STOP

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pCpGfree-basic-Lucia

A Lucia® reporter plasmid without a promoter and devoid of CpG dinucleotides

Catalog # pcpgf-baslc

For research use only

Version 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-basic-Lucia plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

- Products are shipped at room temperature.
- Store lyophilized DNA at -20°C.
- Resuspended DNA is stable 6 months when stored at -20°C.
- Bacteria should be stored at -20°C and are stable up to 1 year.
- Store Zeocin™ at 4 °C or at -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. Viability of the lyophilized bacteria upon resuspension has been verified.

GENERAL PRODUCT USE

Methylation of CpG dinucleotides within the promoter/enhancer region of genes is often associated with transcriptional silencing. This epigenetic event plays an important role in the regulation of gene activity in normal and cancer cells. Recently, it has been confirmed that the activity of enhancers is correlated with DNA methylation[1].

InvivoGen provides pCpGfree-basic-Lucia, a secreted luciferase reporter plasmid that is completely devoid of CpG dinucleotides and lacks the entire promoter region. It contains a multiple cloning site upstream of the L ucia luciferase reporter gene. Expression of Lucia luciferase in cells transfected with this plasmid depends on the insertion of a functional promoter or enhancer/promoter cassette upstream from the Lucia luciferase gene. Thus, pCpGfree-basic-Lucia allows to study the effect of CpG methylation on a promoter, alone or combined with enhancer elements.

PLASMID FEATURES

All the elements required for replication and selection of the plasmid in *E. coli* and gene expression in mammalian cells are completely devoid of CpG dinucleotides. Furthermore, all Dam methylation sites (GATC) have been removed to prevent prokaryotic methylation.

Elements for expression in *E. coli*

• Origin of replication: The *E. coli* R6K gamma ori has been modified to remove all CpGs. This origin is activated by the R6K specific initiator protein π, encoded by the *pir* gene[2].

• Bacterial promoter: EM2K is a CpG-free version of the bacterial EM7 promoter.

• Selectable marker: The Zeocin™ resistance gene is a small gene (<400 bp) that contains numerous CpG dinucleotides. A synthetic new allele was created that contains no CpGs.

Elements for expression in mammalian cells

• Lucia lucifrase is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.

ORF size (from the ATG to the stop codon): 634 bp

• Polyadenylation signal: The polyadenylation signal is a CpG-free form of the late SV40 polyadenylation signal.

• MAR: Matrix attached regions (MARs) are sequences typically AT-rich that are able to form barriers between independently regulated domains[3]. pCpGfree plasmids contains two MARs, from the 5' region of the human IFN-β gene or β-globin gene that were chosen because they are naturally CpG-free. The MARs are placed between the bacterial and mammalian transcription units.

• MCS: The multiple cloning site contains several commonly used restriction sites for convenient cloning of a gene of interest.

5' Sda I, Bsp 120I, Avr II, Nsi I, Ppu 10I, Sca I, Bam HI, Spe I, Hind III 3'

Due to the presence of the R6Kγ origin of replication, pCpG plasmids can only be amplified in *E. coli* mutant strain expressing a *pir* mutant gene. They will not replicate in standard *E. coli* strains. Therefore, pCpG plasmids are provided with the *E. coli* GT115 strain, a pir mutant also deficient in *Dcm* methylation.

1. Hoivik EA. et al., 2011. DNA Methylation of Intronic Enhancers Directs Tissue-Specific Expression of Steroidogenic Factor 1/Adrenal 4 Binding Protein (SF-1/Ad4BP). Endocrinology. 152(5):2100-12. 22. **2. Wu F. *et al.* 1995.** A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. J Bacteriol. 177(22):6338-45. **3. 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_2O. 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 Luria-Bertani (LB) medium in the tube containing the paper disk. Let sit for 5 minutes.
2. Mix gently by vortexing for 1-2 minutes.
3. Streak bacteria taken from this suspension on a LB agar plate.
4. Place the plate in an incubator at 37˚C overnight.
5. Isolate a single colony and grow the bacteria in LB or terrific broth (TB) medium.
6. Prepare competent cells utilizing protocol of choice.

Plasmid amplification and cloning

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

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media.

TECHNICAL SUPPORT
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InvivoGen Europe: +33 (0) 5-62-71-69-39
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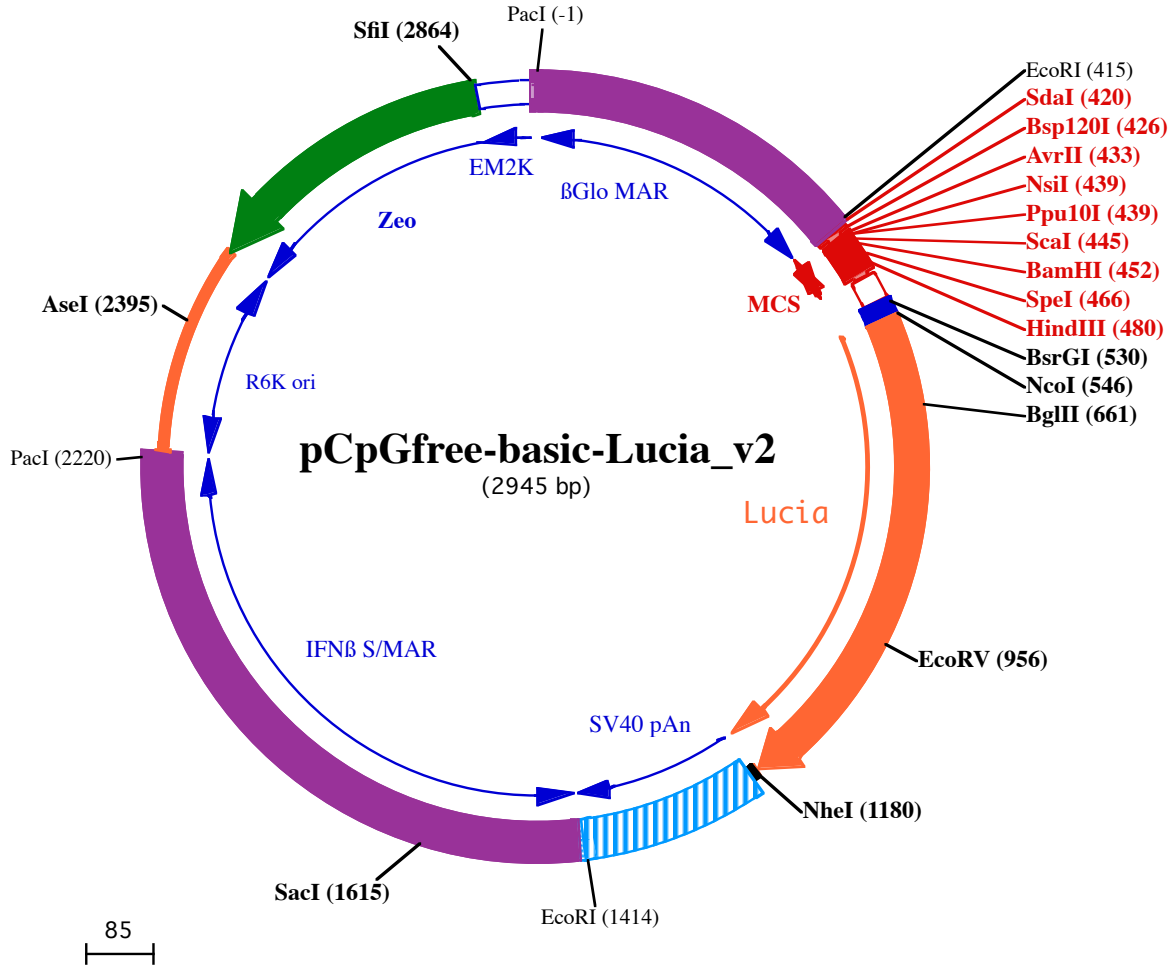
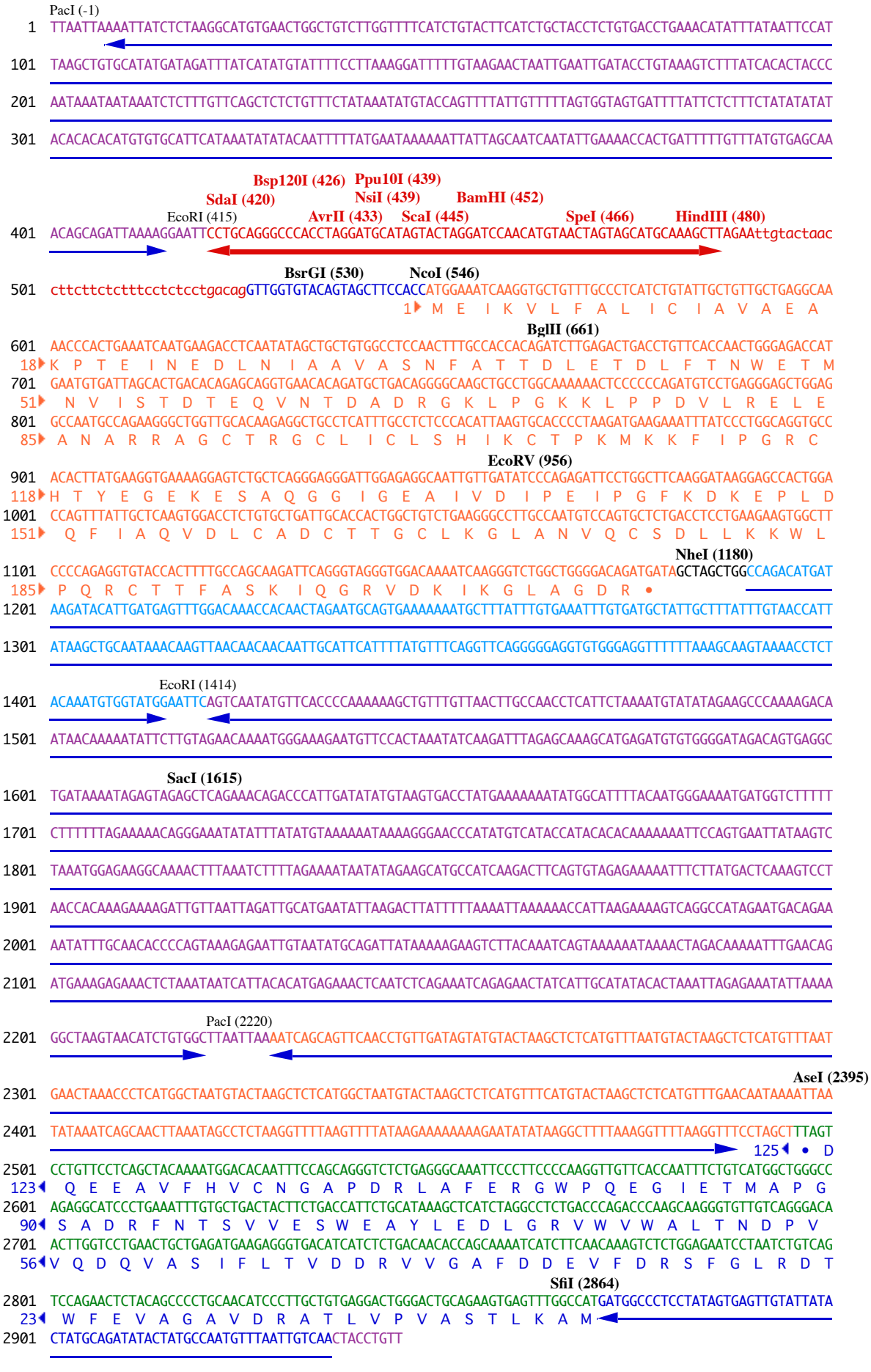
E-mail: info@invivogen.com

RELATED PRODUCTS

Product	Catalog Code
QUANTI-L uc™	rep-qlc1
ChemiComp GT115	gt115-11
pCpGfree-promoter-Lucia	pcpgf-promlc
pCpGfree-promoter (mSEAP)	pcpgf-prom
pCpGfree-basic (mSEAP)	pcpgf-bas


pCpGfree-basic-Lucia_v2
(2945 bp)
SfiI (2864)
PacI (-1)
EcoRI (415)
SdaI (420)
Bsp120I (426)
AvrII (433)
NsiI (439)
Ppu10I (439)
ScaI (445)
BamHI (452)
SpeI (466)
HindIII (480)
BsrGI (530)
NcoI (546)
BglII (661)
EM2K
ßGlo MAR
Zeo
MCS
AseI (2395)
R6K ori
PacI (2220)
Lucia
IFNß S/MAR
EcoRV (956)
SV40 pAn
NheI (1180)
SacI (1615)
EcoRI (1414)
85


PacI (-1)
1 TTAATTAAAATTATCTCTAAGGCATGTGAACTGGCTGTCTTGGTTTTCATCTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCC
201 AATAAATAATAAATCTCTTTGTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA
Bsp120I (426)
Ppu10I (439)
SdaI (420)
NsiI (439)
BamHI (452)
EcoRI (415)
AvrII (433)
ScaI (445)
SpeI (466)
HindIII (480)
401 ACAGCAGATTAAAAGGAATTCCTGCAGGGCCCACCTAGGATGCATAGTACTAGGATCCAACATGTAACTAGTAGCATGCAAAGCTTAGAAttgtactaac
BsrGI (530)
NcoI (546)
501 cttcttctctttcctctcctgacagGTTGGTGTACAGTAGCTTCCACCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAA
1 M E I K V L F A L I C I A V A E A
BglII (661)
601 AACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACCAACTGGGAGACCAT
18 K P T E I N E D L N I A A V A S N F A T T D L E T D L F T N W E T M
701 GAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCCTGAGGGAGCTGGAG
51 N V I S T D T E Q V N T D A D R G K L P G K K L P P D V L R E L E
801 GCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCC
85 A N A R R A G C T R G C L I C L S H I K C T P K M K K F I P G R C
EcoRV (956)
901 ACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTGGA
118 H T Y E G E K E S A Q G G I G E A I V D I P E I P G F K D K E P L D
1001 CCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGAAGAAGTGGCTT
151 Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D L L K K W L
NheI (1180)
1101 CCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATAGCTAGCTGGCCAGACATGAT
185 P Q R C T T F A S K I Q G R V D K I K G L A G D R •
1201 AAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATT
1301 ATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCT
EcoRI (1414)
1401 ACAAATGTGGTATGGAATTCAGTCAATATGTTCACCCCAAAAAAGCTGTTTGTTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCCAAAAGACA
1501 ATAACAAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGC
SacI (1615)
1601 TGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAAATATGGCATTTTACAATGGGAAAATGATGGTCTTTTT
1701 CTTTTTTAGAAAAACAGGGAAATATATTTATATGTAAAAAATAAAAGGGAACCCATATGTCATACCATACACACAAAAAAATTCCAGTGAATTATAAGTC
1801 TAAATGGAGAAGGCAAAACTTTAAATCTTTTAGAAAATAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCCT
1901 AACCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTAAAAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAA
2001 AATATTTGCAACACCCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAAACTAGACAAAAATTTGAACAG
2101 ATGAAAGAGAAACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGAGAAATATTAAAA
PacI (2220)
2201 GGCTAAGTAACATCTGTGGCTTAATTAAAATCAGCAGTTCAACCTGTTGATAGTATGTACTAAGCTCTCATGTTTAATGTACTAAGCTCTCATGTTTAAT
AseI (2395)
2301 GAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAACAATAAAATTAA
2401 TATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCCTAGCTTTAGT
125 • D
2501 CCTGTTCCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCCTTCCCCAAGGTTGTTCACCAATTTCTGTCATGGCTGGGCC
123 Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G
2601 AGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGCCTCTGACCCAGACCCAAGCAAGGGTGTTGTCAGGGACA
90 S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V
2701 ACTTGGTCCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAATCTGTCAG
56 V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D T
SfiI (2864)
2801 TCCAGAACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGCCCTCCTATAGTGAGTTGTATTATA
23 W F E V A G A V D R A T L V P V A S T L K A M
2901 CTATGCAGATATACTATGCCAATGTTTAATTGTCAACTACCTGTT