STOP

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pCpGfree-mcs

A cloning vector completely devoid of CpG dinucleotides

Catalog # pcpgf-mcs

For research use only

Version 21C31-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-mcs 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.
- Lyophilized DNA is stable 1 year when stored 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

InvivoGen has developed a new family of plasmids that are completely devoid of CpG dinucleotides. These plasmids yield high levels of transgene expression both *in vitro* and *in vivo*, and in contrast to CMV-based plasmids allow sustained expression *in vivo*.

pCpGfree plasmids contain elements that naturally lack CpG dinucleotides, were modified to remove all CpGs, or entirely synthesized such as genes encoding selectable markers or reporters.

pCpGfree plasmids represent valuable tools to study the effects of CpGs on gene expression *in vivo* and *in vitro*, using cell lines expressing TLR9, as well as their effects on the innate and acquired immune systems. Furthermore, for RNAi applications, InvivoGen has designed pCpG-siRNA a plasmid that allows long term production of siRNAs *in vivo*.

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[1].

• Bacterial promoter: E2MK 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

• Mammalian promoter: The CpG-free promoter combines the mouse CMV enhancer, the human elongation factor 1 alpha core promoter and 5'UTR containing a synthetic intron.

• 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[2]. 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' Bsr GI, Stu I, Bgl II, Acc65 I, Bsp 120, Eco O109I, Nco I, Nhe I 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. 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_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.

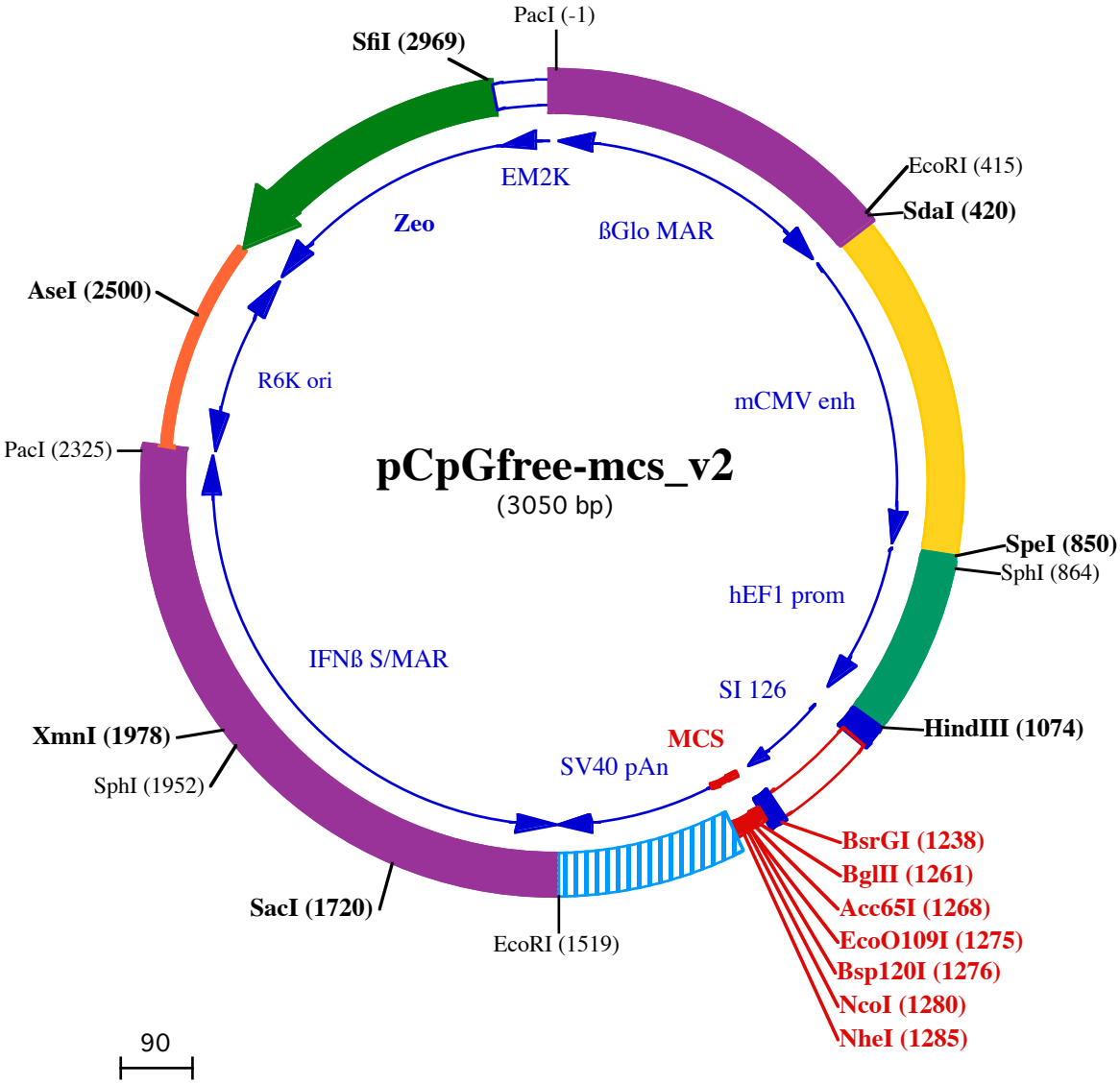
Plasmid amplification and cloning

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
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 3-622-34-80
E-mail: info@invivogen.com


pCpGfree-mcs_v2
(3050 bp)
PacI (-1)
SfiI (2969)
EcoRI (415)
SdaI (420)
SpeI (850)
SphI (864)
HindIII (1074)
BsrGI (1238)
BglII (1261)
Acc65I (1268)
EcoO109I (1275)
Bsp120I (1276)
NcoI (1280)
NheI (1285)
EcoRI (1519)
SacI (1720)
XmnI (1978)
SphI (1952)
PacI (2325)
AseI (2500)
EM2K
Zeo
ßGlo MAR
mCMV enh
hEF1 prom
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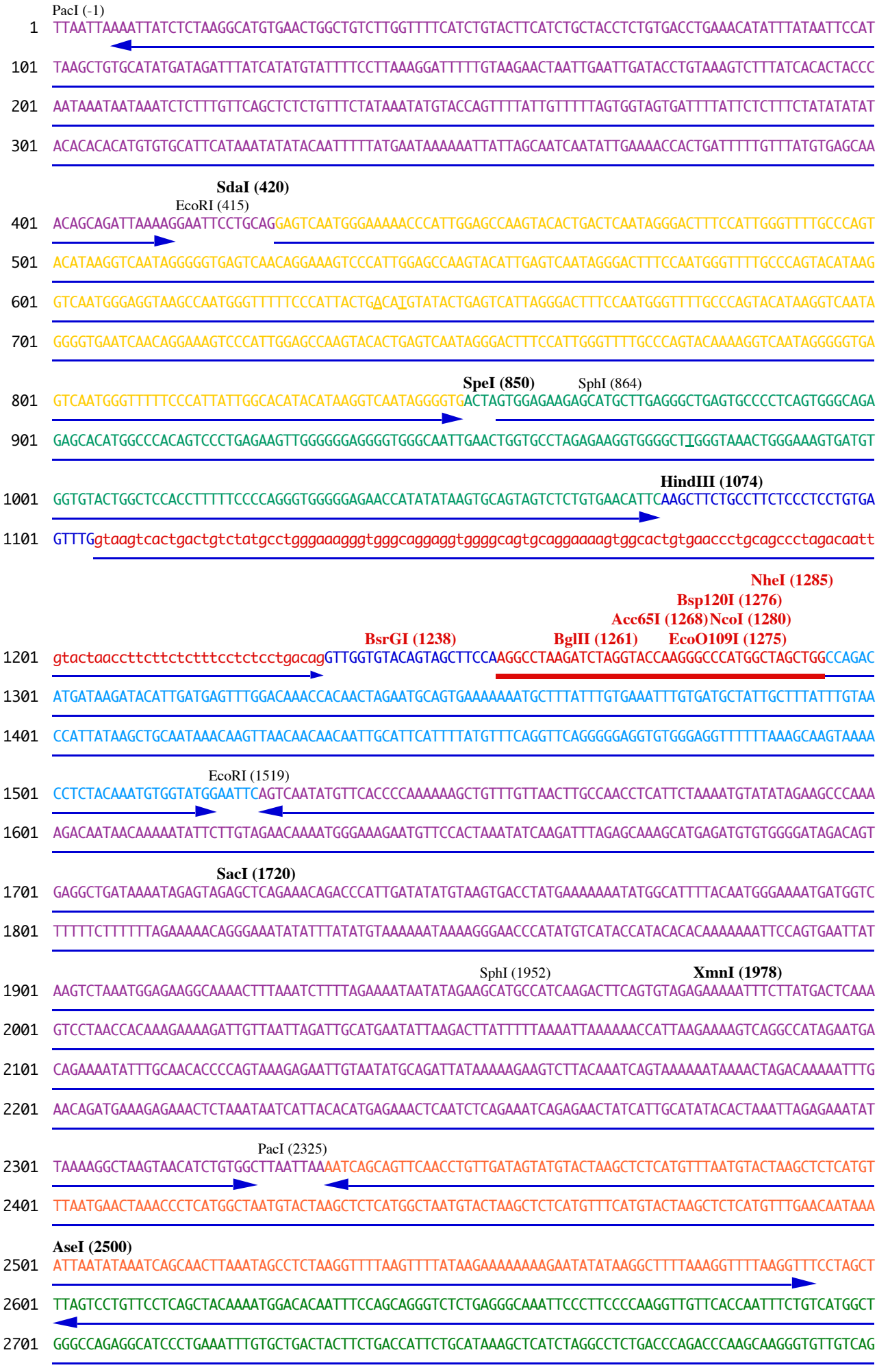
MCS
SV40 pAn
IFNß S/MAR
R6K ori
90


PacI (-1)
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201 AATAAATAATAAATCTCTTTGTTCAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
301 ACACACACATGTGTGCATTCATAAATATATACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTATGTGAGCAA
SdaI (420)
EcoRI (415)
401 ACAGCAGATTAAAAGGAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCCAGT
501 ACATAAGGTCAATAGGGGGTGAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCCAGTACATAAG
601 GTCAATGGGAGGTAAGCCAATGGGTTTTTCCCATTACTGACATGTATACTGAGTCATTAGGGACTTTCCAATGGGTTTTGCCCAGTACATAAGGTCAATA
701 GGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCCAGTACAAAAGGTCAATAGGGGGTGA
SpeI (850)
SphI (864)
801 GTCAATGGGTTTTTCCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTGCCCCTCAGTGGGCAGA
901 GAGCACATGGCCCACAGTCCCTGAGAAGTTGGGGGGAGGGGTGGGCAATTGAACTGGTGCCTAGAGAAGGTGGGGCTIGGGTAAACTGGGAAAGTGATGT
HindIII (1074)
1001 GGTGTACTGGCTCCACCTTTTTCCCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCCTGTGA
1101 GTTTGgtaagtcactgactgtctatgcctgggaaagggtgggcaggaggtggggcagtgcaggaaaagtggcactgtgaaccctgcagccctagacaatt
NheI (1285)
Bsp120I (1276)
Acc65I (1268)
NcoI (1280)
BsrGI (1238)
BglII (1261)
EcoO109I (1275)
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1301 ATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA
1401 CCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAA
EcoRI (1519)
1501 CCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTCACCCCAAAAAAGCTGTTTGTTAACTTGCCAACCTCATTCTAAAATGTATATAGAAGCCCAAA
1601 AGACAATAACAAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGT
SacI (1720)
1701 GAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAAATATGGCATTTTACAATGGGAAAATGATGGTC
1801 TTTTTCTTTTTTAGAAAAACAGGGAAATATATTTATATGTAAAAAATAAAAGGGAACCCATATGTCATACCATACACACAAAAAAATTCCAGTGAATTAT
SphI (1952)
XmnI (1978)
1901 AAGTCTAAATGGAGAAGGCAAAACTTTAAATCTTTTAGAAAATAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAA
2001 GTCCTAACCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAAATTAAAAAACCATTAAGAAAAGTCAGGCCATAGAATGA
2101 CAGAAAATATTTGCAACACCCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAAACTAGACAAAAATTTG
2201 AACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAAACTCAATCTCAGAAATCAGAGAACTATCATTGCATATACACTAAATTAGAGAAATAT
PacI (2325)
2301 TAAAAGGCTAAGTAACATCTGTGGCTTAATTAAAATCAGCAGTTCAACCTGTTGATAGTATGTACTAAGCTCTCATGTTTAATGTACTAAGCTCTCATGT
2401 TTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTTTCATGTACTAAGCTCTCATGTTTGAACAATAAA
AseI (2500)
2501 ATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAAAGAATATATAAGGCTTTTAAAGGTTTTAAGGTTTCCTAGCT
2601 TTAGTCCTGTTCCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCCTTCCCCAAGGTTGTTCACCAATTTCTGTCATGGCT
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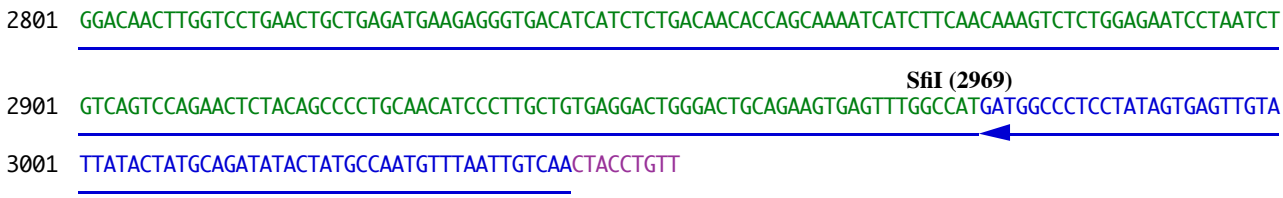
2801 GGACAACTTGGTCCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATCATCTTCAACAAAGTCTCTGGAGAATCCTAATCT
SfiI (2969)
2901 GTCAGTCCAGAACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGTTTGGCCATGATGGCCCTCCTATAGTGAGTTGTA
3001 TTATACTATGCAGATATACTATGCCAATGTTTAATTGTCAACTACCTGTT