

# 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

Toll free (US): 888-457-5873

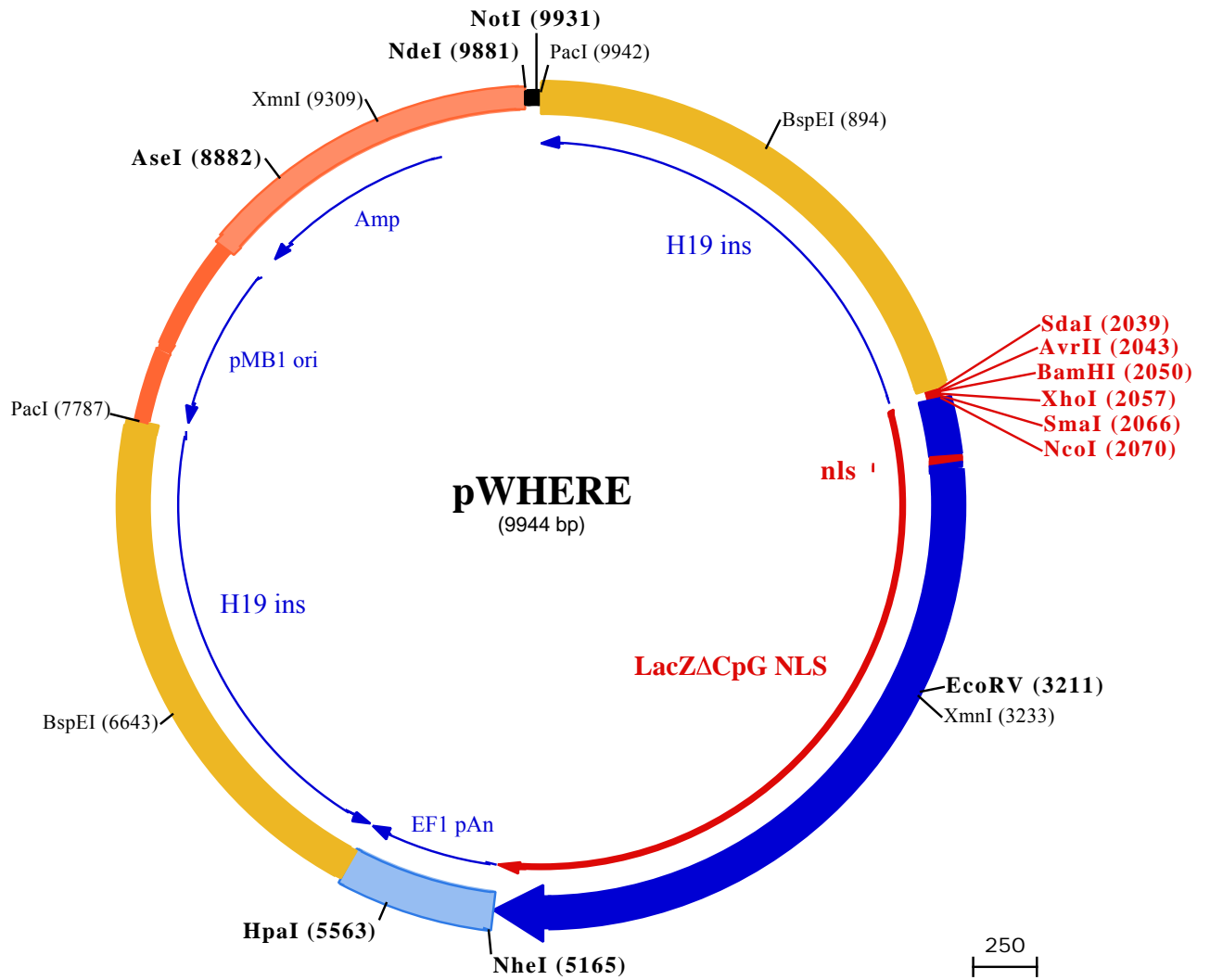
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1 CAGCTTTGTACAGCGGACCCCAACCTATGCCGGTCTGCCGAGCAATATGTAGTATTGTACTGCCACCACGCGGCATCGTCTGTCCATTTAGCTATAGC  
101 CAAATCTGCACAGCGTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAAGGAGCTACCCAAGAAATGTGTGTTGTACCACCCCATGACCCCTTA  
201 TGAATCATTGAATGCTATGCCTGAGTGACCCATGAGTTTGCCATAGGTGAACCGCAATTTTGGTACACCTCTATGATAAAGTATGTGGGACTGCACTGGTC  
301 GCTGCTCGGCAACTTCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTAGGGAATGAGTCAAGTTCTCTGGTTCAAGTGTGTAAGGGAACCATTCCAGA  
401 GGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCTGGGATATTGCTGGGAATGAATCGCTCCCCCAAGTTGGCAGCATTGGGCCAC  
501 GATATATAGGAGTATGCTGCCACCGCGGTAGCATCCGTTCCCTTGTGACATAACAGCTTCTATGCCTTCTATAGTGAGCCACACTGGCTGGTTTT  
601 GGGTTCAAGTGACCAAAGGGACCCCTCCAGAAACACAAGTGTTCACACCTCTATAAACCATATCGACCACTGAGGCATAGCGGCTTCGGACATTGCTG  
701 TGGGCAACCCGAACCTTGGCCCTTGGACATTGTCATGGGCAACCTTAACTTTGGTGTCTCTGGAACATAATGTTAAGATGACAGTACCAGCGCAG  
801 CAATTTGGTCTTTCCACTCACAACGGCTTTTGTGCTTTCTGGCATCGAACCATGCACTGGTTTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGAAG  
901 GGCATAGGTGCTCTGCCTTCTGCTTTTAAACAAGGCTCTCCGGGACAGTGCAAAACAGGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAAAC  
1001 CAGCCAGGGTCTTACCACCTCTTCAATTGATTTGGGGTGCACACCAAGGCTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCATTTGA  
1101 GAGAGAATGCAGTTTCAAGATTGTTTGCAGCCCTGAGCCGGAGATCATTAGCATCTGAACGCCCAATTAGAATACGAAAGCGCAATCACCAGACTTTC  
1201 TTGTTGGCGGTTCTTAAAGTATTCTTTGGGTAGGGAGTCAAGTGTCTCATAGATGTGCAGAATTTGAGGACCATGCTTGTGGGGTCTGCATTATGG  
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1401 ATAGCTCTTGAGAACGTTTTATCAAGGACTAGCATGAACCCCTGGCCTCATGAAGCCATGACTATGGGATCATAGATGGTGATAGGGAGAAAACTCAA  
1501 TCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCCGCTGCGGCAACCCCTGGTCTTTACACACAAAGGATTTTTCAGAGAGTAAG  
1601 CCGACCTTGTGATTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAATGCATGTGCTGCCCCCTAGTGGCATTGTGACCCCCCTGAGGACT  
1701 GAACTTGGGTGACCCACAGCATTGCCATTTGTGAATCCAATACCAGGGGTGGGGGGCTCTTTAGGTTTGGCGCAATCGATTTTGTGCCACCACGCGG  
1801 CAACTCCCGCTATAAACCCCAACTGATTACAGCAGAGCTCCAAGAATAGGGCATGGTCTCCTTGAGAATTCTTATGCCTCTGGATGCTCGTGTGAA  
1901 TGTAGCATGTTCTTTGAGTCTGGGTGTAAGTGCCTGCACGCTCATCCCCGGACATGAAAATAGAATCTCTATTTTCTACCAACCTTTTCTTTCC

BspEI (894)

2001 TTGTGGTATTCCGGAACTGTAGGCAATGGCTCTCGAGGTCTAGGTGGATCTCTCGAGTCCCGGCCATGGACCTGTTGTGCTGCAAAGGAGAGAC  
2101 TGGGAAACCCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCTTTGGAGAACTCTGAGGAAGCCAGACAGACAGGCCAGCC  
2201 AGCAGCTCAGGTCTCTCAATGGAGAGTGGAGTTTGCCTGGTCCCTGCCCTGAAGCTGTGCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCAGT  
2301 TCCAAGAAGAAGAGGAAAGTTGAGGCTGACACTGTTGTGGTGCAAGCAACTGGCAGATGCATGGCTATGATGCCCCCTCTACACCAATGTCACCTAC  
2401 CCCACTCTGTGAACCCCTTTTGTGCCACTGAGAACCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGA  
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2801 TGCTGGAGGCTGAGGTGCAGATGTGTGGAGAAGTACAGACTACCTGAGAGTCAAGTGTGAGCCTCTGGCAAGGTGAGACCAGGCTGGCCTCTGGCACAGC  
2901 CCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGTGAGATC  
3001 CCCAACCTCTACAGGCTGTTGTGGAGTGCACACTGCTGATGGCACCTGATTGAAGCTGAAGCCTGTGATGTTGGATTGAGAGAAGTCAGGATTGAGA  
3101 ATGGCCTGTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGAGTCAACAGGCATGAGCACCCTCTGCATGGCAAGTGTGGATGGAACAGACAAT  
3201 GGTGCAAGATATCTGCTAATGAAGCAGAACAACCTTCAATGCTGTCAAGTGTCTCACTACCCCAACCACCTCTCTGGTACACCCTGTGTGACAGGTAT  
3301 GGCCTGTATGTTGTTGATGAAGCAACATTTGAGACACATGGCATGGTCCCATGAACAGGCTCACAGATGACCCAGGTTGGCTGCCATGCTGAGA  
3401 GAGTGACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGTGATCATCTGGTCTCTGGCAATGAGTCTGGACATGGAGCCAAACCATGATGCTCTCTA  
344▶ rgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTy

AvrII (2043) XhoI (2057) NcoI (2070)

SdaI (2039) BamHI (2050) SmaI (2066)

1▶ MetAspProValValLeuGlnArgArgAsp

11▶ TrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerG

44▶ InGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaVa

77▶ IProLysLysLysArgLysValGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyr

111▶ ProIleThrValAsnProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnT

144▶ hrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAs

177▶ pLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMet

211▶ SerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspPheSerArgAlaV

244▶ alLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAl

277▶ aProPheGlyGlyGluIleIleAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIle

311▶ ProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluA

344▶ snGlyLeuLeuLeuAsnGlyLysProLeuLeuIleArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMe

EcoRV (3211)

XmnI (3233)

377▶ tValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyr

411▶ GlyLeuTyrValValAspGluAlaAsnIleGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluA

444▶ rgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTy

3501 CAGGTGGATCAAGTCTGTGACCCAGCAGACCTGTGCAGTATGAAGGAGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGG  
477▶ rArgTrpIleLysSerValAspProSerArgProValGlnTyrGluGlyGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArg  
3601 GTTGATGAGGACCAGCCCTTCCCTGCTGTGCCCAAGTGGAGCATCAAGAAGTGGCTCTCTCTGCCTGGAGAGACCAGACCTGATCCTGTGTGAATATG  
511▶ ValAspGluAspGlnProPheProAlaValProLysTrpSerIleLysLysTrpLeuSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrA  
3701 CACATGCAATGGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGGCAAGCCTTCAGACAGTACCAGAGTGGCAAGGAGATTGTGTGGGAGTGGTGGAA  
544▶ IahisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAs  
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3901 CTGGTCTTTGCAGACAGGACCCCTCACCTGCCTCACAGAGGCCAAGCACCAGCAACAGTTCTTCCAGTTCAGGCTGCTGGACAGACCATTGAGGTGA  
611▶ LeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrlIleGluValT  
4001 CATCTGAGTACCTTTCAGGCACCTCTGACAATGAGCTCCTGCAGTGGTGGCCTGGATGGCAAGCCTCTGGCTTCTGGTGAGGTGCCTCTGGATGT  
644▶ hrSerGluTrpLeuPheArgHisSerAspAsnGluLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspVa  
4101 GGCCCTCAAGGAAAGCAGCTGATTGAACTGCCTGAGCTGCCTCAGCCAGAGTCTGTGGCAACTGTGGCTAACAGTGAGGTGGTTCAGCCCAATGCA  
677▶ IAlaProGlnGlyLysGlnLeuIleGluLeuProGluLeuProGlnProGlnProLysArgTrpGlnPheAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAla  
4201 ACAGCTTGGTCTGAGGCAGGCCACATCTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCCTCTCTGTGACCCTGCCTGCTGCCTCTCATGCCATCCCTC  
711▶ ThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisAlaIleProH  
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4401 CAAGAAGCAGCTCCTCACCCCTCTCAGGACCAATTCACCAGGCTCCTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCT  
777▶ pLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspAsnAspIleGlyValSerGluAlaThrArgIleAspProAsnAla  
4501 TGGTGGAGAGGTGGAAGGCTGTGGACACTACCAGGCTGAGGCTGCCCTGCTCCAGTGCACAGCAGACACCCTGGCTGATGCTGTTCTGATCACCACAG  
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4601 CCCATGCTTGGCAGCACCAGGCAAGCCTGTTCATCAGCAGAAAAGCCTACAGGATTGAGCTCTGGACAGATGGCAATCACAGTGGATGGTGGAGGT  
844▶ IahisAlaTrpGlnHisGlnGlyLysThrLeuPheIleSerArgLysThrTyrArgIleAspGlySerGlyGlnMetAlaIleThrValAspValGluVa  
4701 TGCCTCTGCACACCTCACCCCTCAAGGATTGGCCTGAACGTCAACTGGCACAGGTGGCTGAGAGGTGAACCTGGCTGGGCTTAGGCCCTCAGGAGAAC  
877▶ IAlaSerAspThrProHisProAlaArgIleGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsn  
4801 TACCCTGACAGGCTGACAGCTGCCTGCTTGGACAGTGGGCTGCCTCTGTCTGACACTACACCCTTATGTGTTCCTTCTGAGAATGACCCCTGAGGT  
911▶ TyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgC  
4901 GTGGCACCAGGGAGCTGAACTATGGTCTCACCAGTGGAGGGGAGACTTCCAGTCAACATCTCCAGTACTCTCAGCAACAGCTCATGAAACCTCTCA  
944▶ ysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnIleSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHi  
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**NheI (5165)**

5101 GAGTTCAGTTATCTGCTGGCAGGTACCACTATCAGCTGGTGTGGTGGCCAGAAGTAAACCTGAGCTAGCATTATCCCTAATACCTGCCACCCCACTTTA  
1011▶ GluPheGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCysGlnLys•••

5201 ATCAGTGGTGAAGAACGGTCTCAGAACTGTTTGTTCATTTGCCATTAAAGTTTGTGTTAGTAAAGACTGGTTAATGATAACAATGCATCGTAAAACCT

5301 TCAGAAGGAAAGGAGAATGTTTTGTGGACCCTTTGGTTTTCTTTTTTCGCTGTGGCAGTTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGGA

5401 AACAACTTGACAAAATTTGTACAGAATTTTGAGACCCATTAAGTAAGTTAATGAGAACTGTGTGTTCTTTGGTCAACACCAGACATTTAGGT

**HpaI (5563)**

5501 GAAAGACATCTAATCTGTTTTACGAATCTGGAACTTCTTGAAATGTAATCTTGGTAAACACTCTGGGTGGAGAATAGGGTGTGTTTTCCCCCA

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6001 CCGCAATTTTGGTCACTCTATGATAAAGTATGTGGGACTGCACCTGGTGTCTGCTCGGCAACTTCCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTA

6101 GGGAAATGAGTCAAGTTCTCTGGTTCAGTGTGAAGGAAACCATTCCAGAGGTGCACACATCTTACCACCCTATGAATCCCTATTTGGGTGACCCTGGGA

6201 TATTGCTGGGAATGAATCGCTCCCCAAGTTGGCAGCATTGGGCCACGATATATAGGAGTATGCTGCCACCAGCGGGTAGCATCCGTTCCCTTGTGCT

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6401 CTTATAAACCATATCGACCACTGAGGCATAGCGGCTTGGGACATTTGCTGTGGGCAACCCGAACCTTGGCCCTTGGACATTTGTCATGGGCAACCTTAAC

6501 TTTGGCTGTCTCTGGAACATAATGTTTAAGATGACAGTACCAGCGCAGCAATTTGGTCTTTCCACTCACACCGGCTTTGTGCTTTCTGGCATCGAAC

**BspEI (6643)**

6601 ACATGCACCTGGTTTATGGGGTCTGAGACCAAGGAGACCATTCCGGAAGGCATAGGTGTCTGCTTTTAACAAGGCTCTCCGGGACAGTGCA

6701 AAACAGGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAACACCAGGCCAGGGTCTACCACCTCTCAATTGATTTGGGCTGACACCCAAAG

6801 CTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCATTTGAGAGAGATGCAGTTTCAAAATTGTTTGCAGCCCTGAGCGGAGATCATTAG

6901 CATCTGAACGCCCAATTAAGAAACGAAAGCGCAAATCACCAGACTTTCTTGTGGCGGTTCTTAAGTGATTTTGGGTAGGAGGTCAGGTGCTCTC

7001 ATAGATGTGCAGAAATTTGAGGACCATGCTTACTGGGCTGTGATATTGTTACTGCAATACATTCCATGATCACCACACATAGTACTATACTTCAATTTT

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PacI (7787)

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8701 CCTGACTCCCCTGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCACGCTCACCGGCTCCAGA

AseI (8882)

8801 TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCT  
8901 AGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTAGCT  
9001 CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTAAAGTTGGC  
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9201 TCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCTGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTAAAAAGTGTCTATCA

XmnI (9309)

9301 TTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATC  
9401 TTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTC  
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9601 GCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGC  
9701 GCGTTCCGTGATGACGGTGAACCTCTGACACATGCAGTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA

NdeI (9881)

9801 GGCGCGTCAGCGGTGTTGGCGGGTGTGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTAAGTGTGAGAGTGCACCATATGGATCTCGATAACAAAA

PacI (9942)

NotI (9931)

9901 AACCCCGCCCCGGCGGGTTTTTTGTTAGCGGCCGCTTAATTA