E. coli* carrying a pMOD2 plasmid.

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H_2O. 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 Amp:

E. coli Fast-Media® Amp 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® Amp is a liquid or solid based medium with ampicillin, and contains stabilizers.

E. coli Fast-Media® Amp can be ordered separately (#fas-am-b (liquid), #fas-am-s (solid)).

Method:

1. Pour the contents of a pouch into a 0.5 L or 1 L glass flask.
2. Add 200 ml of distilled water to the flask.
3. Heat in a microwave on MEDIUM power setting (about 400 Watts), 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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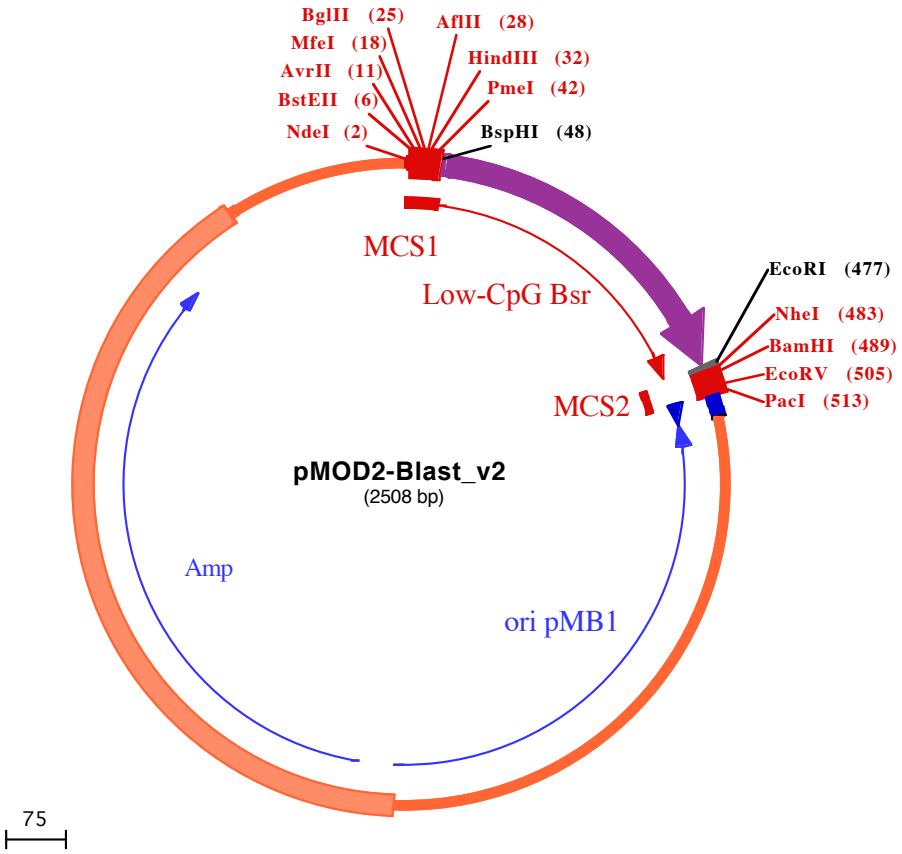
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AflII (28)
MfeI (18)
HindIII (32)
AvrII (11)
PmeI (42)
BstEII (6)
NdeI (2)
BspHI (48)
MCS1
Low-CpG Bsr
EcoRI (477)
NheI (483)
BamHI (489)
EcoRV (505)
PacI (513)
MCS2
pMOD2-Blast_v2
(2508 bp)
Amp
ori pMB1
75

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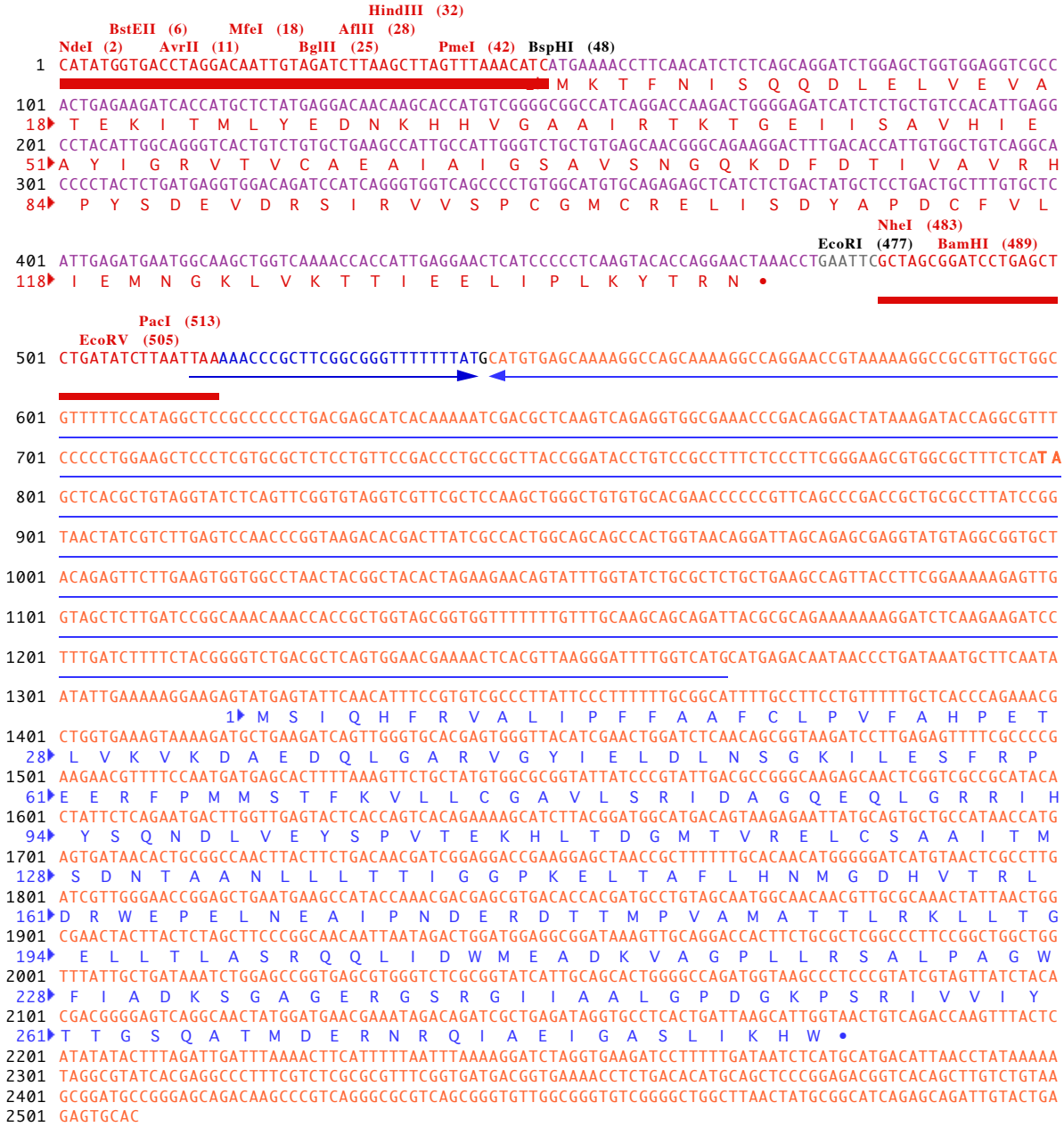
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