pSELECT-NGFP-blasti

Plasmid for the expression of GFP-N-terminal tagged proteins

Catalog code: psetb-ngfp

https://www.invivogen.com/pselect-gfp-tag

For research use only

Version 20I11-MM

PRODUCT INFORMATION

Contents

- 20 µg of **pSELECT-NGFP-blasti** plasmid provided as lyophilized DNA
- 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

pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human ß-globin polyA terminates the transcription of the selectable marker.

pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. The GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm). pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest. The N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS).

PLASMID FEATURES

First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **MCS:** The multiple cloning site contains the following restriction sites:

5' - Bam HI, Eco 47III, Nco I, Nhe I - 3'

Each restriction site is compatible with many other enzymes.

- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

Second expression cassette

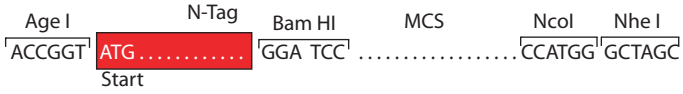
- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr:** Resistance to Blasticidin S is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

CLONING STRATEGY

For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Bam HI/Nhe I restriction site combination for cloning into plasmids with the N-Tag. For the plasmids with N-Tag, check whether your gene of interest and the start codon of the N-Tag are in the correct reading frame.

Note: The Bam HI restriction site is compatible with Bgl II.

If it is not possible to use the Nhe I restriction site, it is possible to another restriction site such as Nco I.



Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is downstream of the Bam HI site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.

TECHNICAL SUPPORT

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

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

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

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.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

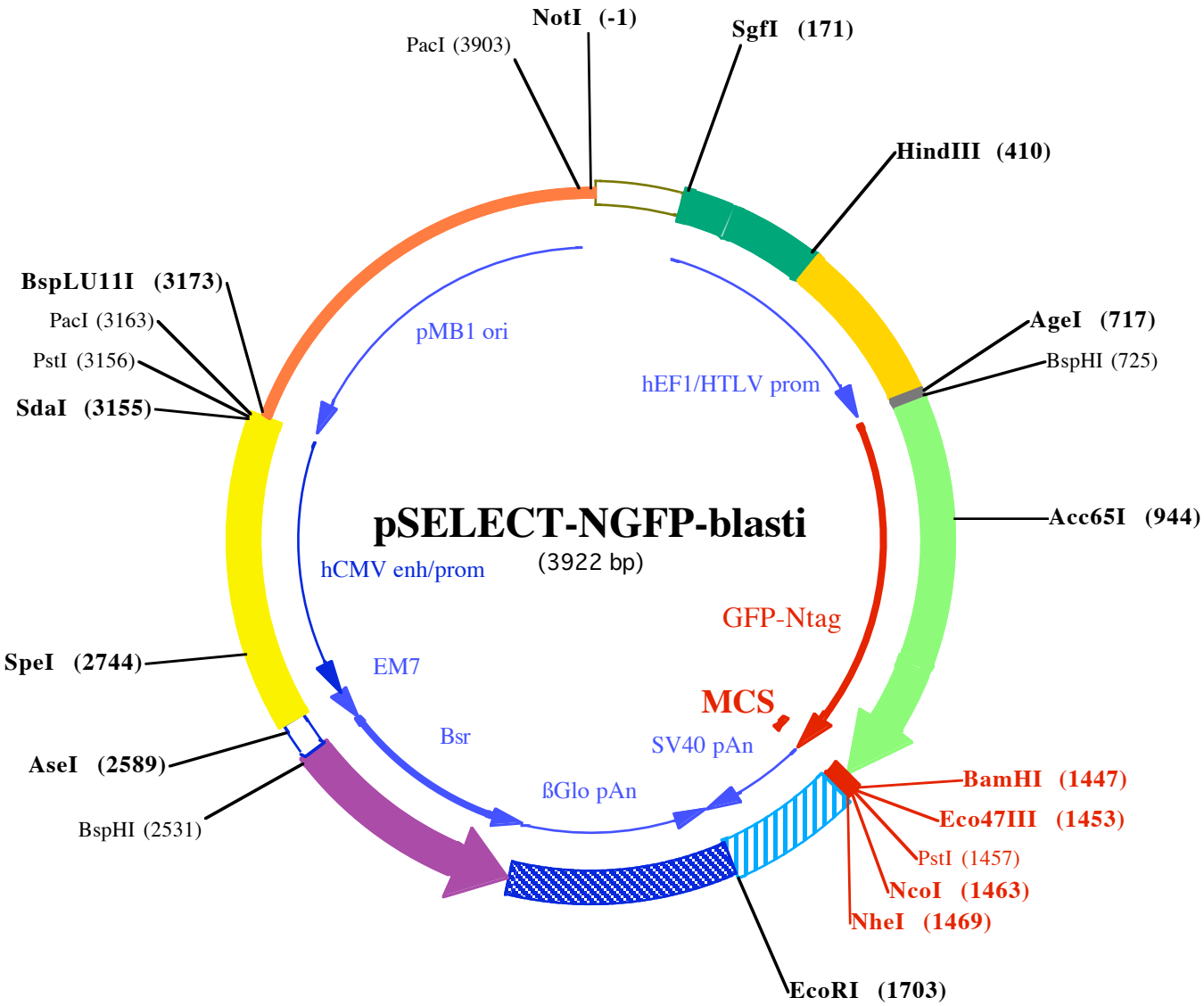
Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

References:

1. Kim, D.W. et al., 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 2: 217-223. **2. Takebe, Y. et al., 1988. S**R alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol. Cell Biol. 1: 466-472. **3. Carswell, S., & Alwine, J.C., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences.Mol. Cell Biol. 10: 4248-4258. **4. Yu J & Russell JE., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA.Mol Cell Biol, 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-CGFP-zeo	psetz-cgfp
pSELECT-NHA-blasti	psetb-nha
pSELECT-CHA-blasti	psetb-cha
pSELECT-NHA-zeo	psetz-nha
pSELECT-CHA-zeo	psetz-cha
pSELECT-CHis-blasti	psetb-chis
pSELECT-NHis-zeo	psetz-nhis
pSELECT-CHis-zeo	psetz-chis


NotI (-1)
SgfI (171)
PacI (3903)
HindIII (410)
BspLU11I (3173)
PacI (3163)
PstI (3156)
SdaI (3155)
AgeI (717)
BspHI (725)
pMB1 ori
hEF1/HTLV prom
pSELECT-NGFP-blasti
(3922 bp)
Acc65I (944)
hCMV enh/prom
GFP-Ntag
SpeI (2744)
EM7
MCS
Bsr
SV40 pAn
AseI (2589)
ßGlo pAn
BamHI (1447)
Eco47III (1453)
BspHI (2531)
PstI (1457)
NcoI (1463)
NheI (1469)
EcoRI (1703)

InvivoGen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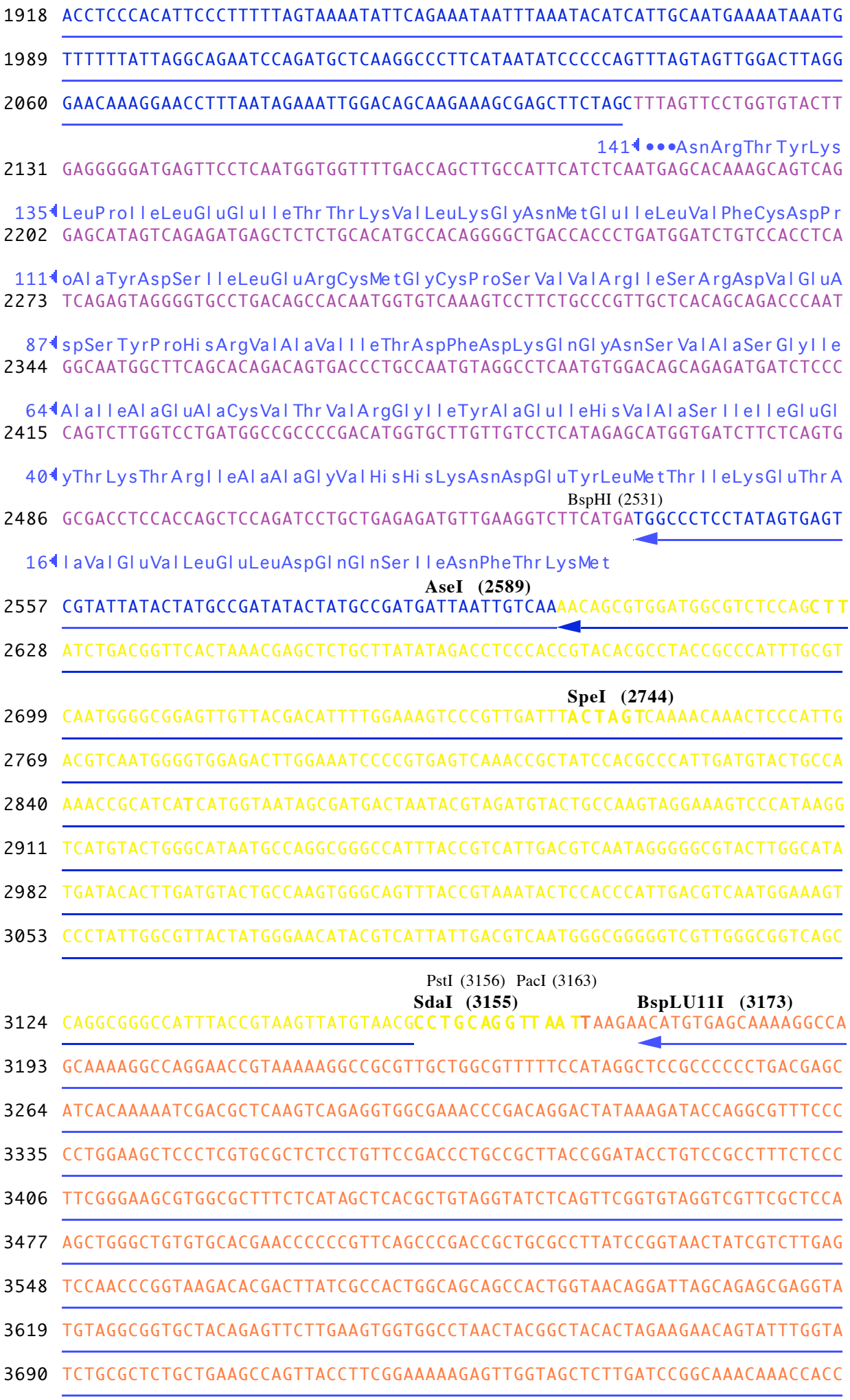
NheI (1469)
BamHI (1447)
NcoI (1463)
EcoRI (170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1989 TTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGG
2060 GAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTT
141 ●●●AsnArgThrTyrLys
2131 GAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAG
135 LeuProIleLeuGluGluIleThrThrLysValLeuLysGlyAsnMetGluIleLeuValPheCysAspPr
2202 GAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTCCACCTCA
111 oAlaTyrAspSerIleLeuGluArgCysMetGlyCysProSerValValArgIleSerArgAspValGluA
2273 TCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCAAT
87 spSerTyrProHisArgValAlaValIleThrAspPheAspLysGlnGlyAsnSerValAlaSerGlyIle
2344 GGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCC
64 AlaIleAlaGluAlaCysValThrValArgGlyIleTyrAlaGluIleHisValAlaSerIleIleGluGl
2415 CAGTCTTGGTCCTGATGGCCGCCCCGACATGGTGCTTGTTGTCCTCATAGAGCATGGTGATCTTCTCAGTG
40 yThrLysThrArgIleAlaAlaGlyValHisHisLysAsnAspGluTyrLeuMetThrIleLysGluThrA
BspHI (2531)
2486 GCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCTCCTATAGTGAGT
16 laValGluValLeuGluLeuAspGlnGlnSerIleAsnPheThrLysMet
AseI (2589)
2557 CGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTT
2628 ATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGT
SpeI (2744)
2699 CAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTG
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PstI (3156) PacI (3163)
SdaI (3155)
BspLU11I (3173)
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3548 TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
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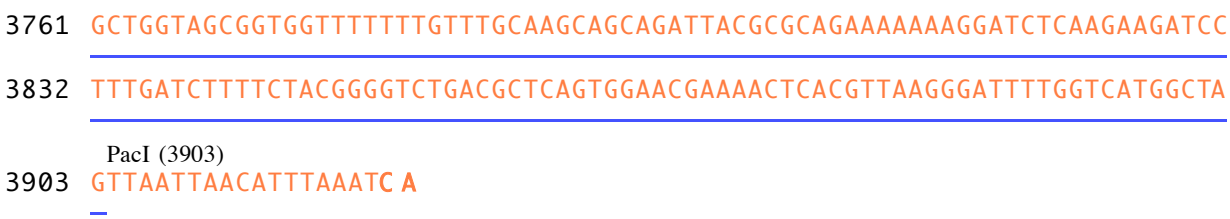
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PacI (3903)
3903 GTTAATTAACATTTAAATC A