

# STOP

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### TECHNICAL SUPPORT

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# pCpGfree-vitroNmcs

A CpG-free expression plasmid, containing a multiple cloning site, selectable with G418/Kanamycin

Catalog code: pcpgvtn-mcsg2

<https://www.invivogen.com/pcpgfree-vitro-neomycin>

For research use only

Version 19L12-MM

## PRODUCT INFORMATION

### Contents:

- 20 µg of pCpGfree-vitroNmcs plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk

### Storage and stability:

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.

### Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

## GENERAL PRODUCT USE

pCpGfree-vitro plasmids represent innovative tools to study the effects of CpG dinucleotides in numerous 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. 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 family of expression vectors devoid of CpG dinucleotides that are selectable in mammalian cells. 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 $\alpha$  core promoter and 5'UTR containing a synthetic intron (I 126). This composite promoter yields high and ubiquitous expression of the LacZ gene.
- **MCS:** The multiple cloning site contains the following restriction sites: 5' - BsrG I, Sca I, Bgl II, ApaL I, Bsp120 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  $\beta$ -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 Neo resistance gene (Neo- $\Delta$ CpG):** The CpG-free Neo gene is active both in *E. coli* and mammalian cells and confers resistance to Kanamycin in *E. coli* and G418 in 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  $\pi$ , 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.

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.

## 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 H<sub>2</sub>O. Store resuspended plasmid at -20°C.

### Reconstitution of *E. coli* GT115 strain under sterile conditions

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.

### Plasmid amplification and cloning:

Plasmid amplification and cloning can be performed in competent *E. coli* GT115.

### Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

### Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

## RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT115 cells G418	Competent <i>E. coli</i> cells Selection antibiotic	gt115-11 ant-gn-1

### TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

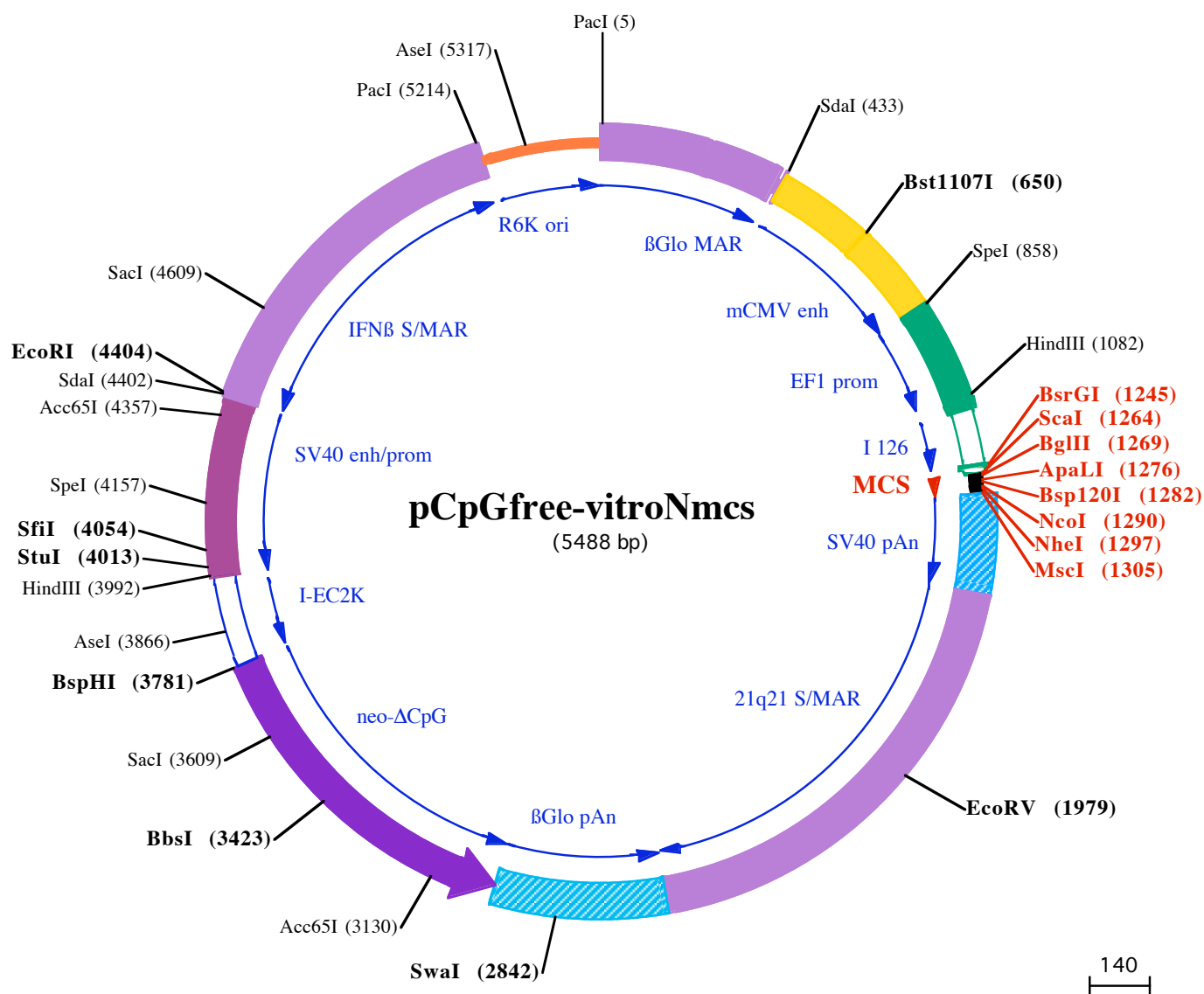
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PacI (5)

1 TTAATTAATAATCTCTAAGGCATGTGAAGTGGCTGCTTGGTTTTTCATCTGACTTCTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT

101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTCCCTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC

201 AATAAATAATAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACCAAGTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATAT

301 ACACACACATGTGTGCATTCAAAATATATAAATTTTTATGAATAAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

401 ACAGCAGATTAAGGAATTTCAATTGCCTGCAGGAGTCAATGGGAAAAACCATTGGAGCCAAGTACTGACTCAATAGGGACTTTCCATTGGGTTTT

SdaI (433)

501 GCCCAGTACATAAGGTCAATAGGGGGTGAAGTCAACAGGAAAGTCCATTGGAGCCAAGTACTGACTGAGTCAATAGGGACTTTCCAAATGGGTTTTGCCAGT

Bst1107I (650)

601 ACATAAGGTCAATGGGAGTAAGCCAATGGGTTTTCCATTACTGACATGTATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG

701 GTCAAATAGGGGTGAATCAACAGGAAAGTCCATTGGAGCCAAGTACTGACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAG

801 GGGGTGAGTCAATGGGTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGAGAAGAGCATGCTTGAGGGCTGAGTGCCTCCAGT

SpeI (858)

901 GGGCAGAGAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGGTGGCAATTGAAGTGGTGCCTAGAGAAGGTGGGCTTGGGTAAGTGGGAAA

HindIII (1082)

1001 GTGATGTGGTACTGGCTCCACCTTTTTCCACAGGTGGGGGAGAACCATATAAGTGCAGTAGTCTCTGTGAACATTCAGCTTCTGCCTTCCCTC

1101 CCTGTGAGTTGgtaagtcactgactgtctatgcttgggaaaggggtggcaggagatggggcagtgaggaaaagtgccactatgaaccTGCAGCCCTA

BsrGI (1245) ScaI (1264) BglIII (1269) Bsp120I (1282) NheI (1297)

1201 GAcAattgtactaaccttcttctcttctcctctcctgacagGTTGGTGTACAGTAGCTTCCAAGTACTAAGATCTAGTGCACAGGGCCACCATGGAGCTA

MscI (1305)

1301 GCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAACCACAAC TAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGC

1401 TTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAA

1501 AGCAAGTAAAACTCTACAATGTGGTATGGAATTGGAGCCCACTGTGTTTCATCTTACAGATGAAATACTGACATTGAGAGGAGTTAGTTAACTTGCC

1601 TAGGTGATTCAGCTAATAAGTGAAGAAAGATTTCAATCCAAGGTGATTGATTCTGAAGCCTGTGCTAATCACATTACCAAGCTACAACCTCATTTA

1701 TAAATAATAAGTCAAGTTTCAAGGGCTTTCAGGTGCTCTGCACCTTCTACAAGCTGTGCCATTTAGTGAACACAAAATGAGCCTTCTGATGAAGTAGTCT

1801 TTTCAATTTTTCAGATATTAGAACAATAAATTTCTTAGCTGCCAGCTGATTGAAGGCTGGGACAAAATTCAAACATGCATCTACAACAATATATATCTCA

EcoRV (1979)

1901 ATGTTAGTCTCAAATTTCTATTGACTTCAACTCAAGAGAATATAAAGAGCTAGTCTTTATACACTCTTTAAGGTATGATATCATCTGAAAAGTAACAAA

2001 TTGATGCAAAATTTGAATGAACCTTATCATGGTGTATTACACAATGTGTTTCTTCCCTGCAATGTATTTCTTCTCTAATTTCTCCATTTGATCTTT

2101 CATAACAATCTGGTTCTGATGTATGTTTTTGGATGCACCTTTCAACTCAAAAGACAGAGCTAGTTACTTTCTTCTGGTGTCCAAGCACTGTATTT

2201 GTATCTGTATTCAAGCCCTTGAATATTGACTGGATCATTATTTACCTCTAGGATGGCTTCCCAAGCAACTGTGTTACCCAGAGACTACATTTT

2301 GTATCTTGTGACCTTTGAACCTCCACCAGTGTCTAAAAATAATATGTATGCAAAATTAAGTGTATGAGAATGTATAATTAACAATATAAAAAGGAGA

2401 AGCAAGGAGAGAAACACAGGTGTGATTTGTGTTTGTGCTTAAAAGGCAGTGTGAAAAGGAAAGAAATGCCATTTATAGTGAAGGAGACAAAAGTTATAT

2501 TACCTCTTATCTGGCTTTAAGGAGATTTTGTGAGCTAAAATCTATATTCATAGAAAAGCCTTACCTGAGTTGCCAATCTCAATTTCAAAAATACA

2601 GCATAGCAAAAATTTAACTCCAAATCAAGCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATAGGGCTGTTGCCAATGTGCATTA

2701 GCTGTTTGACGCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGGTTGAACTAGCTCTTCAATTTCTTTATGTTTTAAATGCAGTGA

SwaI (2842)

2801 CCTCCACATTCCTTTTTAGTAAAAATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAA

2901 GGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGAACTTTAATAGAATTTGGACAGCAAGAAAGCTTAGCTTTAGAAGAACT

3001 CATCAAGAAGTCTGTAGAAGCAATCTCTGGGAGTCAGGGGCTGCAATGCCATAGAGCACTAGGAACCTGTCTGCCACTCTCCCCTAGCTCTCTTGC

265 PhePheGI

261 uAspLeuLeuArgTyrPheAl a l eArgGI nSer AspP roAl aAl a l eGI yTyrLeuVal l LeuPheArgAspAl aTrpGI uGI yGI yLeuGI uGI uAl a

Acc65I (3130)

3101 TATGTCCCTGGTTGCTAGGGCAATGTCTGGTACCTGTGACCCACTCCAGCCTGCCACAGTCTATGAAGCCAGAGAACCCTTCCATTTTCAACCATGATG

228 l l eAspArgThr Al aLeuAl a l eAspGI nTyrArgAspAl aVal GI yLeuArgGI yCysAsp l l ePheGI ySer PheArgGI yAsnGI uVal l Me l l eA

3201 TTGGGAAGGCAGGCATCCCCATGAGTCAACCTAGGTCCTCACCATCTGGCATGGATGCCTTGGACCTGGCAAAATAGTTGAGCAGGGGCCAGGCCCTGGT

194 s nP roLeuCysAl aAspGI yHi s Thr Val Val LeuAspGI uGI yAspP roMeT ser Al aLysLeuArgAl aPheLeuGI uAl aP roAl aLeuGI yGI nHi

3301 GTTCTTCACTCAAGTCTCTGGTCCACCAGGCCAGCTCCATCTGTTTCTGGCCCTCTCTATCTGTGCTTGGCCTGGTGGTCAAAGGGCAGGTGGC

161 sGI uGI uAspLeuAspAspGI nAspVal l LeuGI yAl aGI uMeTArgThr ArgAl aArgGI u l l eArgHi sLysAl aGI nHi sAspPheP roCysThr Al a

BbsI (3423)

3401 TGGGTCAAGGTGTGGAGTCTTCTCATGGCATCAGCCATGATTGACACTTTCTCAGCTGGAGCTAGGTGAGAGGAAAGGAGTCTGCCAGGCACCTCA

128 P roAspLeuThr Hi sLeuArgArgMe tAl aAspAl aMe t l l eSer Val l LysGI uAl aP roAl aLeuHi sSer Ser LeuLeuAspGI nGI yP roVal l GI uG

3501 CCTAGTAGGAGCCAGTCCCTCCAGCTTCTGTGACCACATCAAGGACAGCTGCACAGGGGACCCAGTTGTTGCCAACCGAGAGTCTGGCAGCCTCAT

94 l yLeuLeuLeuTrpAspArgGI yAl aGI uThr Val ValAspLeuValAl aAl aCysP roVal GI yThr ThrAl aLeuTrpSer LeuArgAl aAl aGI uAs

SacI (3609)

3601 CCTGGAGCTCATTGAGAGCCCACTGAGTGTCTGCTTTACAAAAGGACTGGCCTGCCTGGGCTGAAAGCTGAAAAGTCTGATCATCAGAGCAACCAAT

61 pGI nLeuGI uAsnLeuAl aGI ySer LeuAspThr LysVal l PheLeuVal l P roArgGI yGI nAl aSer LeuArgPheValAl aAl aAspSer CysGI y l l e

**BspHI (3781)**  
 3701 GGTCTGCTGTGCCAGTCATAGCCAACAGTCTCTCAACCCAGGCAGCTGGAGAACCTGCATGTAGGCCATCTTGTTCAATCATGATGGCTCCTCctgtc  
 28 Thr Gl nGl nAl aTrpAspTyrGl yPheLeuArgGl uVal TrpAl aAl aProSer Gl yAl aHi sLeuGl yAspGl nGl ul l eMe t ←

3801 aggagaggaaagagaagaagggttagtacaattgCTATAGTGAGTTGATTACTATGCTTATGATTAATTGTCAAAGCTAGGCTGCAgggttcatagtg  
 AseI (3866)

3901 ccacttttctgcactgcccatctcctgccacccttccaggcatagacagtcagtgacttacCAAACCTCACAGGAGGGAGAAGGCAGAGCTTTTT ←  
 HindIII (3992)

**StuI (4013)** **SfiI (4054)**  
 4001 GCAAAAGCCTAGGCCTCAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCcAGGgGGCCTgGGCCTCTGCATAATAAAAAAATTAGTCAG

4101 CCTGGGgctggggtgggggaggggtggggggccaactgggCAGGGGTGGGGGCCACTAGTGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTG  
 SpeI (4157)

4201 CATACTTCTGCCTGCTGGGAGCCTGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGAGCCTGGG

4301 GACTTTCACACCCTAACTGACACACATTCCACAGCTGGTCTTTTCAGCCTCAGAAGGTACCTAACCAAGTTCCTCTTTCAGAGTTATTTTCAGGCCCTG  
 Acc65I (4357) SdaI (4402)

**EcoRI (4404)**  
 4401 CAGGAATTCAGTCAATATGTTCAACCCAAAAAGCTGTTTGTAACTTGTCAACCTCATTCTAAAAATGTATATAGAAGCCAAAAAGACAATAACAAAAAT  
 ←

4501 ATTCTTGTAGAACAAAAATGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGATAGACAGTGAAGCTGATAAAATAG

4601 AGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAATGATGGTCTTTTTCTTTTTTAGAA  
 SacI (4609)

4701 AAACAGGGAAATATATTTATATGTAATAAATAAAAGGGAACCATATGTCATACCATACACAAAAAATTCAGTGAATTATAAGTCTAAATGGAGAA

4801 GGCAAACTTTAAATCTTTTAGAAAAATAATAGAAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCTAACCAACAAGA

4901 AAAGATTGTAATTAGATTGCATGAATATTAAGACTTATTTTTAAATTAATAAAACCATTAAAGAAAGTCAGGCCATAGAATGACAGAAAAATTTGCAA

5001 CACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAGTCTTACAATCAGTAAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAA

5101 ACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACATCATTGCATATACACTAAATTAGAGAAATATTAAGGCTAAGTAAC

5201 ATCTGTGGCTTAATTAAGTTATCCTAGGAAACCTTAAACCTTTAAAGCCTTATATATTCTTTTTTTCTTATAAAACTTAAACCTTAGAGGCTATTT  
 PacI (5214)

5301 AAGTTGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACATGAAACATGAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGC  
 AseI (5317)

5401 CATGAGGGTTTAGTTCATTAACATGAGAGCTTAGTACATTAACATGAGAGCTTAGTACATACTATCAACAGGTTGAAGTCTGATT →