

STOP

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pCpGfree-vitroHmcs

A CpG-free expression plasmid, containing a multiple cloning site, selectable with hygromycin

Catalog # pcpgvth-mcsg2

For research use only

Version # 09E11-MM

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-vitroHmcs plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 4 pouches of Fast-Media® Hygro (2 TB and 2 Agar)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA is stable for 12 months when stored at -20°C.
- Resuspended DNA is stable for 6 months when stored at -20°C.
- Bacteria should be stored at -20°C and are stable up to 1 year.
- Store Fast-Media® Hygro 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.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.
- Viability of the lyophilized bacteria upon resuspension has been verified.

GENERAL PRODUCT USE

pCpGfree-vitro plasmids represent innovative tools to study the effects of CpG dinucleotides in a number of applications. DNA vaccination exploits the immunostimulatory character of certain CpG motifs to prime and boost the immune response. However, these immunostimulatory CpG motifs are antagonized by CpG dinucleotides in certain distinct base contexts, termed neutralizing CpG motifs. Both types of CpG motifs are usually present in plasmidic DNAs, and therefore may lead to an unfavorable immune response. pCpGfree-vitro is the ideal tool to overcome this problem, and may be used to study the effects of these two types of CpG motifs by adding them in different configurations to the pCpGvitro backbone.

CpG dinucleotides are key elements in a number of cellular functions associated with chromatin. Many recent findings have altered our vision of chromatin and its role in the regulation of cellular processes such as transcription regulation, DNA replication and repair, cell cycle control, and cell aging. Several large multisubunit complexes, consisting of methyl-CpG binding (MBD) proteins and histone deacetylases, have been implicated in the regulation of chromatin dynamics. These complexes are recruited to methylated CpG dinucleotides by DNA methyl transferases (DNMTs) and induce chromatin remodelling. However the specific roles of these complexes are still to be explored. Due to the absence of CpG dinucleotides within its backbone, pCpGfree-vitro is not the target of DNMTs and thus MBD proteins. Therefore, it provides a useful model to study the other proteins involved in these complexes, in particular the histone deacetylases. It can also be used to analyze the effects of CpG methylation on the regulation and duration of gene expression.

PLASMID FEATURES

pCpGfree-vitro is a new family of expression vectors completely devoid of CpG dinucleotides that are selectable in mammalian cells. Similarly to the other pCpGfree plasmids (i.e. pCpGfree-lacZ, pCpGfree-mcs, and pCpGfree-siRNA), all the elements required for replication and selection of the plasmids in bacteria, and gene expression in mammalian cells have been modified to remove all CpG dinucleotides.

- **Composite CpG-free promoter** combining the mouse CMV enhancer, the human elongation factor 1 α core promoter and 5'UTR containing a synthetic intron (I 126). This composite promoter yields high and ubiquitous expression of the gene cloned into the mcs.
- **MCS:** The multiple cloning site contains the following restriction sites: 5' - *Bsr* I, *Sea* I, *Bgl* II, *Apa* I, *Bsp* I20 I, *Nco* I, *Nhe* I, *Msc* I - 3' Each restriction site is compatible with several other enzymes, increasing the cloning options.

- **CpG-free polyadenylation signals (pAn):** The polyadenylation signals utilized are CpG-free versions of the SV40 late and human β -globin polyadenylation signals. These polyA enable efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **CpG-free matrix attached regions (MARs)** are AT-rich sequences that are able to form barriers between independent expression cassettes.
- **CpG-free Hygromycin resistance gene (hph- Δ CpG):** The CpG-free Hygro[®] gene is active both in *E. coli* and mammalian cells.
- **CpG-free SV40 promoter** works in tandem with a bacterial promoter located within a synthetic intron (I-EC2K). This composite promoter drives the expression of the resistance gene in both mammalian cells and *E. coli*.
- **CpG-free *E. coli* R6K gamma origin of replication:** This origin is activated by the R6K specific initiator protein π , encoded by the *pir* gene. Expression of the *pir* gene is necessary for the replication and amplification of pCpGvitro plasmids. *E. coli* GT115 strain expresses a *pir* mutant gene that allows higher plasmid copy number.

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 H₂O. Store resuspended plasmid at -20°C.

Reconstitution of *E. coli* GT115 strain

Use sterile conditions to do the following:

- 1- Reconstitute *E. coli* GT115 by adding 1 ml of LB medium in the tube containing the paper disk. Let sit for 15 minutes. Mix gently by inverting the tube several times. Let sit 5 more minutes.
- 2- Streak bacteria taken from this suspension on a LB agar plate.
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in *E. coli* growth medium.
- 5- Prepare competent cells utilizing your preferred protocol.

Preparation of Fast-Media Hygro

Fast-Media[®] is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media[®] Hygro can be ordered separately [#fas-hg-1 (TB), #fas-hg-s (Agar)].

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. Wu F. *et al.* 1995., A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. *J Bacteriol.* 177(22):6338-45.
2. Bode J. *et al.*, 1996. Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. *Crit Rev Eukaryot Gene Expr.* 6(2-3):115-38.

TECHNICAL SUPPORT

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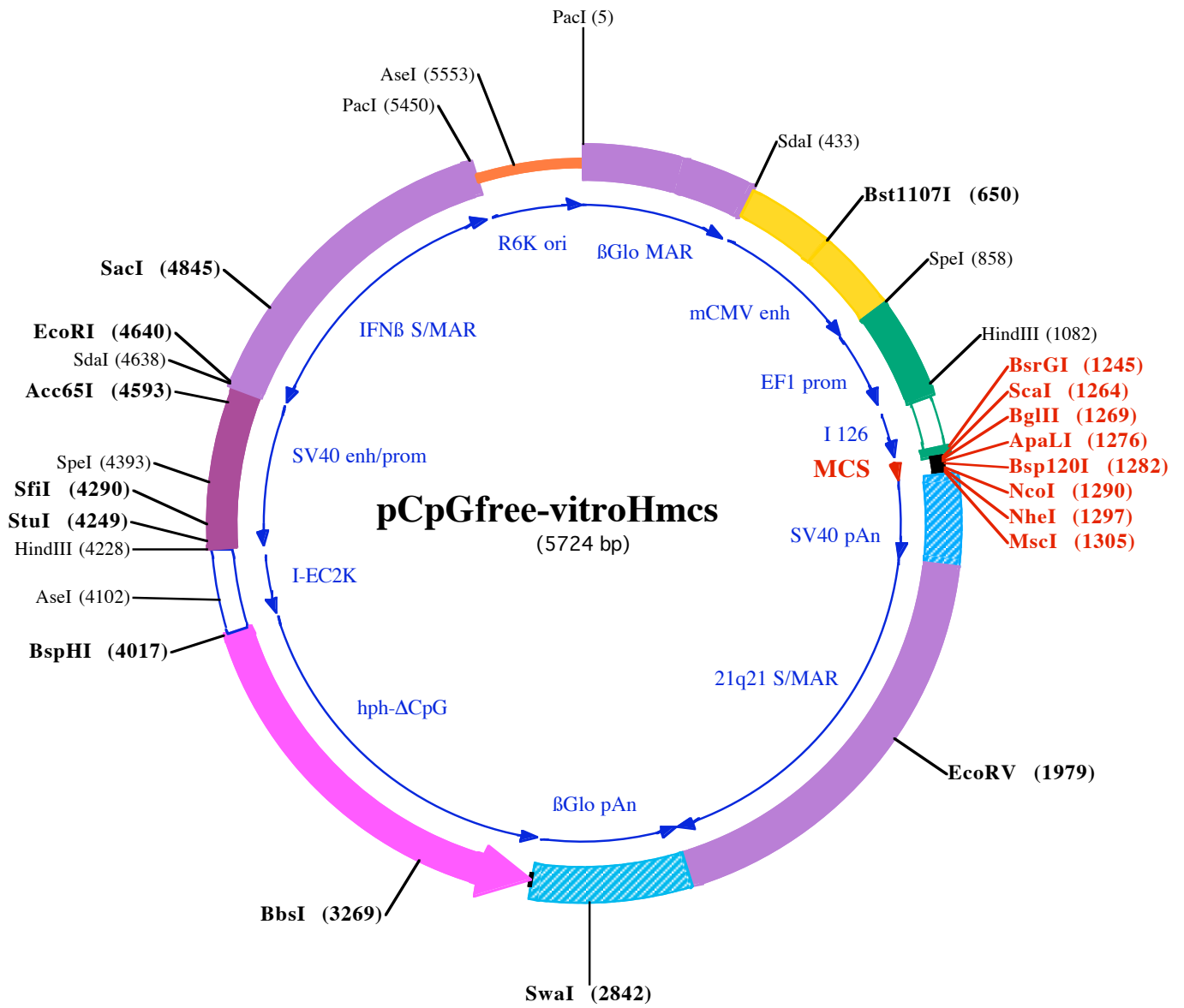
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150

PaeI (5)
1 TTAATTAAAATATCTCTAAGGCATGTGAAGCTGGCTGCTTGGTTTCATCTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCCTTAAAGGATTTTGTGAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC
201 AATAAATAATAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATAT
301 ACACACACATGTGTGCATTCAATAATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA
SdaI (433)
401 ACAGCAGATTAAGGAATTTCAATTGCCTGCAGGAGTCAATGGGAAAAACCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTT
501 GCCCAGTACATAAGGTTCAATAGGGGGTGAAGTCAACAGGAAAGTCCCATTTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAAATGGGTTTTGCCAGT
Bst1107I (650)
601 ACATAAGGTCATGGGAGGTAAGCCAATGGGTTTTCCCACTACTGACATGTATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG
701 GTCATAGGGGTGAATCAACAGGAAAGTCCCATTTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCATAG
SpeI (858)
801 GGGGTGAGTCAATGGGTTTTTCCATTATTGGCACATACATAAGGTCATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTGCCCTCAGT
901 GGGCAGAGACACATGGCCACAGTCCCTGAGAAGTTGGGGGGAGGGTGGGCAATTGAATGGTGCCTAGAGAAGTGGGGCTTGGGTAACTGGGAAA
HindIII (1082)
1001 GTGATGTGGTACTGGCTCCACTTTTTCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGCCTTCCCT
1101 CCTGTGAGTTTgtaagtcaactgactgtctatgctctggaaaggggtgggcaggagatggggcagtcaggaaaagtgccactatgaaccTGACGCCTA
BsrGI (1245) BglIII (1269) Bsp120I (1282) NheI (1297)
1201 GAcattgtactaaccttcttctcttctcctcctgacagGTTGGTGTACAGTAGCTTCCAGTACTAAGATCTAGTGCACAGGGCCACCATGGAGCTA
MseI (1305) ScaI (1264) ApaLI (1276) NcoI (1290)
1301 GCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAGTGAAGTGCAGTGAAGAAATGCTTTATTTGTGAAATTTGTGATGCTATTGC
1401 TTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTACAGGGGAGGTGTGGGAGGTTTTTAA
1501 AGCAAGTAAAACCTCTACAATGTGGTATGGAATTGGAGCCCACTGTGTTTCATCTTACAGATGGAATACTGACATTACAGAGGAGTTAGTTAACTTGCC
1601 TAGGTGATTACAGTAAAGTGAAGAAAGATTTCAATCCAAGGTGATTTGATTCTGAAGCCTGTGCTAATACATTACACCAAGCTACAACCTTCAATTA
1701 TAAATAAAGTCAAGTCTTCAAGGCCTTTCAAGTGTCTGCACCTTCTACAAGCTGTGCCATTTAGTGAACACAAAATGAGCCTTCTGATGAAGTAGTCT
1801 TTTCAATTTTTCAGATATTAGAACAATAAAATCTTAGCTGCAGCTGATTGAAGGCTGGGACAAAATCAAACATGCATCTACAACAATATATATCTCA
EcoRV (1979)
1901 ATGTTAGTCTCAAATCTATTGACTTCAACTCAAGAGAATATAAAGAGCTAGTCTTTATACACTCTTAAAGGTATGATATCATCTGGAAAGTAACAAA
2001 TTGATGCAAAATTTGAATGAACCTTATCATGGTGTATTTACACAATGTGTTTCTTCCCTGCAATGTATTTCTTCTAATTCCTTCCATTGATCTTT
2101 CATAACAATCTGGTTCTGATGATGTTTTTTGGATGCACCTTTCAACTCCAAAAGACAGAGCTAGTTACTTTCTTCTGCTCCAAGCACTGTATTT
2201 GTATCTGATTCAAGCCCTTGAATATTGACTGGATCATTATTTACCTCTAGGATGGCTTCCCAGGCAACTGTGTTCAACCAGAGACTACATTTT
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2601 GCATAGCAAACTTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTA
2701 GCTGTTGACGCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGGTTGAACTAGCTCTTCAATTTCTTATGTTTTAAATGCACTGA
SwaI (2842)
2801 CCTCCACATTCCTTTTTAGTAAAAATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAA
2901 GGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCTCTAGTaatatTCATTG
3001 CTTGGCTCTGGGCTCTGTGGAGGCTCTCTGTTTCCAGAGTCAGCCAGAATCTCAACACATCCATCAGTCCAACAGCAGCAGACTCTTGCATTTGGA
340 LysAl aArgP roArgThr Ser P roArgArgAsnGl ySer AspAl aLeuVal l Gl uVal l CysGl yAspThr TrpVal l Al aAl aSer ArgArgAl a l l eGl nT
3101 GTTCTTCCAACAGCTTCCAGCACCAGACCTCAATGGCATCACATCTCTTGTGCCCCAAGCAGCATCATCAAAGTTCCATCAACAGAGATGATACA
306 hrArgGl yVal l Thr Gl yAl aGl ySer ArgVal l l eAl aAspCysArgGl yGl nAl aT rpAl aAl aAspAspPheAsnGl yAspVal l LeuSer Gl nTyrLe
BbsI (3269)
3201 GTTGGTCCAGGCCAATCTGAGCATGTAGGCTCTCAGTCTGGGGAAACCAGCCAGTCTGGGTGCTTCTTTCAAATATCTTGTGTTGTTCCATGCA
273 uGl nAspLeuGl y l l eArgLeuMetTyrAl aArgLeuArgP roSer Gl yAl aLeuGl uP roHi sArgArgGl uPheTyrArgThr Gl nGl n l uMetCys
3301 AGCCAGCAAGGCTTCCAAAAAATGTTGGCAACCTCATATTGAGAATCTCCAAACATGGCTTCAGACAGTCAATGACTGCAGTGAATTCGCATTG
240 Al aLeuT rpP roArgT rpPhePhe l l eAsnAl aVal l Gl uTyrGl nSerAspGl yPheMetAl aGl uSer T rpAsp l l eVal l Al aThr l l eArgGl yAsnA
3401 TCTGTGAGAACATTGTTGCTTCCAAAATCAGCATGGACCAGGTGCTGACTTCAGGACAATCTTCTGCCACAGCATGAGTTCCAGTGCCTGAGCAA
206 spThr LeuVal l AsnAsnSer Gl yPheAspAl aHi sVal l LeuHi sArgVal l Gl uP roCysAspGl uAl aT rpLeuMetLeuGl uAspLeuAl aGl nAl aVa
3501 CAGAAGCAGAACTGTGTCATCCATCACAGTCTGCCAGTGTAGACATGAGGATCAGCAATGGCACAATGAAATCCCTTCCAAAGTGGTACTGACCAAT
173 l l SerAl aSer Val l ThrAspAspMetVal l Thr Gl nT rpHi sTyrVal l Hi sP roAspAl a l l eAl aCys l l ePheAspArgT rpThr Thr TyrGl nGl y l l e
3601 GCCTTGGGACCAAAAGGACCAATCCAGAGTTTGGCTCAGATCAGTCTGCTGCAATGGCATCCATTGCTTCAGCAACAGGTTGCAGAACAGCTGGCAGC
140 Gl yGl nP roGl yPheP roGl yPheGl ySer Thr Gl nSer LeuAspAl aAl aAl a l l eAl aAspMetAl aGl uAl aVal l P roGl nLeuVal l Al aP roLeuG

3701 TCAGTTTCAGGGAGATCCTGGAGAGTACTCCTTGTGCTCTTCTGCTGATGAGTGGAGAGATTGAGAAAATTCCTCAATGTCCAGAAGCTTCTGGAA
 106 I uThr Gl uP roLeuAspGl nLeuThr Val Gl yGl nAl aArgArgSer l l eCysTyrThr LeuSer Gl uSer PheGl uGl yI l eAspLeuVal Gl uProI l
 3801 TTGGCAGAGCAGCAGAGGCAAAGTGTCTGTAACATATCTGTCTTTGTA AAAACCATCAGCACAGAATTGACCCCTCAGAACATAACCTCTTCTCCAAAC
 73 eP roLeuAl aAl aSer Al aPheHi sArgTyrVal T yrArgAspLysTyrPheGl yAspAl aCysSerAsnVal l ArgLeuVal TyrGl yArgGl yGl yVal
 3901 ATCAAAAAGAAAAGGCTCTGCTTTCTCACCTTCAGACAGCTGCATGAGATCAGAAACAGAATCAAATTTTTCAATGAGAACTTCTCAACAGAAGTTGCT
 40 AspPheSer PheAl aArgSer Gl uGl uGl yGl uSer LeuGl nMe tLeuAspSer Val SerAspPheLysGl uI l eLeuPheLysGl uVal Ser Thr Al aT
BspHI (4017)
 4001 GTCAGTTCAGGTTTCTTCA^TGATGGCTCCTCctgtcaggagaggaagaagaaggtagtacaattgCTATAGT GAGTTGATTATACTATGCTTATG
 6 hr LeuGl uProLysLysMet
 AseI (4102)
 4101 ATTAATTGTCAAAC TAGGGCTGCAGgggttcatagtgccacttttctgcactgccccatctctgccccacctttccaggcatagacagtcagtgactt

 HindIII (4228) **StuI (4249)** **SfiI (4290)**
 4201 acCAAAC TACAGGAGGAGAAGGCAG^{AAGCTTTTTGCAAAA}GCCTAGGCC TCCAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCcAGGgG

 SpeI (4393)
 4301 GCCTgGGCCTCTGCATAAATAAAAAAATTAGTCAGCC TGGGcctgggggtgggggcagggtggggggc caactgggCAGGGGTGGGGGCCACTAGTGG
 4401 GACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGAGCCCTGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGC

Acc65I (4593)
 4501 ATGCTTTGCATACTTCTGCCTGCTGGGAGCCTGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGGTCTTTTCAGCCTCAGAAGGTACCTA

EcoRI (4640)
 SdaI (4638)
 4601 ACCAAGTTCCTCTTT CAGAGGTTATTT CAGGCCCTGCAGGAATTCAGTCAATATGTTACCCCAAAAAAGCTGTTTGTTAACTTGTCACCTCATTCTAA
 4701 AATGTATATAGAAGCCAAAAGACAATAACAAAAATATTCTTGTAGAACAAAATGGGAAAAGATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGA

SacI (4845)
 4801 GATGTGTGGGATAGACAGTGAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATT
 4901 ACAATGGGAAAATGATGGTCTTTTTCTTTTTAGAAAAACAGGAAATATATTTATATGTA AAAAATAAAAGGGAACCCATATGTCATACCATAACACACA
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 5201 AAAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAA
 5301 TAAAAC TAGACAAAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACTATCATTGCATAT

 PacI (5450)
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 AseI (5553)
 5501 TTTTTCTTATAAAACCTTAAACCTTAGAGGCTATTTAAGTTGCTGATTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACATGAAACATGAGAG
 5601 CTTAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAACATGAGAGCTTAGTACATTAACATGAGAGCTTAGTACATAC
 5701 TATCAACAGGTTGAACTGCTGATT