

# pWHERE

An optimized vector for mouse and rat transgenesis

Catalog # pwhere

For research use only

Version 05B11SV

## PRODUCT INFORMATION

### Content:

pWHERE is provided as 20 µg of lyophilized DNA.

### Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA is stable for 12 months when stored at -20°C
- Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles

### 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

The pWHERE plasmid was designed for studies of temporal expression and tissue distribution of **your promoter of interest**, cloned within an insulated LacZ cassette, in transgenic mice and rats.

A multiple cloning site (MCS) has been added upstream of the LacZ gene for convenient cloning of your promoter of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the *E. coli* region is flanked on either side by the well cutting 8 bp-recognizing restriction enzyme *Pac* I that enables linearization and easy excision of the *E. coli* region.

## PLASMID FEATURES

• **mH19 insulators** on either side of the lacZ transcription unit. Both insulators are expected to protect the integrated transcriptional LacZ unit from negative as well as positive influences from neighboring sequences. Insulator elements can be functionally identified by their ability to shield promoters from regulators in a position-dependent manner or by their ability to protect adjacent transgenes from position effects. The fragment of the differential methylated region (DMD) located between the mouse *Igf2* and H19 acts as a powerful insulator<sup>1</sup>.

The enhancer blocking activity of the DMD fragment is dependent upon four responsive elements to the vertebrate enhancer-blocking protein CTCF<sup>2</sup>. Two mouse DMD fragments have been introduced in opposite orientation in the pWHERE plasmid to insulate your promoter of interest cloned upstream of the new CpG-free LacZ gene from the 5' and 3' adjacent regions at the integrated site in transgenic mice.

• **MCS:** The multiple cloning site, located downstream of the mH19 insulator, contains the following restriction sites:

*Sda* I, *Avr* II, *Bam* HI, *Xho* I, *Sma* I and *Nco* I

*Sda* I is compatible with *Nsi* I and *Pst* I

*Avr* II is compatible with *Nhe* I, *Spe* I and *Xba* I

*Bam* HI is compatible with *Bgl* II and *Bcl* I

*Xho* I is compatible with *Ava* I and *Sal* I

*Nco* I is compatible with *Bsp* HI and *Bsp* LU111

*Sma* I is compatible with any blunt end restriction enzyme

• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size but with the same activity as the longer Ori.

• **Amp:** The ampicillin resistance gene allows the selection of transformed *E. coli* carrying a pWHERE plasmid.

• **LacZ-ΔCpG NLS:** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. A nuclear localization signal of SV40 large T has been inserted in the 5' end of the *lacZ* gene to allow the targeting of the chimeric protein to the nucleus. To reduce the immunogenicity of this bacterial gene, InvivoGen has engineered a synthetic *lacZnls* gene that is entirely free of CpG motifs, whereas the wild type *lacZ* gene contains 298 CpG dinucleotides.

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

## EXPERIMENTAL OUTLINE

Clone your promoter into pWHERE mcs



Select and isolate recombinant pWHERE



Linearize recombinant pWHERE with *Pac* I



Purify *Pac* I/*Pac* I fragment containing your transgene



Prepare DNA for microinjection



Generate transgenic lines

## 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.

### **Pac I linearization of recombinant pWHERE:**

1- Digest 10 µg recombinant pWHERE plasmid with 1 to 5 units of *Pac* I restriction enzyme.

**Note:** *Pac* I may be purchased from New England Biolabs and used at 0.1-0.5 unit per µg plasmid DNA.

2- Incubate at 37°C for 1-2 hours.

3- Purify the fragment containing the LacZ expression cassette by agarose gel following your usual protocol.

### References:

1. Kaffer CR. et al. 2000. A transcriptional insulator at the imprinted H19/*Igf2* locus. *Genes Dev.* 14:1908-19.
2. Bell AC. and Felsenfeld G. 2000. Methylation of a CTCF-dependent boundary controls imprinted expression of the *Igf2* gene. *Nature.* 405:482-485.

## TECHNICAL SUPPORT

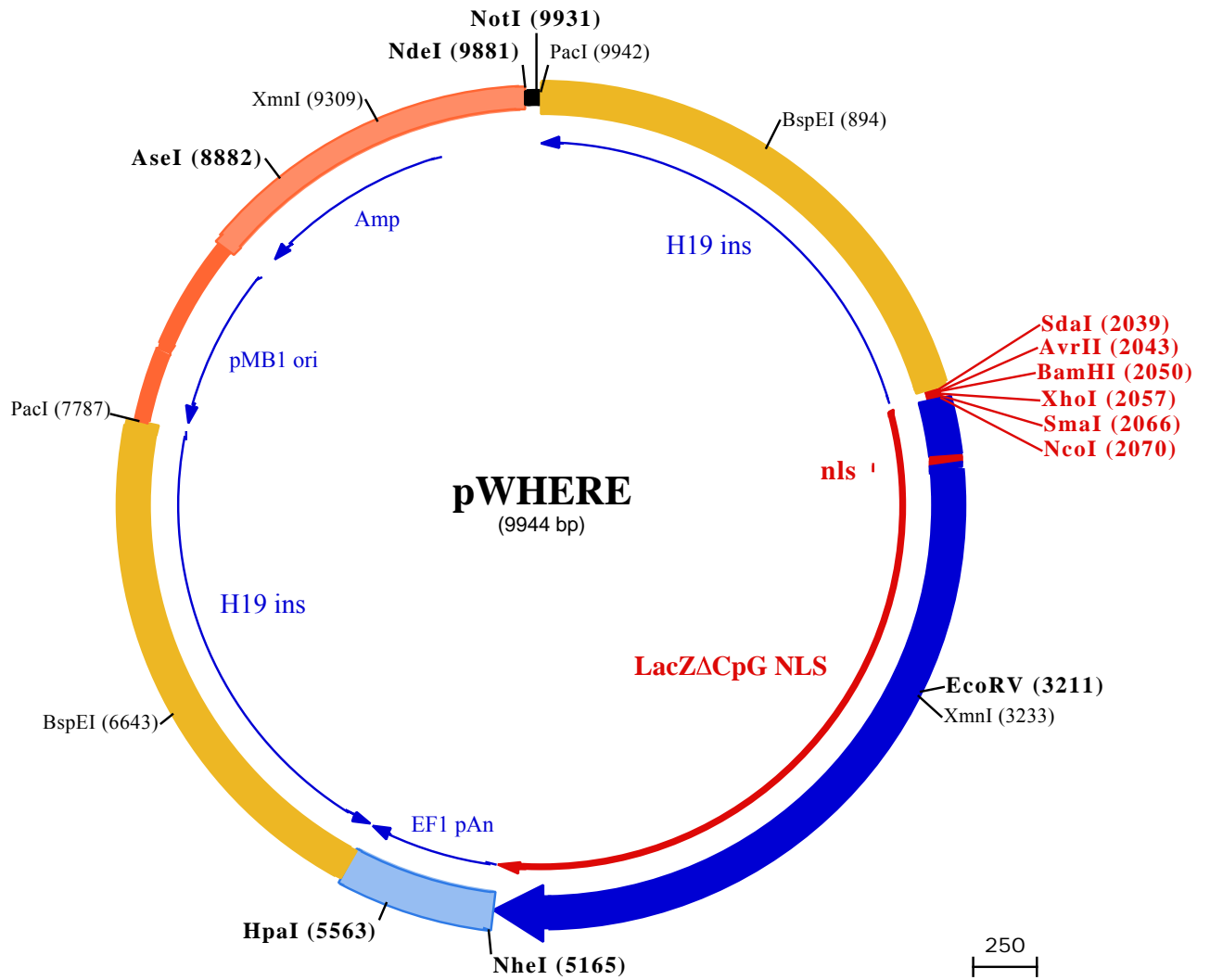
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1 CAGCTTTGTACAGCGGACCCCAACCTATGCCGCTCTGCCGAGCAATATGTAGTATTGTACTGCCACCACGCGGCATCGTCTGTCCATTTAGCTATAGC  
101 CAAATCTGCACAGCGTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAAGGAGCTACCCAAGAAATGTGTGTTGTACCACCCCATGACCCCTTA  
201 TGAATCATTGAATGCTATGCCTGAGTGACCCATGAGTTTGCCATAGGTGAACCGCAATTTTGGTACACCTCTATGATAAAGTATGTGGGACTGCACTGGTC  
301 GCTGCTCGGCAACTTCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTAGGGAATGAGTCAAGTTCTCTGGTTCAGTGTGTAAGGGAACCATTCCAGA  
401 GGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCTGGGATATTGCTGGGAATGAATCGCTCCCCCAAGTTGGCAGCATTGGGCCAC  
501 GATATATAGGAGTATGCTGCCACCGCGGTAGCATCCGTTCCCTTGTGACATAACAGCTTCTATGCCTTCTATAGTGAGCCACACTGGCTGGTTTT  
601 GGGTTTCAGTGACCAAAGGGACCCCTCCAGAAACACAAGTGTTCACACCTCTATAAACCATATCGACCACTGAGGCATAGCGGCTTCGGACATTGCTG  
701 TGGGCAACCCGAACCTTGGCCCTTGGACATTGTCATGGGCAACCTTAACTTTGGTGTCTCTGGAACATAATGTTAAGATGACAGTACCAGCGCAG  
801 CAATTTGGTCTTTCCACTCACAACGGCTTTTGTGCTTTCTGGCATCGAACCATGCACTGGTTTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGAAG  
901 GGCATAGGTGCTCTGCCTTCTGCTTTTAAACAAGGCTCTCCGGGACAGTGCAAAACAGGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAAAC  
1001 CAGCCAGGGTCTTACCACCTCTTCAATTGATTTGGGCTGACACCAAGGCTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCATTTGA  
1101 GAGAGAATGCAGTTTCAGAATTGTTTGCAGCCCTGAGCCGGAGATCATTAGCATCTGAACGCCCAATTAGAATACGAAAGCGCAAATCACCAGACTTTC  
1201 TTGTTGGCGGTTCTTAAGTGATTCTTTGGGTAGGGAGTCAAGTGTCTCATAGATGTGCAGAATTTGAGGACCATGCTTGTGGGCTGCAATTATGG  
1301 TACTGCAATACATTCATGATCACCACACATAGTAGCTATACTTCAATTTTACACAATAGCGCTGATGGCCCCAGAACCTATAAGTCAGATACCTGAG  
1401 ATAGCTCTTGAGAACGTTTTATCAAGGACTAGCATGAACCCCTGGCCTCATGAAGCCATGACTATGGGATCATAGATGGTGATAGGGAGAAAACTCAA  
1501 TCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCCGCTGCGGCAACCCCTGGTCTTTACACACAAAGGATTTTTCAGAGAGTAAG  
1601 CCGACCTTGTGATTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAATGCATGTGCTGCCCCCTAGTGGCATTGTGACCCCCCTGAGGACT  
1701 GAACTTGGGTGACCCACAGCATTGCCATTTGTAATTCCAATACCAGGGGTGGGGGGCTCTTTAGGTTTGGCGCAATCGATTTTGTGCCACCACGCGG  
1801 CAACTCCCGCTATAAACCCCAACTGATTACAGCAGAGCTCCAAGAATAGGGCATGGTCTCCTTGAGAATTCTTATGCCTCTGGATGCTCGTGTGAA  
1901 TGTAGCATGTTCTTTGAGTCTGGGTGTAAGTGCCTGACCGTCTATCCCGGACATGAAAATAGAATCTCTATTTTCTACCAACCTTTTCTTTCC

BspEI (894)

AvrII (2043) XhoI (2057) NcoI (2070)  
SdaI (2039) BamHI (2050) SmaI (2066)  
2001 TTGTGGTATTCCGGAACTGTAGGCAATGGCTCTCGAGGTCTAGGTGGATCTCTCGAGTCCCGGCCATGGACCTGTTGTGCTGCAAAGGAGAGAC  
2101 TGGGAGAACCCTGGAGTGACCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCTTTGGAGAACTCTGAGGAAGCCAGACAGACAGGCCAGCC  
11 MetAspProValValLeuGlnArgArgAsp  
11 TrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerG  
2201 AGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGCCTGGTCCCTGCCCTGAAGCTGTGCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCAGT  
44 InGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaVa  
2301 TCCAAGAAGAAGAGGAAAGTTGAGGCTGACACTGTTGTGGTGCCAAGCAACTGGCAGATGCATGGCTATGATGCCCCCTCTACACCAATGTCACCTAC  
77 IProLysLysLysArgLysValGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProI leTyrThrAsnValThrTyr  
2401 CCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGA  
111 ProI leThrValAsnProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnT  
2501 CCAGGATCATCTTGTGAGTCAACTCTGCCTTCCACCTCTGGTCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTGA  
144 hrArgI leI lePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAs  
2601 CCTCTCTGCCTTCTCAGAGCTGGAGAGAACAGGCTGGCTGTATGGTGTCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGTGGAGGATG  
177 pLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMet  
2701 CTGGCATCTCAGGATGTGAGCCTGTGCACAAGCCACCACCCAGATTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTG  
211 SerGlyI lePheArgAspValSerLeuLeuHisLysProThrThrGlnI leSerAspPheHisValAlaThrArgPheAsnAspPheSerArgAlaV  
2801 TGCTGGAGGCTGAGTGCAGATGTGTGGAGAAGCTCAGAGACTACCTGAGAGTCAAGTGTGAGCCTCTGGCAAGGTGAGACCAGGCTGGCCTCTGGCACAGC  
244 alLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAl  
2901 CCCCTTTGGAGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGTGAGATC  
277 aProPheGlyGlyGluI leI leAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluI le  
3001 CCAACCTCTACAGGCTGTTGTGGAGTGCACACTGCTGATGGCACCTGATTGAAGCTGAAGCCTGTGATGTTGGATTGAGAGAAGTCAGGATTGAGA  
311 ProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuI leGluAlaGluAlaCysAspValGlyPheArgGluValArgI leGluA  
3101 ATGGCCTGTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGAGTCAACAGGCATGAGCACCCTCTGCATGGCAAGTGTGGATGAAACAGACAAT  
344 snGlyLeuLeuLeuAsnGlyLysProLeuLeuI leArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMe  
EcoRV (3211) XmnI (3233)  
3201 GGTGAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTGTCAAGTGTCTCACTACCCCAACCACCTCTCTGGTACACCCTGTGTGACAGGTAT  
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3301 GGCCTGTATGTTGTTGATGAAGCAACATTTGAGACACATGGCATGGTGGCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCCTGCCATGCTGAGA  
411 GlyLeuTyrValValAspGluAlaAsnI leGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgLeuThrProAlaMetSerGluA  
3401 GAGTGACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAACCATGATGCTCTCTA  
444 rgValThrArgMetValGlnArgAspArgAsnHisProSerValI leI leTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyr

3501 CAGGTGGATCAAGTCTGTTGACCCAGCAGACCTGTGCAGTATGAAGGAGGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGG  
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3601 GTTGATGAGGACCAGCCCTTCCCTGCTGTGCCAAGTGGAGCATCAAGAAGTGGCTCTCTCGCTGGAGAGACCAGACCTGTATCCTGTGTGAATATG  
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611▶ LeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValT  
4001 CATCTGAGTACCTCTTCAGGCAGCTCTGACAATGAGCTCCTGCAGTGGATGGTGGCCTGGATGGCAAGCCTCTGGCTTCTGGTGGAGTGCCTCTGGATGT  
644▶ hrSerGluTrpLeuPheArgHisSerAspAsnGluLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspVa  
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677▶ IaIaProGlnGlyLysGlnLeuIleGluLeuProGluLeuProGlnProGlnProSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAla  
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4801 TACCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTGGGCTGCCTCTGTCTGACACTTACACCCTTATGTTTCCTCTGAGAATGACCCCTGAGGT  
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977▶ sArgHisLeuLeuHisAlaGluGluGlyThrTrpLeuAsnIleAspGlyPheHisMetGlyIleGlyGlyAspAspSerTrpSerProSerValSerAla

**NheI (5165)**

5101 GAGTTCAGTATCTGCTGGCAGGTACCACTATCAGCTGGTGTGGTGCCAGAAGTAAACCTGAGCTAGCATTATCCCTAATACCTGCCACCCCACTTTA  
1011▶ GluPheGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys...  
5201 ATCAGTGGTGAAGAACGGTCTCAGAAGTGTGGTTTCAATTGGCCATTAAGTTTAGTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAAACCT  
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**HpaI (5563)**

5501 GAAAGACATCTAATCTGTTTACGAATCTGAAACTTCTTGAAAATGTAATCTTGAGTAAACACTCTGGGTGGAGAATAGGGTGTGTTTCCCCCA  
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**BspEI (6643)**

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PacI (7787)

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8701 CCTGACTCCCCTGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCACGCTCACCGGCTCCAGA

AseI (8882)

8801 TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCT  
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XmnI (9309)

9301 TTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACCTGATCTTCAGCATC  
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9701 GCGTTCCGTGATGACGGTGAACCTCTGACACATGCAGTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAG

NdeI (9881)

9801 GGCGCGTCAGCGGTGTTGGCGGGTGTGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTAAGTGCACCATATGGATCTCGATAACAAAA

PacI (9942)

NotI (9931)

9901 AACCCCGCCCCGGCGGGTTTTTTGTTAGCGGCCGCTTAATTA