

pWHERE Kit

An optimized vector for mouse and rat transgenesis

Catalog # kwhere

For research use only

Version # 03G18-SV

PRODUCT INFORMATION

Content:

- 20 µg of pWHERE is provided as lyophilized DNA.
- 10 µg of pWHERE Control with the rat EF-1α promoter provided as lyophilized DNA.
- 4 pouches of *E. coli* Fast-Media® Amp (2 pouches for liquid and 2 for solid media)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be resuspended upon receipt and stored at -20°C (see Methods). Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
- Bacteria should be stored at -20°C. Bacteria are stable up to one year when properly stored.
- Store *E. coli* Fast-Media® Amp at room temperature. Fast-Media® is stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

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.

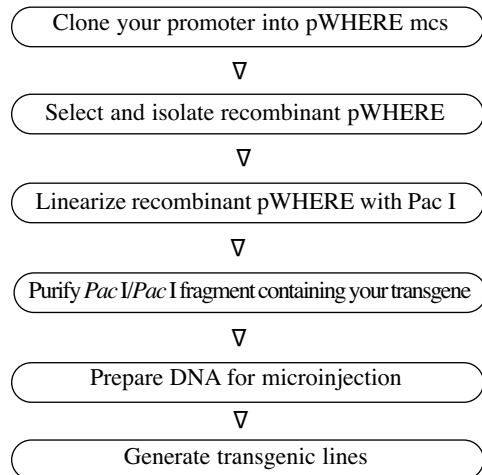
A multiple cloning site (MCS) has been added upstream of the LacZ gene for convenient cloning of your promoter of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the *E. coli* region is flanked on either side by 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 insulator¹. The enhancer blocking activity of the DMD fragment is dependent upon four responsive elements to the vertebrate enhancer-blocking protein CTCF². 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-
- **rEF-1α promoter (pWHERE Control):** EF-1α is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The promoter of this 'housekeeping' gene exhibits a strong activity, higher than viral promoters such as SV40 and RSV promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*.
- **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



TECHNICAL SUPPORT

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METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at $1\mu\text{g}/\mu\text{l}$, resuspend the DNA in $20\mu\text{l}$ of sterile H_2O . Store resuspended plasmid at -20°C .

Selection of bacteria with *E. coli* Fast-Media Amp:

E. coli Fast-Media® Amp is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Amp is a liquid or solid based medium with ampicillin and contains stabilizers.

E. coli Fast-Media® Amp can be ordered separately (catalog code # fas-am-b, fas-am-s).

Method:

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

Pac I linearization of recombinant pWHERE:

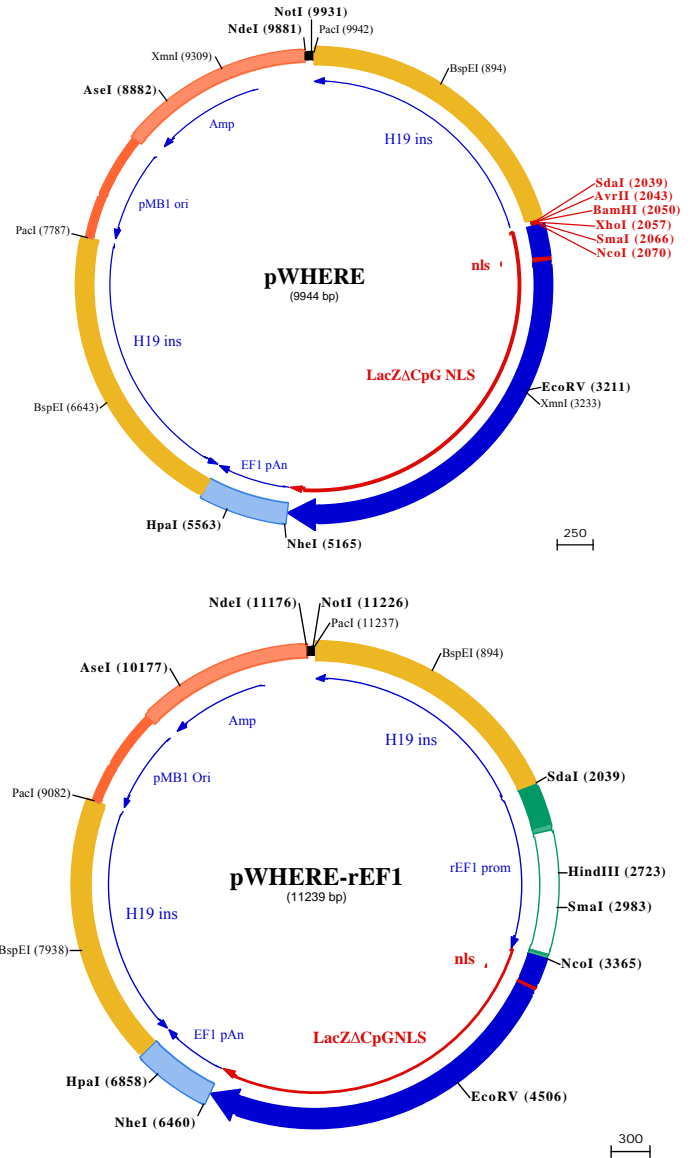
- 1- Digest $10\mu\text{g}$ recombinant pWHERE plasmid with 1 to 5 units of Pac I restriction enzyme.

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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pWHERE sequence

1 CAGGTTTGTACAGCGGACCCCAACCTATGCCGGCTCTGCCGAGCAATATGTAGTATTGTACTGCCACCACGCGGCATCGTCTGCCATTTAGCTATAGC
101 CAAATCTGCACAGCGTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAAGGAGCTACCCAAGAAATGTGTGTTGTACCACCCCATGACCCTTA
201 TGAATCATTGAATGCTATGCCTGAGTGACCCATGAGTTTGCCATAGTGTAACCGCAATTTTGGTCCACTCTATGATAAGTATGTGGACTGCACTGGTC
301 GCTGCTCGGCAACTTCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTAGGGAATGAGTCAAGTTCTCTGGTTTCACTGTGTAAGGGAACATTCCAGA
401 GGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCCTGGGATATTGCTGGGAATGAATCGCTCCCCCAAGTTGGCAGCATTGGGCCAC
501 GATATATAGGAGTATGCTGCCACCGCGGTAGCATCCGTTCCCTTGTGTCACATAACAGCTTCTATGCCTTCTATAGTGAGCCACACTGGCTGGTTTT
601 GGGGTTCACTGACCAAAGGGACCCCTCCAGAAACAAGTGTTCACACCTTTATAAACCATATCGACCACTGAGGCATAGCGGCTTCGGACATTGCTG
701 TGGGCAACCCGAACCTTTGGCCCTTGGACATTGTCATGGGCAACCTTAACCTTTGGTGTCTCTGGAACATAATGTTAAGATGACAGTCAACGCGCAG

BspEI (894)

801 CAATTTGGTCTTTCCACTCACAACGGCTTTTGTGCTTTCTGGCATCGAACCCATGCCTGGTTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGGAAG
901 GGCATAGGTGCTCTGCCTTCTGCTTTTAAACAAGGCTCTCCGGGACAGTGCAAAACAGGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAAAC
1001 CAGCCAGGGTCTTACCACCTTCTCAATTGATTTTGGGCTGACACCAAGGCTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCAATTGA
1101 GAGAGAATGCAGTTTACAATGTTTGCAGCCCTGAGCCGGAGATCATTAGCATCTGAACGCCCAATTAGAATACGAAAGCGCAATACCAGACTTTC
1201 TTGTTGGCGGTTCTTAAGTGATTCTTTGGGTAGGGAGTGCAGTGTCTCATAGATGTGCAGAATTTGAGGACCATGCTTAGTGGGGTCTGCATTATGG
1301 TACTGCAATACATTCCATGATCACCACACATAGTAGCTATACTTCAATTTTACACAATAGCGCTGATGGCCCCAGAACCTATAAGTCAGATACCTGAG
1401 ATAGCTCTTGAGAAGCTTTTATCAAGGACTAGCATGAACCCCTGGCCTCATGAAGCCCATGACTATGGGATCATAGATGGTGATAGGGGAGAAAACCTCAA
1501 TCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCCGCTGCGGCAACCTGGTCTTTACACACAAAGGATTCTTTGCAGAGAGTAAG
1601 CCGACCTTGTGATTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAATGCATGTGTCTGCCCCCTAGTGGCATTGTGACCCCCCTGAGGTACT
1701 GAACTTGGGTGACCCACAGCATTGCCATTTGTGAATCCAATACCAGGGGTGGGGGGCTCTTTAGGTTTGGCGCAATCGATTTTGTGCCACCACGCGG
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1901 TGTAGCATGTTCCCTTTGAGTCTGGGTGTAAGTGCCCTGCAGGCTCATCCCCGGACATGAAAATAGAATCTCTATTTTCTACCAACCTTTTTCTTTCC

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

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

2001 TTGTGGTATTTCGGAACTGTAGGCAATGGCTCCTCGAGGTCTAGGTGGATCCTCTCGAGTCCCGGCCATGGACCTGTGTGCTGCAAAGGAGAGAC
1 1 MetAspProValValLeuGlnArgArgAsp
2101 TGGGAAACCCTGGAGTGACCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCCTTTGGGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCC
11 TrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerG
2201 AGCAGCTCAGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTCCCTGCCCTGAAGCTGTGCTGAGTCTTGGCTGGAGTGTGACCTCCAGAGGCAGT
44 InGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaVa
2301 TCCAAGAAGAAGAGGAAAGTTGAGGCTGACACTGTTGTGGTGCAAGCAACTGGCAGATGCATGGCTATGATGCCCATCTACACCAATGTCACCTAC
77 IProLysLysLysArgLysValGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProl IeTyrThrAsnValThrTyr
2401 CCCATCACTGTGAACCCCTTTTGTGCCACTGAGAACCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGA
111 ProI IeThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnT
2501 CCAGGATCATCTTTGATGGAGTCAACTCTGCCTTCCACCTCTGGTGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGA
144 hrArgI IeI IePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAs
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177 pLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMet
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211 SerGlyI IePheArgAspValSerLeuLeuHisLysProThrThrGlnI IeSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaV
2801 TGCTGGAGGCTGAGGTGACAGTGTGTGGAAGCTCAGAGACTACCTGAGAGTCAAGTGCAGCTTGGCAAGGTGAGACCCAGGTTGGCCTCTGGCACAGC
244 aLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpLeuGlnGlyGluTrpGlnValAlaSerGlyThrAl
2901 CCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCAACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGCTGTGAGATC
277 aProPheGlyGlyGluI IeI IeAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluI Ie
3001 CCCAACCTTACAGGGCTGTGTGGAGCTGCACACTGCTGATGGCACCTGATTGAAGCTGAAGCCTGTGATGTTGGATTACAGAGAAGTCAGGATTGAGA
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3101 ATGGCCTGCTGTGCTCAATGGCAAGCCTGCTGCATCAGGGGAGTCAACAGGCATGAGCACCACCTCTGCATGGACAAGTGGATGGATGAACAGACAAT
344 snGlyLeuLeuLeuLeuAsnGlyLysProLeuLeuI IeArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMe
EcoRV (3211) XmnI (3233)
3201 GGTGCAAGATATCTGCTAATGAAGCAGAACAACCTCAATGCTGTGAGGTGCTCTCACTACCCCAACCACCTCTCTGGTACACCCTGTGTGACAGGTAT
377 tValGlnAspI IeLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyr
3301 GGCCTGTATGTTGTTGATGAAGCCAAACATTGAGACACATGGCATGGTGGCCATGAACAGGCTCACAGATGACCCAGTGGCTGCCATGTCTGAGA
411 GlyLeuTyrValValAspGluAlaAsnI IeGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluA
3401 GAGTGACCAGGATGGTGCAGAGAGACAGGAACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGGACATGGACCAACCATGATGCTCTCTA
444 rgValThrArgMetValGlnArgAspArgAsnHisProSerVal I IeI IeTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTy

7201 GAAGCCCATGACTATGGGATCATAGATGGTGATAGGGGAGAAAACCTCAATCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCGCCG
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7601 GGCATGGTCTCCTTGCAAGTCTTATGCCTCTGGATGCTCGTGTGAATGTAGCATGTTCTTTGAGTCTGGGTGTAAGTGCCCTGCACGCTCATCCC

PacI (7787)

7701 CCGGACATGAAAATAGAATCTCTATTTTCTACCAACCTTTTTCTTTCTTGTGGTGATTCCGGAACTGTAGGCAATGGCTTTAATTAATAAACCCGCTTC
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8701 CCTGACTCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCACGCTCACCGGCTCCAGA

AseI (8882)

8801 TTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCCGAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCT
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XmnI (9309)

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9601 GCACATTTCCCGAAAAGTGCCACCTGACGCTAAGAAACCATTTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTCGTCTCGC
9701 GCGTTCCGTGATGACGGTGAACCTCTGACACATGCAGTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA

NdeI (9881)

9801 GGCGCGTCAGCGGTGTTGGCGGGTGTGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTAAGTGTGAGAGTGCACCATATGGATCTCGATAACAAAA

PacI (9942)

NotI (9931)

9901 AACCCCGCCCGGGGGTTTTTTGTTAGCGGCCGCTTAATTA

pWHERE-rEF1 sequence

1 CAGCTTTGTCACAGCGGACCCCAACCTATGCCGCGTCTGCCGAGCAATATGTAGTATTGTACTGCCACCACGCGGCATCGTCTGTCCATTTAGCTATAGC
101 CAAATCTGCACACGCTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAAGGAGCTACCCAAGAAATGTGTGTTGTACCACCCATGACCCCTTA
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301 GCTGCTCGGCAACTTCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTAGGGAATGAGTCAAGTTCTCTGGTTCAGTGTGTAAGGGAACATTCCAGA
401 GGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCCTGGGATATTGCTGGGAATGAATCGTCCCCCAAGTTGGCAGCATTGGGCCAC
501 GATATATAGGAGTATGCTGCCACCGCGCGTAGCATCCGTTCCCTTGTGCACATAACAGCTTCTATGCCTTCTATAGTGAGCCACACTGGCTGGTTTT
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701 TGGGGAACCCGAACCTTTGGCCCTTGGACATTGTCATGGGCAACCTTAACTTTGGCTGTCTCTGGAACATAATGTTAAGATGACAGTACCAGCGCAG
801 CAATTTGGTCTTTCCACTCACAACGGCTTTTGTGCTTTCTGGCATCGAACCCATGCACTGGTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGAAG
901 GGCATAGGTGTCCTGCCTTCTGCTTTTAAACAAGGCTCTCCGGGACAGTGCAAAACAGGTGAACCCCACTTTGCCATAAGTACGATTATCTGCCACAAAC
1001 CAGCCAGGGTCTACCACCTCTCAATTGATTTTGGGTGACACCCAGGCTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCATTTGA
1101 GAGAGAATGCAGTTTCAGAAATGTTTGCAGCCCTGAGCCGGAGATCATTAGCATCTGAACGCCCAATTAGAATACGAAAGCGCAATCACCAGACTTTC
1201 TTGTTGGCGGTTCTTAAGTGATTCTTTGGGTAGGGAGGTCAGGTGTCCTCATAGATGTGCAGAATTTGAGGACCATGCTTAGTGGGGTCTGCATTATGG
1301 TACTGCAATACATTCATGATCACCACACATAGTAGCTATACTTCAATTTTACACAATAGCGCTGATGGCCCAGAACCTATAAGTCAGATACTGAG
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1601 CCGACCTTGTGATTTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAATGCATGTGTCCTGCCCCCTAGTGGCATTGTGACCCCTGAGGTACT
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1801 CAACTCCCGGTATAAACCCACAACCTGATTACGAGACGTCGAAGAATAGGGCATGGTCTCCTTGCAGAATCTTATGCCTCCTGGATGCTCGTGTGAA
1901 TGTAGCATGTTCTTTGAGTCTGGGTGTAAGTGCCCTGCACGCTCATCCCCGGACATGAAAATAGAATCTCTATTTTCTCACCAACCTTTTTCTTTCC
SdaI (2039)
2001 TTGTGGTGATTCCGGAACTGTAGGCAATGGCTCTGCGAGGCCCACTAGTGGAGCCGAGAGTAATTCATACAAAAGGAGGGATCGCCTTCGCAAGGGGAG
2101 AGCCAGGGACCGTCCCTAAATTTCTACAGACCCAAATCCCTGTAGCCGCCACGACAGCGGAGGAGCATGCGCCAGGGCTGAGCGGGGTAGATCA
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HindIII (2723)
2701 ct cact ctt aaggagcccat gaagctt acgt tggat aggaat ggaaggc caggagg ggcgact ggggccc gccgccttcggagc acatgtccgacgcca
2801 cctggatggggcgaggcctgtggctttccgaagcaatcgggctgagtttagcctacctgggccaatgtggccttagcactgggcacggctggcctggcg
SmaI (2983)
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NeoI (3365)
3301 gtcaaaagtattttctaaccgcttccagGTGTTGTGAAAGCCACCCTAATTCAAAGCAACCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGA
3401 GAACCTGGAGTGACCCAGCTCAACAGACTGGCTGCCACCCTCCCTTTGCCTCTTGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCAG
12 ▶ uAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGln
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46 ▶ LeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaValProL

3601 AGAAGAAGAGAAAGTTGAGGCTGACACTGTTGTGGTCCAAAGCAACTGGCAGATGCATGGCTATGATGCCCCATCTACACCAATGTCACCTACCCCAT
79 yslLysLysArgLysValGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProl IeTyrThrAsnValThrTyrProl I
3701 CACTGTGAACCCCTTTGTGCCACTGAGAACCCACTGGCTGTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGACAGG
112 eThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArg
3801 ATCATCTTTGATGGAGTCAACTCTGCCTTCCACCTCTGGTGAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGACCTCT
146 I IeI IePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeu
3901 CTGCCCTTCTCAGAGCTGGAGAGAACAGGCTGGCTGTCAATGGTGTCAAGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGGTGAGGATGCTCTGG
179 erAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGI
4001 CATCTTCAGGGATGTGAGCCTGCTGCACAGCCACCACCCAGATTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTGTGCTG
212 yI IePheArgAspValSerLeuLeuHisLysProThrThrGlnI IeSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeu
4101 GAGGCTAGGTCAGATGTGTGGAGAACCTCAGAGACTACCTGAGAGTCAAGTTCAGGCTTGGCAAGGTGAGACCAGGTTGGCTCTGGCACAGCCCT
246 GluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProP
4201 TTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCCTGACAGAGTCCACCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAA
279 heGlyGlyGluI IeI IeAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIeProAs
4301 CCTCTACAGGCTGTGGAGCTGCACACTGCCTGAGTGGCACCCTGATGAAGCTGAAGCCTGTGATGTTGGATTACAGAACTCAGGATGGAAATGGC
312 nLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuI IeGluAlaGluAlaCysAspValGlyPheArgGluValArgI IeGluAsnGly
4401 CTGCTGTGCTCAATGGCAAGCCTCTGCTCATCAGGGAGTCAACAGGCATGAGCACCACCTCTGCATGGCAAGTGTGATGATGAACAGACAATGGTGC
346 LeuLeuLeuLeuAsnGlyLysProLeuLeuI IeArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetValG

EcoRV (4506)

4501 AAGATATCTGTAATGAAGCAGAACAACCTTCAATGCTGTCCAGGTGCTCTACTACCCCAACCACCTCTCTGGTACACCCTGTGTGACAGGTATGGCCT
379 InAspI IeLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLe
4601 GTATGTTGTTGATGAAGCCAACATTGAGACACATGGCATGGTGCCATGAACAGGCTCACAGATGACCCAGGTGGCTGCCATGTCTGAGAGAGTG
412 uTyrValValAspGluAlaAsnI IeGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgVal
4701 ACCAGGATGGTGAGAGAGACAGGAACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGGACATGGAGCCAAACCATGATGCTCTCTACAGGT
446 ThrArgMetValGlnArgAspArgAsnHisProSerVal I IeI IeTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgT
4801 GGATCAAGTCTGTTGACCCAGCAGACCTGTGCAGTATGAAGGAGGTGGAGCAGACACCACAGCCACAGACATCATCTGCCCATGTATGCCAGGGTTGA
479 rpl IeLysSerValAspProSerArgProValGlnTyrAlaGlyGlyGlyAlaAspThrAlaThrAspI IeI IeCysProMetTyrAlaThrArgVal
4901 TGAGGACAGCCCTTCCCTGTGTGCCAAGTGGAGCATCAAGAAGTGGCTCTCTCTGCTGGAGAGACCAAGCCTCTGATCTGTGTAATGACAT
512 pGluAspGlnProPheProAlaValProLysTrpSerI IeLysLysTrpLeuSerLeuProGlyGluThrArgProLeuI IeLeuCysGluTyrAlaHis
5001 GCAATGGCAACTCTCTGGGAGGCTTTGCCAAGTACTGGCAAGCCTTCAGACAGTACCCCAAGGCTGCAAGGAGGATTTGTGTGGGACTGGGTGGACCAAT
546 AlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnS
5101 CTCTCATCAAGTATGAGAAATGGCAACCCCTGGTCTGCATATGGAGACTTGGTGGACACCCCAATGACAGGCTTCTGCAATGAATGGCTGGT
579 erLeuI IeLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuVa
5201 CTTTGCAGACAGGACCCCTCACCCCTGCCTCACAGAGGCCAAGCACCAGCAACAGTTCTTCCAGTTCAGGCTGTCTGGACAGACCATTGAGGTGACATCT
612 IPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGlnGlnPhePheGlnPheHisGlnLeuSerGlyGlnThrI IeGluValThrSer
5301 GAGTCACTCTCAGGACTCTGACAAATGAGCTCCTGCATGGATGGGAGACTTGGTGGACACCCCAATGACAGGCTTCTGCAATGAATGGCTGGT
646 GluTyrLeuPheArgHisSerAspAsnGluLeuLeuHisTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaP
5401 CTCAAGGAAAGCAGCTGATTGAACCTGCCTGAGCTGCCTCAGCCAGAGTCTGCTGGCAACTGTGGCTAACAGTGAAGGTTGGTTCAGCCCAATGCAACAGC
679 roGlnGlyLysGlnLeuI IeGluLeuProGluLeuProGlnProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAl
5501 TGTGCTGAGGACAGCCACATCTCTGCATGGCAGCAGTGGAGGCTGGCTGAGAACCTCTCTGTGACCCTGCCTGCTGCATGCCATCCCTCACCT
712 aTrpSerGluAlaGlyHisI IeSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisAlaI IeProHisLeu
5601 ACAACATCTGAAATGGAATCTGCAATTGAGCTGGGCAACAAGAGATGGCAGTTCACAGGCAGTCTGGCTTCTCTCAGATGTGGATTGGAGACAAGA
746 ThrThrSerGluMetAspPheCysI IeGluLeuGlyAsnLysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpI IeGlyAspLysL
5701 AGCAGCTCCTCACCCCTCAGGACCAATTCACAGGCTCCTCTGCAATGAGACTTGGAGTGTCTGAGGCCACCAGGCTGACCCAAATGGCTGGG
779 ysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspAsnAspI IeGlyValSerGluAlaThrArgI IeAspProAsnAlaTrpVa
5801 GGAGAGGTGGAAGGCTGCTGGCACTACCAGGCTGAGGCTGCCTGCTCCAGTGCACAGCAGACACCCTGGCTGATGCTGTTCTGATCACCACAGCCCAT
812 IGluArgTrpLysAlaAlaGlyHisTyrGlnAlaGluAlaLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeuI IeThrThrAlaHis
5901 GCTTGGCAGCAGCCAGCAGCTGTTTCATCAGCAGAAAGACCTACAGGACTGATGGCTCTGGACAGATGGCAATCAGAGTGGAGTTGGAGTTGGCT
846 AlaTrpGlnHisGlnGlyLysThrLeuPheI IeSerArgLysThrTyrArgI IeAspGlySerGlyGlnMetAlaI IeThrValAspValGluValAlaS
6001 CTGACACACCTCACCCCTGCAAGGATGGCTGAACTGCAACTGGCACAGGTGGCTGAGAGGTTGAACTGGCTGGGCTTAGGCCCTCAGGAGAATACCC
879 erAspThrProHisProAlaArgI IeGlyLeuAsnCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrPr
6101 TGACAGGCTGACAGCTGCCTGTTGACAGGTGGGACTGCCTCTGTGACATGTACACCCTTATGTGTTCCCTTCTGAGAATGGCCTGAGGTGTGGC
912 oAspArgLeuThrAlaCysPheAspArgTrpAspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGly
6201 ACCAGGGAGCTGAACTATGGTCTCACAGTGGAGGGAGACTCCAGTTCAACATCTCCAGTACTCTCAGCAACAGCTCATGAAAACCTCTCACAGGC
946 ThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnPheAsnI IeSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgH
6301 ACCTGCTCATGCAGAGGAGGAACCTGGCTGAACATTGATGGCTTCCACATGGGCAATGGAGGAGTACTCTTGGTCTCCTCTGTGCTGTGAGT
979 isLeuLeuHisAlaGluGluGlyThrTrpLeuAsnI IeAspGlyPheHisMetGlyI IeGlyGlyAspAspSerTrpSerProSerValSerAlaGluPh

NheI (6460)

6401 CCAGTTATCTGCTGGCAGGTACCCTATCAGCTGGTGTGGTCCAGAAGTAAACCTGAGCTAGCATTATCCCTAATACCTGCCACCCCACTTAAATCAG
1012 eGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValI IeTrpCysGlnLys•••••
6501 TGGTGAAGAAGCAGCTCAGAAGTGTGGTTTCAATGGCTTAAAGTATTAGTAGTAAAGACTGGTAAATGATAACATGCATCGTAAACCTTCAGA

6601 AGGAAAGGAGAAATGTTTGTGGACCACTTGGTTTTCTTTTTTGGCTGTGGCAGTTTTAAGTTATTAGTTTTTAAATCAGTACTTTTTAATGGAAACAA
6701 CTTGACCAAAAAATTTGTCACAGATTTTGGAGCCATTAAAAAAGTAAATGAGAAACCTGTGTCTTCTTGGTCAACACCGGACATTTAGGTTAGG

HpaI (6858)

6801 ACATCTAATTTCTGGTTTTACGAATCTGAAAACCTCTTGAATAATGTAATCTTGAAGTTAACTTCTGGTGGAGAATAGGGTTGTTTTCCCCCACAATA
6901 TTGGAAGGGGAAGGAATATCATTAAAGCTATGGGAGGTTTCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAAC
7001 AGGCCAAAACTGAGTCTTTGGGTTGCATAGAAAAGCTGCCTGCAGAGCTTTGTACAGCGGACCCCAACCTATGCCCGCTTGGCCAGCAATATGTAGTA
7101 TTGTACTGCCACCACCGGCATCGTCTGCCATTTAGCTATAGCCAAATCTGCACAGCGTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAA
7201 GGAGCTACCAAGAAATGTGTGTACCACCCCATGACCTTATGAATCATTGAATGCTATGCCTGAGTGACCCATGAGTTTGCATAGGTGAACCGCA
7301 ATTTTGGTCACTCTATGATAAAGTATGTGGACTGCCTGGCTGCTGGCAACTCTGGTCTTACCAGCCACTGACGATCTGGGCTGTGTAGGGAA

7401 TGAGTCAAGTTCTCTGGTTCAGTGTGTAAGGGAACCATTCCAGAGGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCCTGGGATATTG
7501 CTGGGAATGAATCGCTCCCCCAAGTTGGCAGCATTGGGCCACGATATATAGGAGTATGCTGCCACCGCGCGGTAGCATCCGTTCCCTTGTGCACATA
7601 ACAGCTTCTATGCCTTCTATAGTGAGCCACACTGGCTGGTTTTGGGGTTCAGTGACCAAAGGACCCTCCAGAAACACAAGTGTCCAACCTCTTAT
7701 AAACCATATCGACCACTGAGGCATAGCGGCTTCGGACATTGCTGTGGGCGAACCAGAACTTTGGCCCTTGGACATTGTCATGGGCAAACTTAACCTTGG
7801 CTGTCTCTGGAACATAATGTTAAGATGACAGTCACCAGCGCAGCAATTTGGTCTTTCCACTCACAAACGGCTTTTGTGCTTCTGGCATCGAACCATG

BspEI (7938)

7901 CACTGGTTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGAAGGGCATAGGTGTCCTGCTTTTAAACAAGGCTCTCCGGGACAGTGCAAAACA
8001 GGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAAACCAGCCAGGGTCTACCACCTCTCAATTGATTTTGGGCTGACCCCAAGGCTTGA
8101 TGTAGGATTCTCAGCTGCCAAGCTGGCAGCTGACCCCATGAGAGAGAATGCAGTTTCAGAATTGTTGCAGCCCTGAGCCGGAGATCATTAGCATCT
8201 GAACGCCCAATTAGAATACGAAAGCGCAATCACCAGACTTCTTGTGGCGGTTCTTAAGTGATTCTTGGGTAGGAGGTCAGGTGCTCTCATAGA
8301 TGTGCAGAAATTTAGGACCATGCTTAGTGGGGTCTGCATTATGGTACTGCAATACATCCATGATCACCACACATAGTAGCTATACTTCAATTTTCACAC
8401 AATAGCGCTGATGGCCCAAGCCCTATAAGTCAGATACCTGAGATAGCTCTTGAGAACGTTTTATCAAGGACTAGCATGAACCCCTGGCCTCATGAAGC
8501 CCATGACTATGGGATCATAGATGGTATAGGGGAGAAACTCAATCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCGCCGTGGC
8601 CAACCTGGTCTTTACACACAAAGGATTCTTTGCAGAGAGTAAGCCGACCTTGTGATTTGGGAGTCCGAGTCCACGAGTACCAGCTAGAAAATGCAT
8701 GTGTCTGCCCCCTAGTGGCATTGTGACCCCTGAGGTAAGTGGGTGACCCACAGCATTGCCATTTGTGAATCCAATACCAGGGTGGGGG
8801 GGCTCTTTAGGTTGGCGCAATCGATTTTGTGCCACCACGCGCAACTCCCGGTATAAACCACAACTGATTACGACAGCGTCCAAGAATAGGGCAT
8901 GGTCTCCTTGAGAAATCTTATGCCTCCTGGATGCTCGTGTGAATGTAGCATGTTCTTTGAGTCTGGGTGTAAGTGCCTGCACGCTCATCCCCGGA

PacI (9082)

9001 CATGAAAATAGAATCTCTATTTTCTACCAACCTTTTCTTCTTCTTGTGGTGATTGCGGAACCTGATAGCAATGGCTTTAATTAATAAACCCGCTTCGGCGG
9101 GTTTTTTATGCATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGCGTGTGGCGTTTTCCATAGGCTCGCCCCCTGACGAGC
9201 ATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGTCCCTCGTGCCTCTCTGT
9301 TCCGACCTGCCGCTTACCAGTACCTGTCCGCTTTCTCCCTCGGAAGCGTGGCGTTTTCTCATA GCTCAGCTGTAGGTATCTCAGTTCGGGTAG
9401 GTCGTTGCTCAAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGGCGCTTATCCGGTAACATATCGTCTGAGTCCAACCCGGTAAGAC
9501 ACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCCGGTGTACAGAGTCTTGAAGTGGTGGCCTAACTACGG
9601 CTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACCACCGCT
9701 GGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGT
9801 GGAACGAAAACCTACGTTAAGGATTTTGGTCTAGAGATTATCAAAAAGGATCTTACCTAGATCCTTTAAATTAATAAATGAAGTTTAAATCAATCTA
9901 AAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTCCATAGTTGCCTGA
10001 CTCCCCGCTGTGATAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCAAGTGTGCAATGATACCGGAGACCCACGCTCACCGCTCCAGATTTAT

AseI (10177)

10101 CAGCAATAAACAGCCAGCCGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATAATTGTTGCCGGGAAGCTAGAGT
10201 AAGTAGTTCGCCAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTACAGGCATCGTGTGTCACGCTCGTCTTGGTATGGCTTCACTCAGCTCCGGT
10301 TCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTAAAGTTGGCCGAG
10401 TGTTATCACTCATGTTATGGCAGCACTGCATAATTCTTACTGTGATGCCATCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATT
10501 CTGAGAATAGTGTATGGGCGACCGAGTTGCTCTTCCCGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTAAAAAGTCTCATCATTGGA
10601 AAACGTTCTTCGGGCGCAAACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCCAGCATCTTTTA
10701 CTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTCT
10801 TTTTCAATATTATTGAAGCATTATCAGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGTTCCGCGCACA
10901 TTTCCCGAAAAGTGCACCTGACGTCTAAGAAACATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACAGGCCCCTTTCGTCTGCGCGGTT
11001 TCGGTGATGACGGTGAACCTCTGACACATGCAGCTCCCGGAGCGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCGTCAGGGCGC

NdeI (11176)

11101 GTCAGCGGTGTTGGCGGTGTCGGGGTGGCTTAACTATGCGGCATCAGAGCAGATTGACTGAGAGTGCACCATATGATCTCGATAACAAAAACCC

PacI (11237)

NotI (11226)

11201 CGCCCCGGCGGGTTTTTTGTTAGCGGCGGCTTAATTA