pZERO-mcs

A plasmid containing a multiple cloning site and the puromycin resistance gene

Catalog # pzero-mcs

For research use only

Version # 10C15-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT110 bacteria transformed with pZERO-mcs.
- GT110 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1.*
- 4 pouches of *E. coli* Fast-Media® Puro (2 TB and 2 agar).

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® Puro 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

pZERO-mcs is a ready-made expression vector containing the Puromycin resistance gene, the hybrid EF1α/HTLV promoter and a multiple cloning site.

pZERO-mcs may be used for:

Cloning in a gene of interest. Six unique restriction sites comprise the MCS facilitating cloning of genes. Cloned genes will be under the control of the EF1α/HTLV promoter.

As an "empty" control vector. Since pZERO-mcs does not contain a gene of interest, it can be used in conjunction with other vectors of the pZERO family to serve as an experimental control.

pZERO-mcs is selectable with the potent antibiotic puromycin in both *E. coli* and mammalian cells.

PLASMID FEATURES

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

• **MCS:** The multiple cloning site contains the following restriction sites:
5' - Sal I, BamH I, Eco47 III, Nco I, Nhe I, Msc I - 3'
Each restriction site is compatible with many other enzymes, increasing the cloning options.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

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

• **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

• **Puro:** Resistance to Puromycin is conferred by the *Pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *Pac* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

METHODS

Growth of pZERO-mcs-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 puromycin LB agar plate prepared with the *E. coli* Fast-Media® Puro 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 puromycin using the Fast-Media® Puro TB provided (see below).

5- Extract the pZERO-mcs plasmid DNA using the method of your choice.

Note: *For long-term storage of the pZERO-mcs-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

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic.

Fast-Media® Puro can be ordered separately (#fas-pr-l (liquid), #fas-pr-s (solid)).

Method:

1- Pour the contents of a Fast-Media® 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:

1. Kim, D.W. *et al.* (1990). Gene 2: 217-223.
2. Takebe, Y. *et al.* (1988). Mol. Cell Biol. 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). Mol. Cell Biol. 10: 4248-4258.
4. Yu J & Russell JE. (2001). Mol Cell Biol, 21(17):5879-88.

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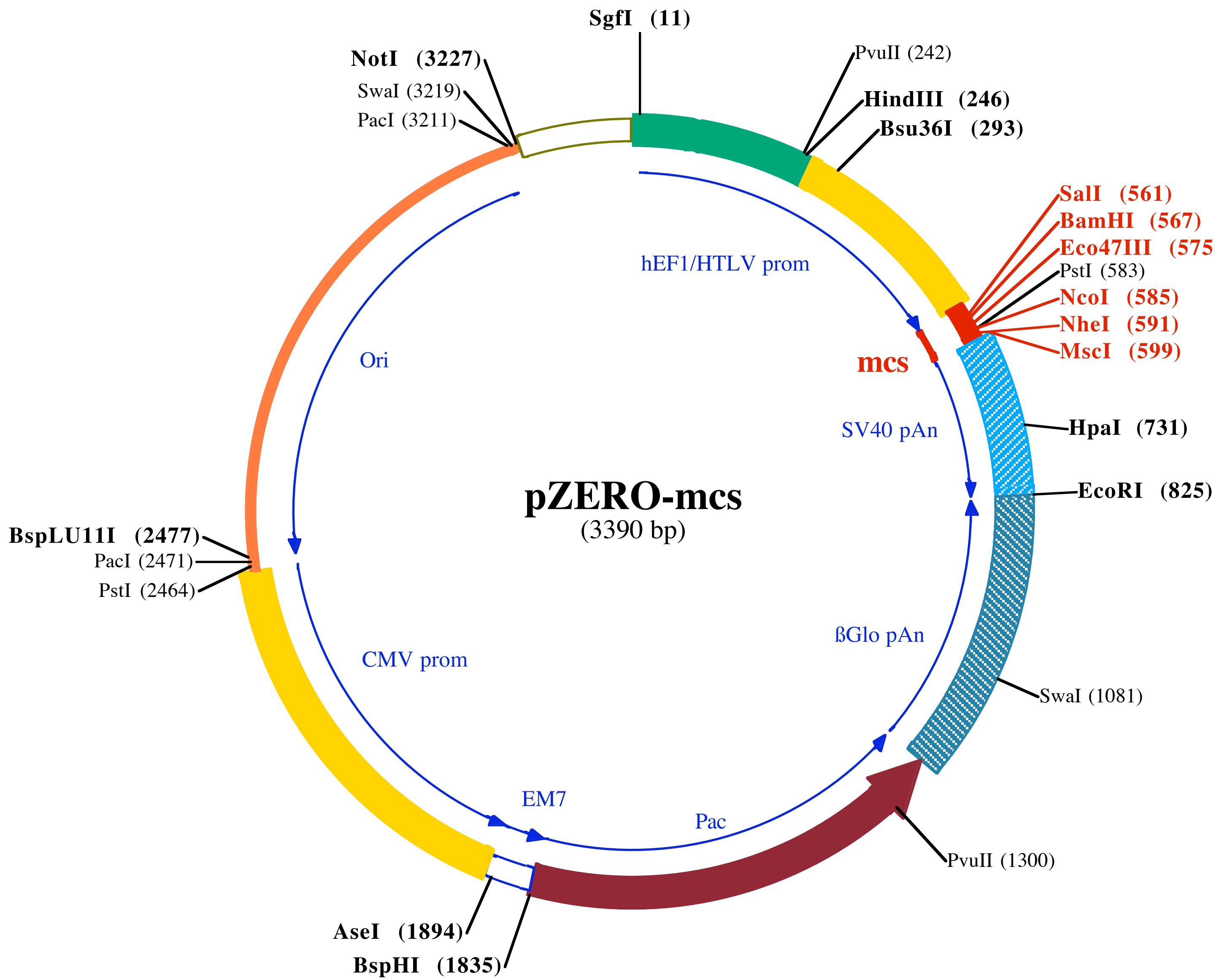

InvivoGen

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            SgfI (11)
   1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

 101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

                                                HindIII (246)
                                         PvuII (242)                                        Bsu36I (293)
 201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

 301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

 401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

                                                            BamHI (567)  PstI (583)      NheI (591)
                                                       SalI (561)   Eco47III (575)NcoI (585)   MscI (599)
 501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCGTGTCGACGGATCCAGCGCTCTGCAGCCATGGGCTAGCTGGC

 601 CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT

                                  HpaI (731)
 701 TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAG

                              EcoRI (825)
 801 TAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAA

 901 TAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTT

                                                                                  SwaI (1081)
1001 GAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGA

1101 AAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATA

                                                                                              PvuII (1300)
1201 GAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTTAAGCTCCAGGCTTCCTTGTCATGCACCAAGTTCTTGGGCCTTCTGGAACCTCAACATCAG
                                     200◄ •••AlaGlyProLysArgThrMetCysTrpThrArgProGlyGluProValGluValAspAl
1301 CTGTCACAGTGAATCCCAGTCTTTCATAAAAAGGCAGGTTTCTGGGAGCAGAAGTTTCCAGAAAGGCAGGAACTCCAGCCCTTTCAGCAGCTTCAACTCC
 179◄ aThrValThrPheGlyLeuArgGluTyrPheProLeuAsnArgProAlaSerThrGluLeuPheAlaProValGlyAlaArgGluAlaAlaGluValGly
1401 AGGCAGAACAACAGCAGATCCCAGACCCTTTCCCTGGTGGTCAGGGCTCACTCCAACAGTTGCCAGAAACCAAGCTGGCTCTTTTGGCCTGTGTGGTGCC
 146◄ ProLeuValValAlaSerGlyLeuGlyLysGlyGlnHisAspProSerValGlyValThrAlaLeuPheTrpAlaProGluLysProArgHisProAlaL
1501 AGCAGACCTTCCATTTGTTGTTGTGCTGCCAGCCTGCTTCCAGAGAGCTCAGCCATTCTTGGTCCAATTTCAGCAAAAACAGCACCAGCTTCAACAGACT
 112◄ euLeuGlyGluMetGlnGlnGlnAlaAlaLeuArgSerGlySerLeuGluAlaMetArgProGlyIleGluAlaPheValAlaGluAlaGluValSerGl
1601 CAGGTGTTGTCCAAACTGCAACAGCAGCTCCATCATCTGCAACCCAAACTTTTCCAATGTCCAGTCCCACTCTGGTGAGGAAGAGTTCTTGCAGTTCTGT
  79◄ uProThrThrTrpValAlaValAlaAlaGlyAspAspAlaValTrpValLysGlyIleAspLeuGlyValArgThrLeuPheLeuGluGlnLeuGluThr
1701 CACCCTCTCAATGTGCCTGTCAGGGTCAACTGTGTGCCTTGTTGCAGGGTAGTCTGCAAAAGCAGCAGCCAGTGTTCTCACAGCTCTTGGAACATCATCT
  46◄ ValArgGluIleHisArgAspProAspValThrHisArgThrAlaProTyrAspAlaPheAlaAlaAlaLeuThrArgValAlaArgProValAspAspA
                                      BspHI (1835)                                          AseI (1894)
1801 CTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTG
  12◄ rgThrAlaLeuArgValThrProLysTyrGluThrMet
1901 TCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCG

2001 TCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCC

2101 CCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGA

2201 AAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTA

2301 CTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAA

                                                                      PacI (2471)
                                                               PstI (2464)     BspLU11I (2477)
2401 TGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAA

2501 AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG

2601 AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC

2701 TTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAAC

2801 CCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA

2901 CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCT

3001 CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGA

3101 TTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCAT

            PacI (3211)  SwaI (3219)  NotI (3227)
3201 GGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACG

3301 CTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
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