

STOP

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E-mail: info@invivogen.com



pCpGfree-vitroNLacZ

A LacZ expression plasmid completely devoid of CpG dinucleotides, selectable with G418/Kanamycin

Catalog code: pcpgvtn-lz

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

For research use only

Version 19L12-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pCpGfree-vitroNLacZ 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 α 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 β -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 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 Neo resistance gene (Neo- Δ 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 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.

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 H₂O. 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

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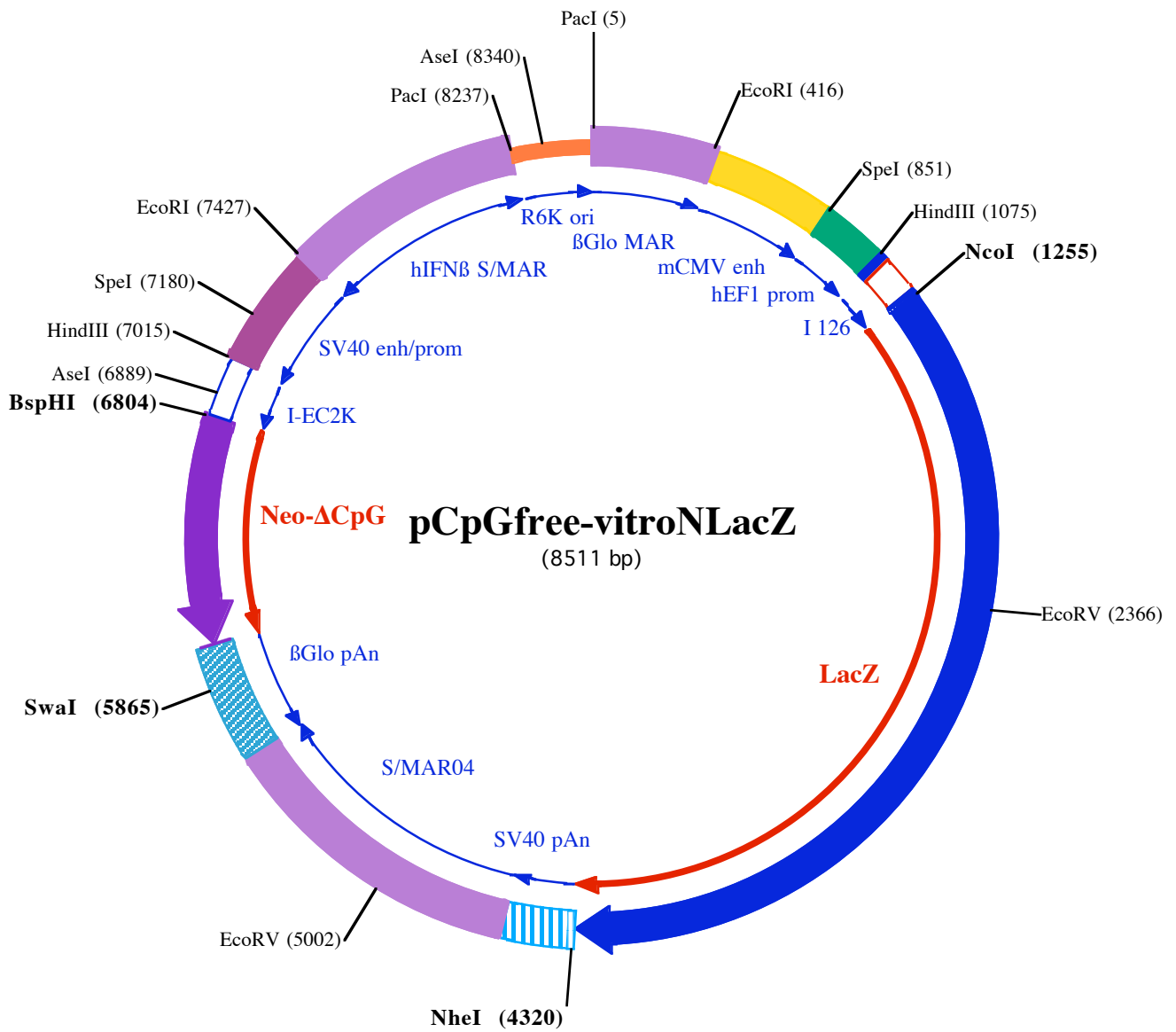
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Pacl (5)
1 TTAATTAATAATCTCTAAGGCATGTGAAGTGGCTGTCTTGGTTTTTCATCTGTACTTCTGCTACCTCTGTGACCTGAAACATATTTATAATCCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCC
201 AATAAATAATAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACAAGTTTTATTGTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATAT
301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA
EcoRI (416)
401 ACAGCAGATTAAGGAATTCTCGAGGAGTCAATGGGAAAAACCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCAGT
501 ACATAAGGTCAATAGGGGGTGTGAGTCAACAGGAAAGTCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG
601 GTCAATGGGAGGTAAGCCAATGGGTTTTTCCATTACTGACATGATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATA
701 GGGGTGAATCAACAGGAAAGTCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGGTGA
SpeI (851)
801 GTCAATGGGTTTTTCCATTATTGGCACATACATAAGGTCAATAGGGGTACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTGCCCTCAGTGGGCAGA
901 GAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGGTGGCAATTGAACTGGTGCTAGAGAAGGTGGGCTGGGTAACCTGGGAAAGTGTATGT
HindIII (1075)
1001 GGTGACTGGCTCCACCTTTTTCCCAAGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCTGTGA
1101 GTTTGtaagtcactgactgtctatgcttgggaagggtgggcaggagatggggcagtgaggaaaagtgaccatgaaccTGACAGCCCTAGAcatt
NcoI (1255)
1201 gtactaacctttcttctctctctctgacagGTTGGTGTACAGTAGCTCCACATGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGG
1301 AGTGACCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCCTCTTGGAGGAACCTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGTC
15▶ yVal Thr Gl nLeuAsnArgLeuAl aAl aHi sP roP rPheAl aSer T rpArgAsnSer Gl uGl uAl aArgThr AspArgP roSer Gl nGl nLeuArgSer
1401 CTCAATGGAGAGTGGAGGTTGGCTGGTCCCTGCCCTGAACTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCTGACACTGTTGTGGTGC
49▶ LeuAsnGl yGl uTrpArgPheAl aTrpPheP roAl aP roGl uAl aVal P roGl uSer T rpLeuGl uCysAspLeuP roGl uAl aAspThr Val Val Val P
1501 CCAGCAACTGCGAGATGCATGGCTATGATGCCCCATCTACACCAATGTACCTACCCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCAC
82▶ roSerAsnTrpGl nMetHi sGl yTyrAspAl aP roI l eTyrThrAsnVal Thr TyrP roI l eThr Val AsnP roP rPheVal P roThr Gl uAsnP roTh
1601 TGGCTGTACAGCTGACCTCAATGTTGATGAGAGCTGGTCAAGAACAGGACAGCCAGGATCACTTTTGTAGGATGGATCAACTGCTTCCCTCCACCTGG
115▶ r Gl yCysTyrSer LeuThr PheAsnVal AspGl uSer T rpLeuGl nGl uGl yGl nThr ArgI l eI l ePheAspGl yVal AsnSer Al aPheHi sLeuTrp
1701 TGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCTCTGAGTTTGACCTCTCTGCCTCTCAGAGCTGGAGAGAACAGGCTGGCTGTCA
149▶ CysAsnGl yArGTrpVal Gl yTyrGl yGl nAspSer ArgLeuP roSer Gl uPheAspLeuSer Al aPheLeuArgAl aGl yGl uAsnArgLeuAl aVal I
1801 TGGTGTCCAGCTGACCTTGGCAAGGTGAGACCCAGGCTGGCCTCTGGCAGGATGGAGGATCTTGGCCTCTCAGGATGAGGCTGGCAGGCTGACCA
182▶ etVal LeuArgTrpSer AspGl ySer TyrLeuGl uAspGl nAspMetT rpArgMeT Ser Gl yI l ePheArgAspVal Ser LeuLeuHi sLysP roThr Th
1901 CCAGATTTCTGACTTCCATGTTGCCACCAGTTCATGATGACTTACGACAGCTGTGCTGGAGGCTGAGGTGCAGATGTGTGGAGAAGCTCAGAGACTAC
215▶ r Gl nI l eSerAspPheHi sVal Al aThr ArgPheAsnAspPheSer ArgAl aVal LeuGl uAl aGl uVal Gl nMeT CysGl yGl uLeuArgAspTyr
2001 CTGAGAGTCAAGCTGACCTTGGCAAGGTGAGACCCAGGCTGGCCTCTGGCAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGG
249▶ LeuArgVal Thr Val Ser LeuTrpGl nGl yGl uThr Gl nVal Al aSer Gl yThr Al aP roPheGl yGl yGl uI l eI l eAspGl uArgGl yGl yTyrAl aA
2101 ACAGAGTCCACCTGAGGCTCAATGTGGAGAACCACCAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGCTGATGG
282▶ spArgVal Thr LeuArgLeuAsnVal Gl uAsnP roLysLeuTrpSer Al aGl uI l eP roAsnLeuTyrArgAl aVal Val Gl uLeuHi sThr Al aAspGl
2201 CACCCTGATTGAAGCTGAAGCTGTGATGTTGGATTGAGAGAGTCAAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGGATGGAGG
315▶ yThr LeuI l eGl uAl aGl uAl aCysAspVal Gl yPheArgGl uVal ArGl l eGl uAsnGl yLeuLeuLeuLeuAsnGl yLysP roLeuLeuI l eArgGl y
EcoRV (2366)
2301 GTCAACAGGCATGAGCACCACCCTCTGCATGGACAAGTGTGGTGAACAGACAATGGTCAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTG
349▶ Val AsnArgHi sGl uHi sHi sP roLeuHi sGl yGl nVal MetAspGl uGl nThr MetVal Gl nAspI l eLeuLeuMetLysGl nAsnAsnPheAsnAl aV
2401 TCAGGTGCTCTCACTACCCCAACCACCCTCTCTGGTACACCCTGTGTGACAGGTATGGCTGTATGTTGTTGATGAAGCAACATTGAGACATGGCAT
382▶ aI ArgCysSer Hi sTyrP roAsnHi sP roLeuTrpTyrThr LeuCysAspArgTyrGl yLeuTyrVal Val AspGl uAl aAsnI l eGl uThr Hi sGl yMe
2501 GGTGCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCCTGCCATGTCTGAGAGAGTGACAGGATGGTGCAGAGAGACAGGAACCCCTCTGTG
415▶ tVal P roMeTAsnArgLeuThr AspAspP roArgT rpLeuP roAl aMeT Ser Gl uArgVal Thr ArgMetVal Gl nArgAspArgAsnHi sP roSer Val
2601 ATCATCTGGTCTCTGGCAATGAGTCTGGACATGGACCAACCATGATGCTCTACAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCAGTATG
449▶ I l eI l eTrpSer LeuGl yAsnGl uSer Gl yHi sGl yAl aAsnHi sAspAl aLeuTyrArgTrpI l eLysSer Val AspP roSer ArgP roVal I Gl nTyrG
2701 AAGGAGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGGTTGATGAGGACCAGCCCTTCCCTGCTGTGCCAAGTGGAGCAT
482▶ I uGl yGl yGl uAl aAspThr Thr Al aThr AspI l eI l eCysP roMeT TyrAl aArgVal AspGl uAspGl nP roPheP roAl aVal P roLysTrpSer I l
2801 CAAGAAGTGGCTCTCTGCCTGGAGAGACCAGACCTCTGATCTGTGAAATGCACATGCAATGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGG
515▶ eLysLysTrpLeuSer LeuP roGl yGl uThr ArgP roLeuI l eLeuCysGl uTyrAl aHi sAl aMeT Gl yAsnSer LeuGl yGl yPheAl aLysTyrTrp
2901 CAAGCTTTCAGACAGTACCCAGGCTGCAAGGAGGATTTGTGGGACTGGTGGACCAATCTCTCATCAAGTATGATGAGAATGGCAACCCCTGGTCTG
549▶ Gl nAl aPheArgGl nTyrP roArgLeuGl nGl yGl yPheVal T rpAspT rpVal AspGl nSer LeuI l eLysTyrAspHi sSer AspAsnGl uLeuLeuHi s
3001 CCTATGGAGGAGACTTTGGTACACCCCAATGACAGGCAGTCTGTCATGAATGGCTGGTCTTTGCAGACAGGACCCCTACCCTGCCCTCACAGAGGC
582▶ I aTyrGl yGl yAspPheGl yAspThr P roAsnAspArgGl nPheCysMeTAsnGl yLeuVal PheAl aAspArgThr ProHi sP roAl aLeuThr Gl uAl
3101 CAAGCACCAGCAACAGTCTCCAGTTCAGGCTGTCTGGACAGACATTGAGGTGACATCTGAGTACCTTTCAGGACTCTGCAATGAGCTCTGCAC
615▶ aLysHi sGl nGl nGl nPheGl nPheArgLeuSer Gl yGl nThr I l eGl uVal Thr Ser Gl uTyrLeuPheArgHi sSer AspAsnGl uLeuLeuHi s
3201 TGGATGGTGGCCCTGGATGGCAAGCCTCTGGCTTCTGGTGGAGTGCCTCTGGATGGGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGCTGCCTC
649▶ TrpMeTVal Al aLeuAspGl yLysP roLeuAl aSer Gl yGl uVal P roLeuAspVal Al aP roGl nGl yLysGl nLeuI l eGl uLeuP roGl uLeuP roG
3301 AGCCAGAGTCTGCTGGCAACTGTGGCTAACAGTGGGTTGAGGCTTCCAGCCCAATGCAACAGCTTGGTCTGAGGCAGGCCACATCTGTCATGGCAGCAGT
682▶ I nP roGl uSer Al aGl yGl nLeuTrpLeuThr Val ArgVal Val Gl nP roAsnAl aThr Al aT rpSer Gl uAl aGl yHi sI l eSer Al aT rpGl nGl nT
3401 GAGGCTGGTGAAGACCTCTCTGTGACCCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGCATTGAGCTGGGCAAC
715▶ pArgLeuAl aGl uAsnLeuSer Val Thr LeuP roAl aAl aSer Hi sAl aI l eP roHi sLeuThr Thr Ser Gl uMeTAspPheCysI l eGl uLeuGl yAsn
3501 AAGAGATGGCAGTTCACAGGCAGTCTGGCTTCTGCTCAGATGTGGATTGGAGACAAGAAGCAGCTCTCACCCCTCTCAGGACCAATTCACAGGG
749▶ LysArgTrpGl nPheAsnArgGl nSer Gl yPheLeuSer Gl nMeTTrpI l eGl yAspLysLysGl nLeuLeuThr P roLeuArgAspGl nPheThr ArgA

3601 CTCTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGGTGGAGAGGTGGAAGGCTGTGGACACTACCAGGCTGAGGC
782▶ I aProLeuAspAsnAspI l eGl yVal Ser Gl uAl aThr ArgI l eAspP roAsnAl aTrpVal Gl uArgTrpLysAl aAl aGl yHi s TyrGl nAl aGl uAl
3701 TGCCTGCTCCAGTGCACAGCAGCACCCCTGGCTGATGCTTCTGATCACCACAGCCCATGCTTGGCAGCACCAAGGCAAGACCCTGTTTCATCAGACA
815▶ aAl aLeuLeuGl nCysThrAl aAspThr LeuAl aAspAl aVal LeuI l eThr ThrAl aHi sAl aTrpGl nHi sGl nGl yLysThr LeuPheI l eSer Arg
3801 AAGACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGTGGCTCTGACACACCTCACCCCTCAAGGATTGGCCTGAACTGTC
849▶ LysThr TyrArgI l eAspGl ySer Gl yGl nMe tAl aI l eThr Val AspVal Gl uVal Al aSerAspThr P roHi sP roAl aArgI l eGl yLeuAsnCysG
3901 AACTGGCACAGGTGGCTGAGAGGGTGAAGTGGCTGGCTTAGGCCCTCAGGAGAACCCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTGGCTGAACTTG
882▶ I nLeuAl aGl nVal Al aGl uArgVal AsnTrpLeuGl yLeuGl yProGl nGl uAsnTyrP roAspArgLeuThr Al aAl aCysPheAspArgTrpAspLe
4001 GCCTCTGTCTGACATGTACACCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGCACCAGGGAGCTGAACTATGGTCTCACAGTGGAGGGGA
915▶ uP roLeuSerAspMe tTyrThr P roTyrVal PheP roSer Gl uAsnGl yLeuArgCysGl yThr ArgGl uLeuAsnTyrGl yProHi sGl nTrpArgGl y
4101 GACTTCCAGTTCACATCTCCAGGTACTCTCAGCAACAGCTCATGGAACCTCTCACAGGCACCTGCTCCATGCAGAGGAGGGAACCTGGCTGAACTTG
949▶ AspPheGl nPheAsnI l eSer ArgTyrSer Gl nGl nLeuMe tGl uThr Ser Hi sArgHi sLeuLeuHi sAl aGl uGl uGl yThr TrpLeuAsnI l eA
4201 ATGGCTTCCACATGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGTCTGTGAGTTCAGTATTCTGCTGGCAGGTACCACATACAGCTGGTGTG
982▶ spGl yPheHi sMe tGl yI l eGl yGl yAspAspSer TrpSer P roSer Val Ser Al aGl uPheGl nLeuSer Al aGl yArgTyrHi s TyrGl nLeuVal Tr

NheI (4320)

4301 GTGCCAGAAGTAACTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTGGTGGACAACCAACTAGAAATGCAGTGAAGAAAAATGCTTTATT
1015▶ pCysGl nLys●●●
4401 TGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCATTTATGTTTCAGGTTCCAGG
4501 GGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTGGAGCCCCACTGTGTTTCATCTTACAGATGGAATACTGACAT
4601 TCAGAGGAGTTAGTTAACTTGCCTAGGTGATTACAGTAATAAGTGAAGAAAGATTTCAATCCAAGGTGATTTGATTCTGAAGCCTGTGCTAATCACATT
4701 ACACCAAGCTACAACCTCATTATAAATAAAGTCAAGCTTTCAAGGCCCTTTCAGGTGTCTGCACCTTACAAGCTGTGCCATTTAGTGAACACAAAA
4801 TGAGCCTTCTGATGAAGTAGCTTTTCATTATTTAGATATTAGAACACTAAAATTTCTAGCTGCCAGCTGATTGAAGGCTGGGACAAAATTCAAACATG
4901 CATCTACAACAATATATATCTCAATGTTAGTCTCCAATTTCTATTGACTTCAACTCAAGAGAATATAAAGAGCTAGTCTTTATACACTCTTTAAGGTATG
5001 ATATCATCTGAAAGTAACAAAATTGATGCAAAATTTGAATGAACCTTTATCATGGTGTATTTACACAATGTGTTTCTTCCCTGCAATGATTTCTTTCT
5101 CTAATTCCTTCCATTTGATCTTTCATACACAATCTGGTCTGATGTATGTTTTTGGATGCATTTTCAACTCCAAAAGACAGAGCTAGTACTTTCTTC
5201 CTGGTGTCCAAAGCACTGATTTGTATCTGTATTCAAGCCCTTTCGAATATTGACTGGATCATTATTTACCTCTAGGATGGCTTCCCAGGCAACTTG
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5401 AATTAACAATATAAAAAGGAGAAGCAAGGAGAGAAACAGGTGTGATTTGTGTTTGTGCTTAAAAGGCAGTGTGAAAAGGAAGAAATGCCATTT
5501 ATAGTGAGGAGACAAAGTTATATTACCTCTTATCTGGCTTTTAAAGGAGATTTGCTGAGCTAAAAATCCTATATTCATAGAAAAGCCTTACTGAGTTGC
5601 CAATACCTCAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCA
5701 GGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTCTTCATT

EcoRV (5002)

SwaI (5865)

5801 TCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAATATTCAGAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTA
5901 TTAGGCAGAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAG
6001 AAAGCTCTAGCTTTAGAAGAACTCATCAAGAAGTCTGTAGAAGCAATTTCTGCGGAGTCAAGGGCTGCAATGCCATAGAGCACTAGGAACCTGTCTGCC
265◀●●●PhePheGl uAspLeuLeuArgTyrPheAl aI l eArgGl nSerAspProAl aAl aI l eGl yTyrLeuVal l eLeuPheArgAspAl aT
6101 CACTCTCCCCCTAGCTCTTCTGCTATGTCCTGGTGTGCTAGGGCAATGTCTGGTACCTGTGACCCACTCCAGCCTGCCACAGTCTATGAAGCCAGAGA
235◀rPgl uGl yGl yLeuGl uGl uAl aI l eAspArgThrAl aLeuAl aI l eAspGl nTyrArgAspAl aVal Gl yLeuArgGl yCysAspI l ePheGl ySer Ph
6201 ACCTTCCATTTTCAACCATGATGTTGGGAAGGCAGGCATCCCATGAGTCAACCTAGGTCCTCACCATCTGGCATGGATGCCTTGAAGCCTGGCAATAG
202◀eArgGl yAsnGl uVal Me tI l eAsnP roLeuCysAl aAspGl yHi s Thr Val Val LeuAspGl uGl yAspP roMe tSer Al aLysLeuArgAl aPheLeu
6301 TTCAGCAGGGGCCAGGCCCTGGTGTCTTCCATCCAAGTCATCTGGTCCACCAGGCCAGCCTCCATCTGGTCTGGCCCTCTATCTGTGCTTGGCC
169◀Gl uAl aP roAl aLeuGl yGl nHi sGl uGl uAspLeuAspAspGl nAspVal l eLeuGl yAl aGl uMe tArgThr ArgAl aArgGl uI l eArgHi sLysAl aG
6401 TGGTGGTCAAAGGGCAGGTGGTGGTCAAGGGTGTGGAGTCTTCTCATGGCATCAGCCATGATTGACACTTTCTCAGCTGGAGCTAGGTGAGAGGAAA
135◀I nHi sAspPheP roCysThr Al aP roAspLeuThr Hi sLeuArgArgMe tAl aAspAl aMe tI l eSer Val l yLysGl uAl aP roAl aLeuHi sSer Ser Le
6501 GGAGTCTGCCAGGCACCTCACCTAGTAGGAGCCAGTCTTCCAGTCTGTGACCACATCAAGGACAGCTGCACAGGGGACCCAGTTGTTGCCAA
102◀uLeuAspGl nGl yP roVal Gl uGl yLeuLeuTrpAspArgGl yAl aGl uThr Val Val l eAspLeuVal Al aAl aCysP roLeuVal l eGl yThr Thr Al aLeu
6601 CCAGGAGAGTCTGGCAGCCTCATCTGGAGCTCATTGAGAGCCCACTGAGGTCTGTCTTTACAAAAGGACTGGCCTGCCTGGGCTGAAAGTCTGAAA
69◀TrpSer LeuArgAl aAl aGl uAspGl nLeuGl uAsnLeuAl aGl ySer LeuAspThr LysVal l ePheLeuVal l eP roArgGl yGl nAl aSer LeuArgPheV
6701 ACTGCTGCATCAGAGCAACCAATGGTCTGTGCTGCCAGTCTATAGCCAAACAGTCTCTCAACCAGGCAGCTGGAGAACCCTGCATGTAGGCCATCTTGT
35◀aAl aAl aAspSer CysGl yI l eThr Gl nGl nAl aTrpAspTyrGl yPheLeuArgGl uVal l eTrpAl aAl aP roSer Gl yAl aHi sLeuGl yAspGl nGl

BspHI (6804)

AseI (6889)

6801 CAATCATGATGGCTCCTCctgtcaggagaggagaagagaaggttagtacaattgCTATAGTGAGTTGTATTACTATGCTTATGATTAATTGCAAA
2◀uI l eMe t
6901 CTAGGGCTGCAGgttcatagtgccacttttctgcactgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTCACAG

HindIII (7015)

7001 GAGGGAGAAGCGAAGCTTTTTGCAAAAGCCTAGGCCTCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCAGGgGCCtGGCCTCTG

SpeI (7180)

7101 CATAAATAAAAAAATTAGTCAGCCTGGGGctgggggtgggggaggggtggggggccaactgggCAGGGGTGGGGGGCCACTAGTGGGACTATGGTTGCT

7201 GACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGAGCCTGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATAC
7301 TTCTGCCTGCTGGGAGCCTGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGGTTCCTTCAGCCTCAGAAGGTACCTAACCAAGTTCCTCT

EcoRI (7427)
7401 TTCAGAGGTTATTTTCAGGCCCTGCAGGAATTCAGTCAATATGTTCCACCCAAAAAAGCTGTTTGTTAACTTGTC AACCTCATTCTAAAAATGTATATAGAA
7501 GCCCAAAAGACAATAACAAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGAT

7601 AGACAGTGAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGGAAAAAT
7701 GATGGTCTTTTTCTTTTTAGAAAAACAGGAAATATATTTATATGTAAAAAATAAAAGGGAACCCATATGTCATACCATACACACAAAAAATTCAGT
7801 GAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTAGAAAAATAATATAGAAGCATGCCATCAA GACTTCAGTGTAGAGAAAAATTTCTTATG
7901 ACTCAAAGTCCTAACCCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTATTTTTAAAATTAAAAACCATTAAGAAAAGTCAGGCCAT
8001 AGAATGACAGAAAAATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGAAGTCTTACAAATCAGTAAAAAATAAACTAGACAA
8101 AAATTTGAACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGA

PacI (8237)
8201 GAAATATTAAGGCTAAGTAACATCTGTGGCTTAATTAAGTTATCCTAGGAAACCTTAAACCTTTAAAAGCCTTATATATTCTTTTTTTCTTATAAA

AseI (8340)
8301 ACTTAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACATGAAACATGAGAGCTTAGTACATTAG
8401 CCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAACATGAGAGCTTAGTACATTAACATGAGAGCTTAGTACATACTATCAACAGGTTG
8501 AACTGCTGATT