

# STOP

Before opening this package, please read the Limited Use License statement below:

## Important Limited Use License information for pCpGfree-vitroBLacZ

The purchase of the pCpGfree-vitroBLacZ vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact InvivoGen, 10515 Vista Sorrento Parkway, San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

---

### TECHNICAL SUPPORT

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

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



# pCpGfree-vitroBLacZ

A LacZ expression plasmid completely devoid of CpG dinucleotides, selectable with Blasticidin

Catalog code: pcpgtb-lz

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

For research use only

Version 20F08-MM

## PRODUCT INFORMATION

### Contents:

- 20 µg of pCpGfree-vitroBLacZ plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 2 x 1 ml blasticidin at 10 mg/ml

### 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.
- Store blasticidin at 4°C or -20°C. \*

\*The expiry date is specified on the product label.

### 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.

- **LacZ** encodes  $\beta$ -galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope. This CpG-free allele of the *lacZ* reporter gene can be easily subcloned and replaced by a gene of interest.

- **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 Blasticidin resistance gene (*bsr- $\Delta$ CpG*):** The CpG-free blasticidin resistance 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  $\pi$ , 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.

### Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

## RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT115 cells	Competent <i>E. coli</i> cells	gt115-11
Blasticidin	Selection antibiotic	ant-bl-05

## TECHNICAL SUPPORT

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

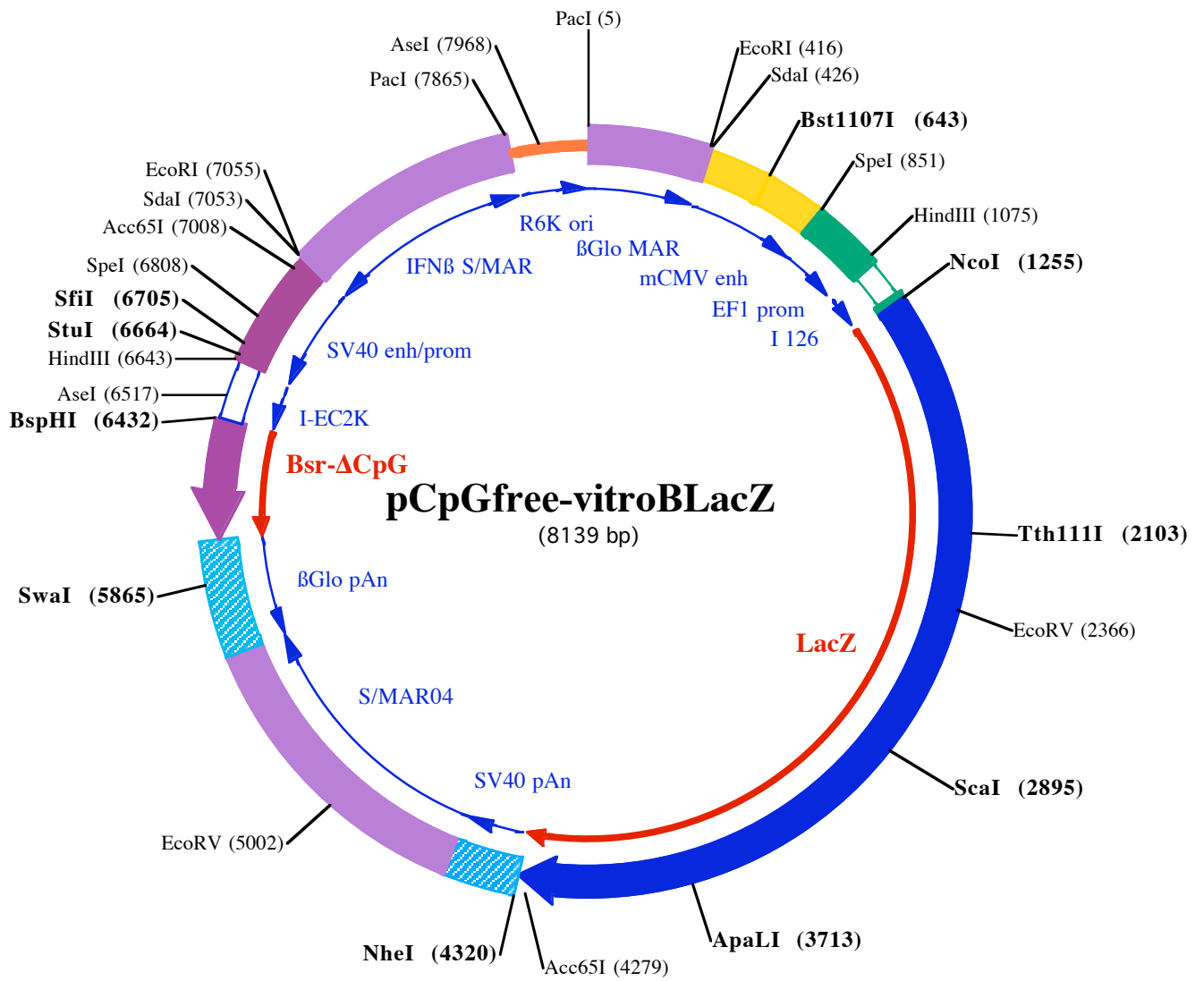
InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

 **InvivoGen**  
www.invivogen.com



PacI (5)  
1 TTAATTAATAATCTCTAAGGCATGTGAAGTGGCTGTCTGGTTTTTCATCTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCAT  
101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCCTAAAGGATTTTGTGAAGAACTAATTGAATTGATACCTGTAAGCTTTATCACACTACCC  
201 AATAAATAATAATCTCTTTGTTTCAGCTCTCTGTTTCTATAAATATGTACAAGTTTTATTGTTTTTAGTGGTAGTGATTTATTCTTTCTATATATAT  
301 ACACACACATGTGTGCATTATAAATATATAAATTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA  
SdaI (426)  
EcoRI (416)  
401 ACAGCAGATTAAGAAATTCCTGCAGGAGTCAATGGGAAAAACCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCAGT  
501 ACATAAGGTCAATAGGGGTGAGTCAACAGGAAAGTCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG  
Bst1107I (643)  
601 GTCAATGGGAGGTAAGCCAATGGGTTTTCCATTACTGACATGTATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATA  
701 GGGGTGAATCAACAGGAAAGTCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGTGA  
SpeI (851)  
801 GTCAATGGGTTTTCCATTATTGGCACATACATAAGGTCATAGGGGTGACTAGTGGAGAAGGACATGCTTAGGGCTGAGTGCCCTCAGTGGGCAGA  
901 GAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGGTGGCAATTGAACGTGGTGCCTAGAGAAGGTGGGGCTGGGTAACCTGGGAAAGTGATG  
HindIII (1075)  
1001 GGTGTACTGGCTCCACCTTTTTCCCAAGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAGCTTCTGCCCTCTCCCTCTGTGA  
1101 GTTTGtaagtactgactgtctatgcctgggaaagggtggcaggagatggggcagtgaggaaaagtgccactatgaaccTGACGCCCTAGAcAaatt  
NcoI (1255)  
1201 gtactaacctttcttctcttctcctcctgacagGTTGGTGTACAGTAGCTCCACCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCTGG  
1301 AGTGACCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCTCTGGAGGAAGTCTGAGGAAGCCAGGACAGCAGGCCAGCCAGCAGCTCAGGTCT  
1501 CCAGCACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTCACTACCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCAC  
1801 TGGTGTCTCAGGTGGTCTGATGGCAGCTACTGGAAGCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCCACCA  
2101 CACGATTTCTGACTTCATGTCGCCACAGTTCAATGATGACTTCAGCAGAGCTGTGCTGGAGGCTGAGGTGACAGATGTGGAGGACTCAGAGTACT  
2401 CTGAGAGTCAAGTGGAGCTCTGGCAAGGTGAGACCCAGTGGCCTCTGGCAGACCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTG  
2701 ACAGAGTCAACCTCAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGCTGATGG  
3001 CACCTGATTGAAGCTGAAGCTGTGATGTTGGATTGAGAAAGTCAAGGATGAGAAATGGCTGCTGCTCAATGGCAAGCTCTGCTCATCAGGGGA  
3301 yVal Thr Gl nLeuAsnArgLeuAl aAl aHi sP rP rPheAl aSer T rpArgAsnSer Gl uGl uAl aArgThr AspArgP rSer Gl nGl nLeuArgSer  
1501 yVal Thr Gl nLeuAsnArgLeuAl aAl aHi sP rP rPheAl aSer T rpArgAsnSer Gl uGl uAl aArgThr AspArgP rSer Gl nGl nLeuArgSer  
1801 LeuAsnGl yGl uTrpArgPheAl aT rpPheP rAl aP rGl uAl aVal P rGl uSer T rpLeuGl uCysAspLeuP rGl uAl aAspThr Val Val Val P  
2101 CysAsnGl yArgT rpVal Gl yTyrGl yGl nAspSer ArgLeuT rpSer Al aGl uGl uP rHeAspLeuSer Al aPheLeuArgAl aGl yGl uAsnArgLeuAl aVal M  
2401 eVal LeuArgT rpSer AspGl ySer TyrLeuGl uAspGl nAspMet T rpArgMetSer Gl y l ePheArgSpVal Ser LeuLeuHi sLysP rThr Th  
2701 rGl n l eSer AspPheHi sVal Al aThr ArgPheAsnAspAspPheSer ArgAl aVal LeuGl uAl aGl uVal Gl nMetCysGl yGl uLeuArgAspTyr  
3001 CTGAGAGTCAAGTGGAGCTCTGGCAAGGTGAGACCCAGTGGCCTCTGGCAGACCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTG  
3301 LeuArgVal Thr Val Ser LeuT rpGl nGl yGl uThr Gl nVal Al aSer Gl yThr Al aP rPheGl yGl yGl u l e l eAspGl uArgGl yGl yTyrAl aA  
3601 ACAGAGTCAACCTCAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGCTGATGG  
3901 spArgVal Thr LeuArgLeuAsnVal Gl uAsnP rO l sLeuT rpSer Al aGl u l eP rAsnLeuTyrArgAl aVal Al aAspGl  
4201 CACCTGATTGAAGCTGAAGCTGTGATGTTGGATTGAGAAAGTCAAGGATGAGAAATGGCTGCTGCTCAATGGCAAGCTCTGCTCATCAGGGGA  
4501 yThr Leu l eGl uAl aGl uAl aCysAspVal Gl yPheArgGl uVal A rGl l eGl uAsnGl yLeuLeuLeuAsnGl yLysP rO leuLeu l eArgGl y  
EcoRV (2366)  
5101 GTCAACAGGCATGAGCACCCTCTGCATGGACAAGTGGATGAACAGACAATGGTGAAGATATCTGCTAATGAAGCAGAACAACCTCAATGTCTG  
5401 Val AsnArgT sGl uHi sHi sP rO leuHi sGl yGl nVal l eMetAspGl uGl nThr MetVal Gl nAsp l eLeuLeuMetLysGl nAsnAsnPheAsnAl aV  
5701 TCAGGTGCTCTCAGTACCACCCCAACCCCTCTCTGGTACACCTGTGGCAGGATGACAGGATGACCTGTATGTTGTTGATGAAGCAACATTCAGCAAGTGGCAT  
6001 a l ArgCysSer Hi sTyrP rO leuHi sP rO leuT rpTyrThr LeuCysAspArgTyrGl yLeuTyrVal Val AspGl uAl aAsn l eGl uThr Hi sGl yMe  
6301 GGTGCCATGAACAGGCTCAGATGACCCAGGTGGCTGCCTGCTGATGCTGAGAGAGTGAACAGGATGGTGCAGAGAGACAGGAACCCCTCTGCTGTG  
6601 tVal P rO leuMetAsnArgLeuThr AspAspP rO rArgT rpLeuP rO l aMetSer Gl uArgVal Thr ArgMetVal Gl nArgAspArgAsnHi sP rSer Val  
6901 ATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCAACCATGATGCTCTCTACAGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCAGTATG  
7201 l e l eT rpSer LeuGl yAsnGl uSer Gl yHi sGl yAl aAsnHi sAspAl aMetTyrArgT rp l eLysSer Val AspP rO rArgP rO l aGl nTyrG  
7501 AAGCAGTGGAGCAGCACCACAGCATCTGCCCCATGTATGCCAGGGTGTGATGAGGACCCCTCTCCCTGCTGGCCCAAGGATGAGTCA  
7801 l uGl yGl yGl yAl aAspThr Thr Al aThrAsp l e l eCysP rO leuTyrAl aArgVal AspGl uAspGl nP rO leuP rO l aVal P rO l sT rpSer l l  
ScaI (2895)  
8101 CAAGAAGTGGCTCTCTGCTGGAGAGACAGACCTCTGATCCTGTGTAATGCACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGG  
8401 eLysLysT rpLeuSer LeuP rO l yGl uThr ArgP rO leu l eLeuCysGl uTyrAl aHi sAl aMetGl yAsnSer LeuGl yPheAl aLysTyrT rp  
8701 CAAGCCTTCAGACAGTACCCAGGCTGCAAGGAGGATTTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCTGGTCTG  
9001 Gl nAl aPheArgAl nTyrP rO rArgLeuGl nGl yGl yPheVal T rpAspT rpAl aAspGl nSer Leu l eLysTyrAspGl uAsnGl yAsnP rO rP rSer A  
9301 CCTATGGAGGAGCTTTGGTACACCCCAATGACAGGAGTCTGATGAATGGCTGGTCTTTGAGAGCAGGACCCCTCACCTCTGCCCTCACAGAGGC  
9601 l aTyrGl yGl yAspPheGl yAspThr P rO leuAspArgGl nPheCysMetAsnGl yLeuVal PheAl aAspArgThr P rO leuP rO l aLeuThr Gl uAl  
9901 CAAGCACCAGCAACAGTCTCCAGTTCAGGCTGTCTGGACAGACATTGAGGTGACATCTGAGTACCTCTCAGGCACTGCAATGAGCTCTGCAC  
10201 LysHi sGl nGl nGl nPhePheGl nPheArgLeuSer Gl yGl nThr l l eGl uVal Thr Ser Gl uTyrLeuPheArgHi sSerAspAsnGl uLeuLeuHi s  
10501 TGGATGTTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGGGTCCTCTGGATGGGCCCTCAAGGAAAGCAGCTGATTGAACCTGCCTGAGCTGCCTC  
10801 T rpMetVal Al aLeuAspGl yLysP rO leuAl aSer Gl yGl uVal P rO leuAspVal Al aP rO l nGl yLysGl nLeu l eGl uLeuP rO l yLeuP rO G  
11101 AGCCAGAGTCTGCTGGCAACTGTGGCTAACAGTGGGTTGGTTCAGCCCAATGCAACAGCTGGTCTGAGGACAGCCACATCTGAGGACAGCCAGTGG  
11401 l nP rO l uSer Al aGl yGl nLeuT rpLeuThr Val A rGVal Val Gl nP rO leuAsnAl aThr Al aT rpSer Gl uAl aGl yHi s l l eSer Al aT rpGl nGl nT r  
11701 GAGGCTGGCTGAGAACCCTCTCTGACCTGCTGCTGCTCATGCCATCCCTCACCTGACAAATCTGAAATGGACTTCTGCATTGAGCTGGGCAAC  
12001 pArgLeuAl aGl uAsnLeuSer Val Thr LeuP rO l aAl aSer Hi sAl a l eP rO leuHi sLeuThr Thr Ser Gl uMetAspPheCys l eGl uLeuGl yAsn  
12301 AAGAGATGGCAGTTCACAGGCAGTCTGGCTTCTGTCTCAGATGGATTGGAGACAAGAGCAGCTCTCACCCCTCTCAGGACCAATTCACAGGG  
12601 LysArgT rpGl nPheAsnArgGl nSer Gl yPheLeuSer Gl nMetT rp l l eGl yAspLysLysGl nLeuLeuThr P rO leuArgAspGl nPheThr ArgA  
12901 CTCTCTGGCAACATGGAGTGTCTGAGGCCACAGGATGACCAACATGCTGGTGGTGGAGAGGTGGAAGGCTGCTGACACTCAGGCTCAGGAGGC  
13201 l aP rO leuAspAsnAsp l eGl yVal Ser Gl uAl aThr Arg l eAspP rO leuAsnAl aT rpVal Gl uArgT rpLysAl aAl aGl yHi sTyrGl nAl aGl uAl  
AlaPI (3713)  
13501 TGCCCTGCTCCAGTGCACAGCAGACCCCTGGCTGATGCTGTTCTGATCACCACAGCCATGCTTGGCAGCACCAGGCAAGACCTGTTTCATCAGCAGA  
13801 aAl aLeuLeuGl nCysThr Al aAspThr LeuAl aAspAl aVal Leu l eThr Thr Al aHi sAl aT rpGl nHi sGl nGl yLysThr LeuPhe l eSer Arg

3801 AAGACCTACAGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCTGAACTGTC  
849 LysThr TyrArgI l eAspGI ySerGI yGInMetAl l eThr Val AspVal GI uValAl aSerAspThr ProHi sProAl aArgI l eGI yLeuAsnCysG  
3901 AACTGGCACAGGTGGCTGAGAGGGTGAACCTGGCTGGGCTTAGGCCCTCAGGAGAACTACCTGCAGGCTGACAGCTGCCTGCTTTGACAGGTGGGACCT  
882 I nLeuAl aGI nValAl aGI uArgValAsnTrpLeuGI yLeuGI yProGI nGI uAsnTyrProAspArgLeuThr Al aAl aCysPheAspArgTrpAspLe  
4001 GCCTCTGTCTGACATGTACACCCCTTATGTGTCCCTTCTGAGAATGGCTGAGGTGGCACCAGGGAGCTGAACATGGCTCACCAGTGGAGGGGA  
915 uP roLeuSerAspMetTyrThr ProTyrVal PheProSer GI uAsnGI yLeuArgCysGI yThr ArgGI uLeuAsnTyrGI yProHi sGI nTrpArgGI y  
4101 GACTTCCAGTTCAAACCTCCAGGTACTCTCAGCAACAGCTCATGAAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGAACTGGCTGAACATTG  
949 AspPheGI nPheAsnI l eSerArgTyrSerGI nGI nLeuMetGI uThr Ser Hi sArgHi sLeuLeuHi sAl aGI uGI uGI yThr TrpLeuAsnI l eA  
4201 ATGGCTTCCACATGGCATTGGAGGAGATGACTCTTGGTCTCCTCTGTGTCTGTGAGTTCAGTTATCTGTGGCAGGTACCACATATCAGCTGGTGTG  
982 spGI yPheHi sMetGI yI l eGI yGI yAspAspSer TrpSer ProSer Val SerAl aGI uPheGI nLeuSerAl aGI yArgTyrHi sTyrGI nLeuVal Tr  
NheI (4320)  
4301 GTGCCAGAAGTAAACCTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAACAAAAATGCTTTATT  
1015 pCysGI nLys●●●  
4401 TGTGAAATTTGTGATGTATTGCTTTATTGTAACCATATAAGCTGCAATAAACAAGTTAAACAACAACAATTCATTCATTTTATGTTTCAGGTTTCAGG  
4501 GGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGGTATGGAATTGGAGCCCACTGTGTTTCATCTTACAGATGAAATACTGACAT  
4601 TCAGAGGAGTTAGTTAACTTGCCTAGGTATTGCTAATAAGTCAAGAAAGATTCAATCCAAGGTATTGATTCTGAAGCCTGTGCTAATCACATT  
4701 ACACCAAGCTACAACCTCATTATAAATAAAGTCAAGCTTCAAGGGCCTTTCAGGTGTCTGCACCTTACAAGCTGTGCCATTTAGTGAACACAAAA  
4801 TGAGCCTTCTGATGAAGTAGTCTTTTATTATTGATATTAGAACACTAAAATTTCTAGCTGCCAGCTGATTGAAGGCTGGGACAAAAATCAACATG  
EcoRV (5002)  
4901 CATCTACAACAATATATATCTCAATGTTAGTCTCCAAATTTCTATTGACTTCAACTCAAGAGAATATAAAGAGCTAGTCTTTATACACTCTTTAAGGTATG  
5001 ATATCATCTGGAAAGTAAACAAATTTGATGCAATTTGAATGAACCTTATCATGGTGTATTTACACAATGTGTTCTTCTCCCTGCAATGTATTTCTTTCT  
5101 CTAATTCCTTCCATTTGATCTTTCATACACAATCTGGTCTGATGTATGTTTTGGATGCACCTTCAACTCCAAGGACAGAGCTAGTTACTTTCTTCTC  
5201 CTGGTGTCCCAAGCACTGATTTGTATCTGTATTCAAGCCCTTGAATATTGACTGGATCATTATTCACCTTAGGATGGCTTCCCAGGCAACTTG  
5301 TGTTCAACCAGAGACTACATTTGTATCTTGTGACCTTGAACCTCCACCAGTGTCTAAAAATAATATGTATGCAAAATTACTTGTCTATGAGAATGTAT  
5401 AATTAACAATATAAAAAGGAGAAGCAAGGAGAGAACACAGGTGTATTTGTGTTGTGTCTAAAAGGCAGTGTGAAAGGAAAGAAATGCCATTT  
5501 ATAGTGAGGAGACAAAGTTATATTACCTCTTATCTGGCTTTAAGGAGATTTGCTGAGCTAAAAATCCTATATTATAGAAAAGCCTTACCTGAGTTGC  
5601 CAATACCTCAATTTAAAATACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCA  
5701 GGGGCTGTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAACAGCTCTTCATT  
SwaI (5865)  
5801 TCTTTATGTTTTAAATGCAGTACCTCCACATTCCTTTTGTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTA  
5901 TTAGGCAGATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAG  
6001 AAAGCTCTAGCTTTAGTTTCTGGTGTATTCAGTGAATCAGCTCCTCAATGGTTGTCTTGACCAGCTTGCCATTCATCTCAATCAGAACAAGCAATCA  
140 AsnArgThr TyrLysLeuP roI l eLeuGI uGI uI l eThr Thr LysVal l eLeuLysGI yAsnMe tGI uI l eLeuVal l ePheCysAspP  
6101 GGGGCATAGTCTGAGATGAGCTCCCTGCACATGCCACAGGGGACACCACTCTGATGGACCTGCCACCTCATCACTGTAGGGGTGCCTCACAGCCACAA  
111 r oAl aTyrAspSer I l eLeuGI uArgCysMetGI yCysProSer Val l eAl aArgI l eSer ArgAspVal l eGI uAspSer TyrProHi sArgVal l eAl aVal l e  
6201 TGGTGTCAAAGTCTTCTGCCATTGGAGACTGCAGACCAATGGCAATGGCCTCTGCACACAGTGCACCTGCCAATGTAGGCTTCAATGTGGACAGC  
78 eThrAspPheAspLysGI nGI yAsnSer ValAl aSer GI yI l eAl aI l eAl aGI uAl aCysVal l eThr Val l eArgGI yI l eTyrAl aGI uI l eHi sValAl a  
6301 AGAGATGATTTCTCCTGCTTGTCTGTGGCAGCACCAACATGGTGTCTTGTCTCCTATAGAGCATGGTATTTCTCAGTGGCACTCCACCAGC  
45 Ser I l eI l eGI uGI yThr LysThrArgI l eAl aAl aGI yVal l eHi sHi sLysAsnAspGI uTyrLeuMetThr I l eLysGI uThr Al aVal l eGI uVal l eLeuG  
BspHI (6432)  
6401 TCAAGTCTGCTGAGAGATGTTGAAGTCTTATGATGGCTCCTCctgtcaggagaggaagagaaggttagtacaattgctATAGTGAAGTGTAT  
11 l uLeuAspGI nGI nSer I l eAsnPheThr LysMet  
AseI (6517)  
6501 TATACTATGCTTATGATTAATTGCAAACTAGGCTGCAGgggttcatagtcaccctttctgcactgcccctctctgccacccttccaggcata  
HindIII (6643) StuI (6664)  
6601 gacagtcagtgaacttacAAACTCACAGGAGGAGAAGGCAGAGCTTTTGCAAAAGCCTAGGCCCTCAAAAAGCCTCCTCACTACTTCTGGAATAGC  
SfiI (6705)  
6701 TCAGAGGCCcAGGgGCCTgGGCCTCTGCATAAATAAAAAAATTAGTACGCTGGGcctggggcaggggtggggggccaactgggCAGGGGTG  
SpeI (6808)  
6801 GGGGGCCACTAGTGGACTATGTTGCTGACTAATTGAGATGCATGCTTTCGATACTTCTGCTGCTGGGAGCCTGGGACTTTCCACACCTGGTTGCT  
6901 GACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGAGCCTGGGACTTTCCACACCTAACTGACACACATTCACAGCTGGTCTTTCAGC  
EcoRI (7055)  
7001 CTCAGAAGGTACCTAACCAAGTCTCTTTTCAGAGGTTATTTTCAGGCCCTGCAGGAATTGAGTCAATATGTTACCCCAAAAAGCTGTTTGTAACTTG  
7101 TCAACCTCATTCTAAAAATGATATAGAAGCCCAAAAGACAATAACAAAAATATTCTGTAGAACAATAAGGAAAGAAATGTTCCACTAAATATCAAGATT  
7201 TAGAGCAAAGCATGAGATGTGTGGGATAGACAGTGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAA  
7301 AAAATATGCCATTTTCAATGGGAAATGATGATCTTTTTCTTTTTTAGAAAAACAGGAAATATATTTATGTAATAAATAAAGGAAACCCATATGT

7401 CATACCATACACACAAAAAATCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTAGAAAATAATATAGAAGCATGCCATCAAG  
7501 ACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCCTAACCAAGAAAAAGATTGTAATTAGATTGCATGAATTAAGACTTATTTTAAAAAT  
7601 AAAAAACCATTAAGAAAAAGTCAGGCCATAGAATGACAGAAAAATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGAAGCTTA  
7701 CAAATCAGTAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGA  
7801 ACTATCATTGCATATACACTAAATTAGAGAAATATTAAGGCTAAGTAACATCTGTGGCTTAATTAAGTTATCCTAGGAAACCTTAAACCTTTAAAAG  
PacI (7865)  
7901 CCTTATATATCTTTTTTTCTTATAAACTTAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTA  
AseI (7968)  
8001 CATGAAACATGAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAACATGAGAGCTTAGTACATTAACATGA  
8101 GAGCTTAGTACATACTATCAACAGGTTGAACTGCTGATT