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 insulator[1].
The enhancer blocking activity of the DMD fragment is dependent upon four responsive elements to the vertebrate enhancer-blocking protein CTCF[2]. 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* LU11I
Sma I is compatible with any blunt end restriction enzyme-
• **rEF-1α promoter(pWHERE Control):** EF-1a 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

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

TECHNICAL SUPPORT
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com

InvivoGen
3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1μg/μl, resuspend the DNA in 20μl 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:

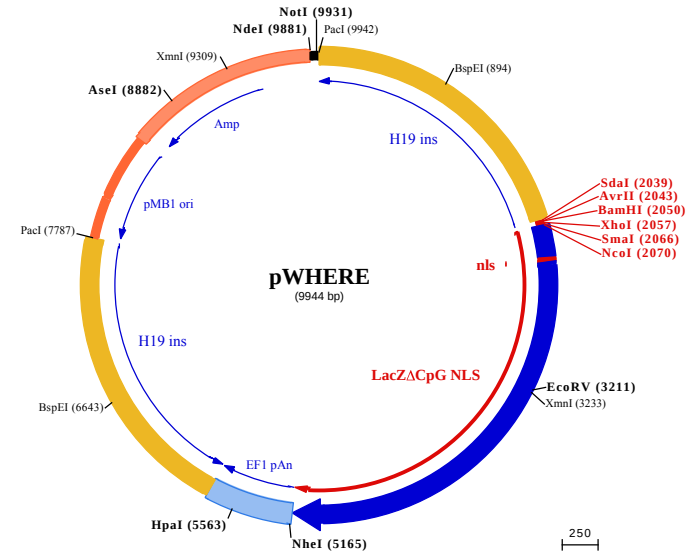
1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
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.*

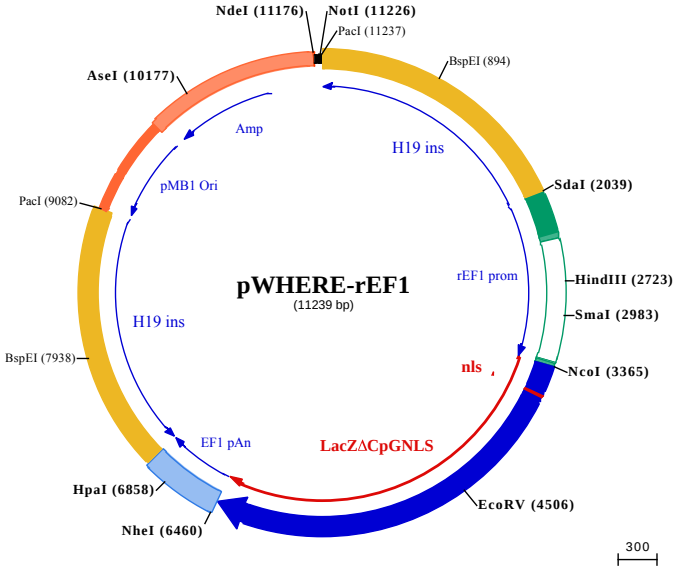
Pac I linearization of recombinant pWHERE:

1- Digest 10 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.



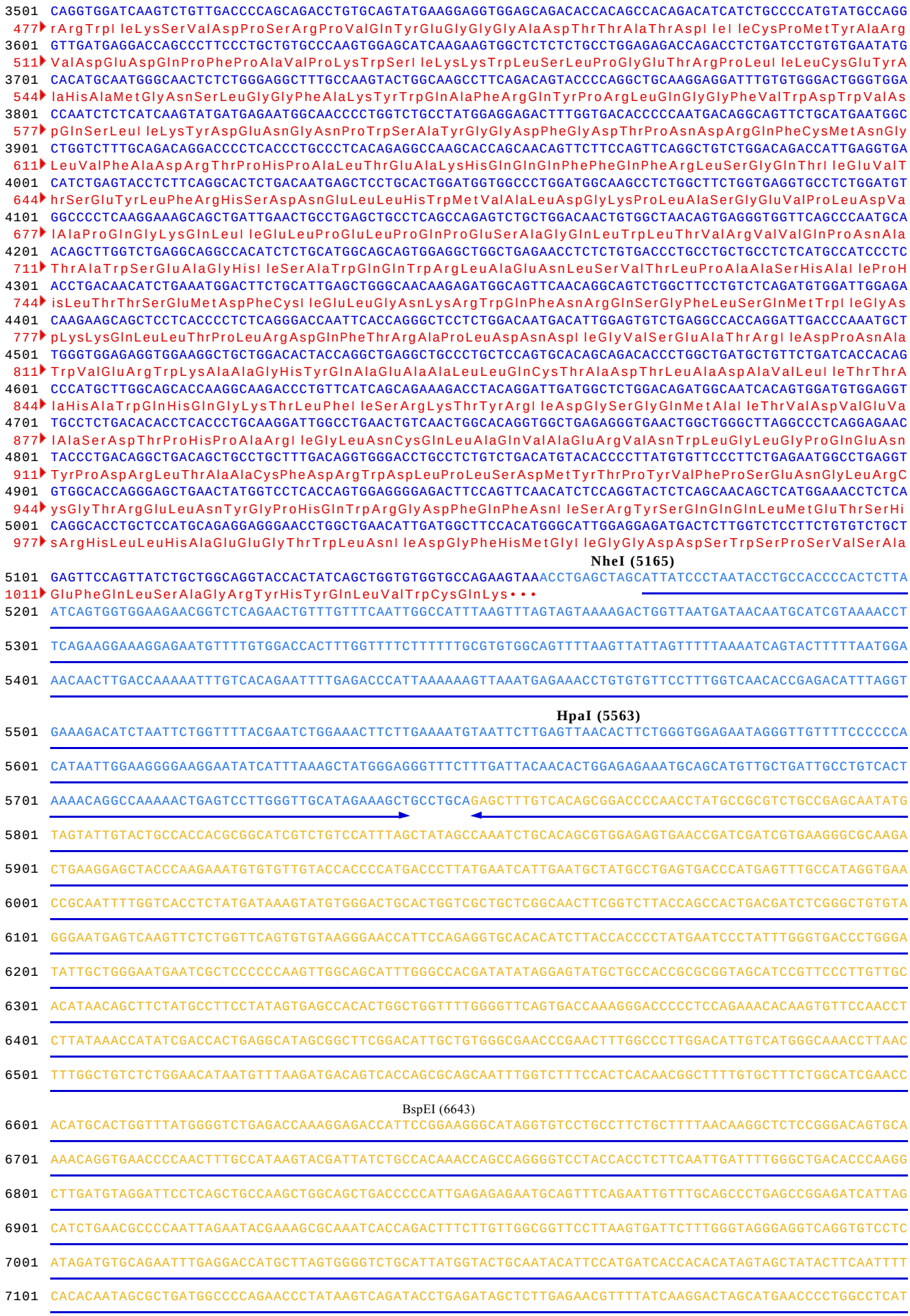


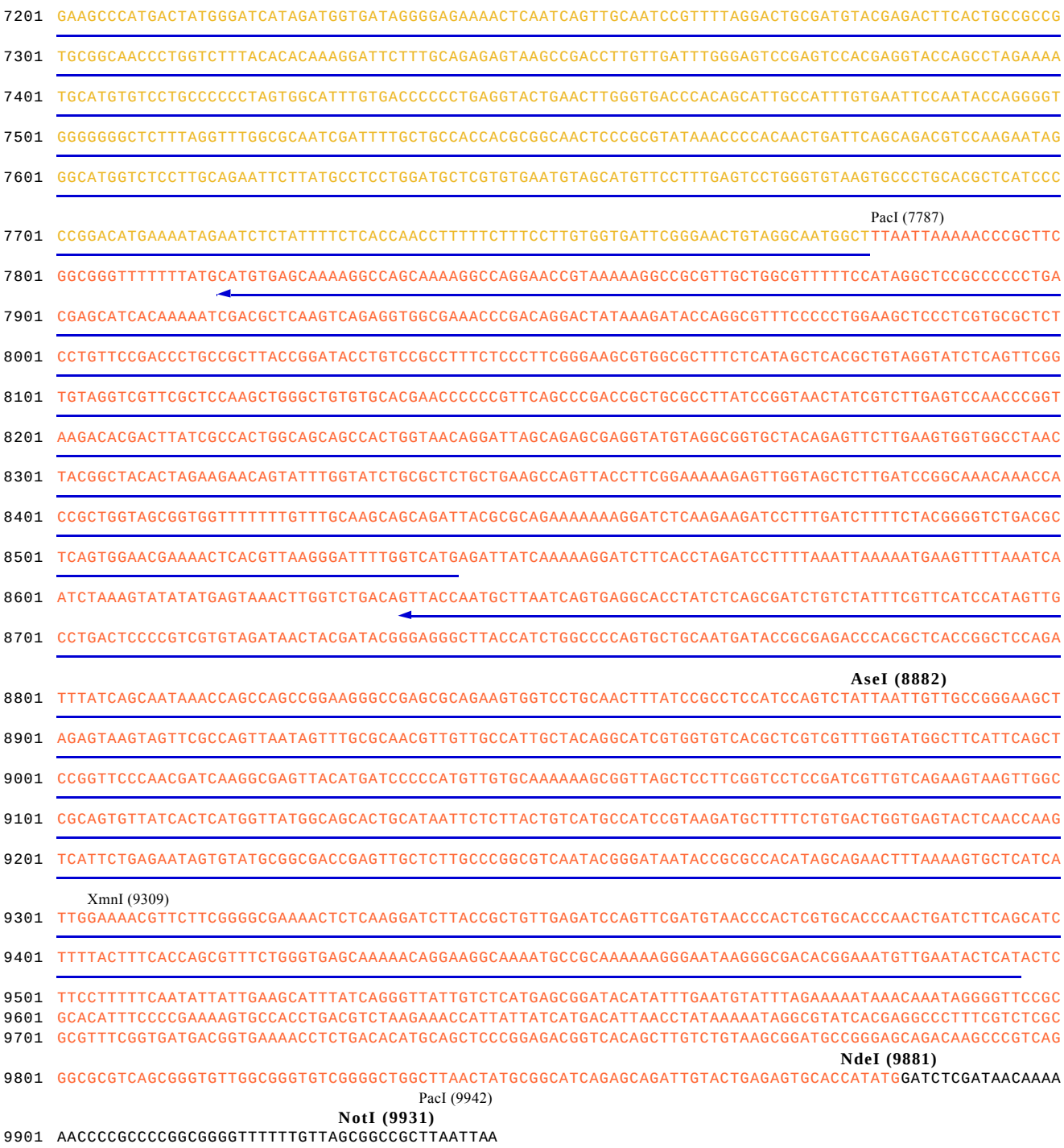
TECHNICAL SUPPORT
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com

InvivoGen
3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA

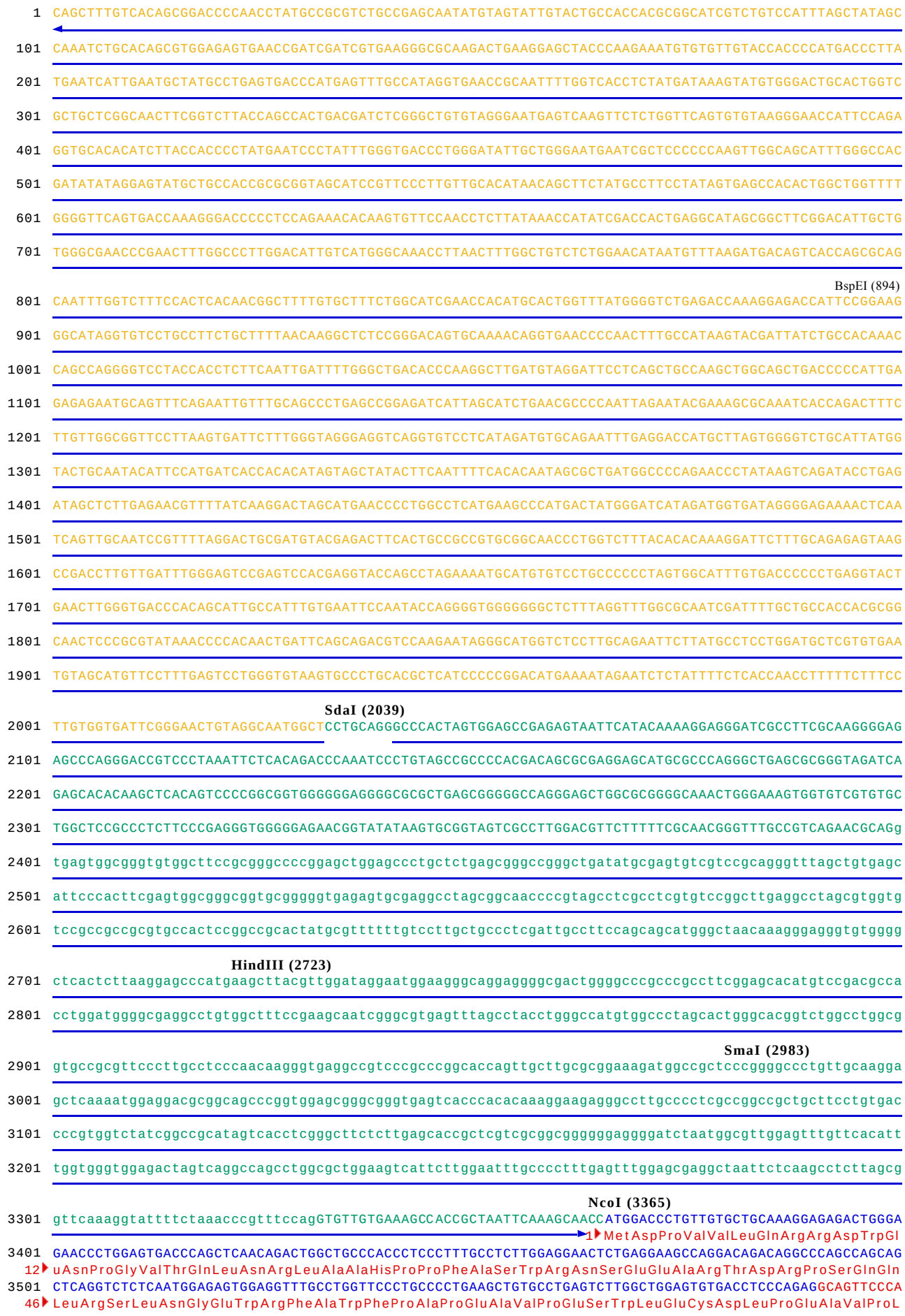
pWHERE sequence

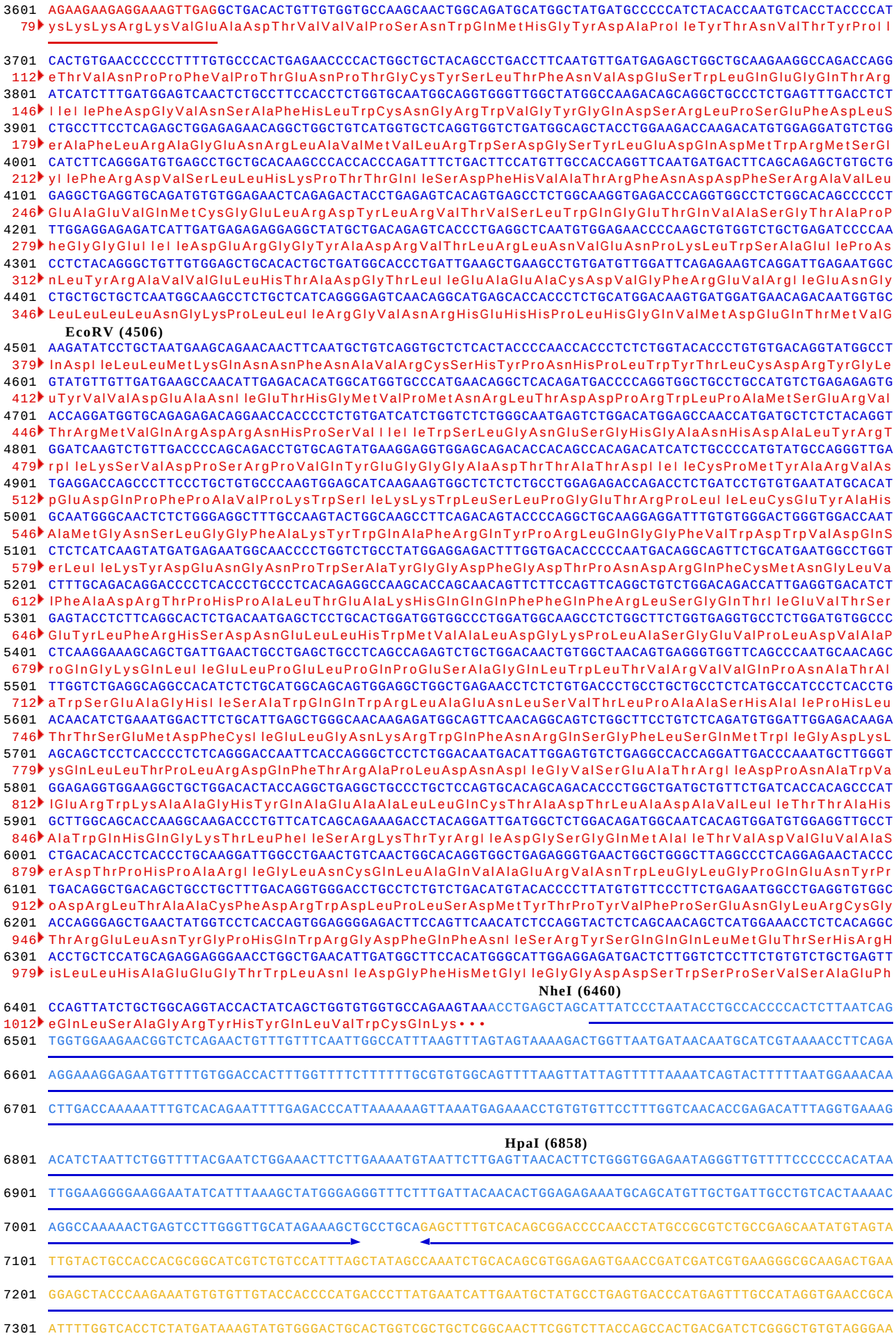
```
   1 CAGCTTTGTCACAGCGGACCCCAACCTATGCCGCGTCTGCCGAGCAATATGTAGTATTGTACTGCCACCACGCGGCATCGTCTGTCCATTTAGCTATAGC
 101 CAAATCTGCACAGCGTGGAGAGTGAACCGATCGATCGTGAAGGGCGCAAGACTGAAGGAGCTACCCAAGAAATGTGTGTTGTACCACCCCATGACCCTTA
 201 TGAATCATTGAATGCTATGCCTGAGTGACCCATGAGTTTGCCATAGGTGAACCGCAATTTTGGTCACCTCTATGATAAAGTATGTGGGACTGCACTGGTC
 301 GCTGCTCGGCAACTTCGGTCTTACCAGCCACTGACGATCTCGGGCTGTGTAGGGAATGAGTCAAGTTCTCTGGTTCAGTGTGTAAGGGAACCATTCCAGA
 401 GGTGCACACATCTTACCACCCCTATGAATCCCTATTTGGGTGACCCTGGGATATTGCTGGGAATGAATCGCTCCCCCCAAGTTGGCAGCATTTGGGCCAC
 501 GATATATAGGAGTATGCTGCCACCGCGCGGTAGCATCCGTTCCCTTGTTGCACATAACAGCTTCTATGCCTTCCTATAGTGAGCCACACTGGCTGGTTTT
 601 GGGGTTCAGTGACCAAAGGGACCCCCTCCAGAAACACAAGTGTTCCAACCTCTTATAAACCATATCGACCACTGAGGCATAGCGGCTTCGGACATTGCTG
 701 TGGGCGAACCCGAACTTTGGCCCTTGGACATTGTCATGGGCAAACCTTAACTTTGGCTGTCTCTGGAACATAATGTTTAAGATGACAGTCACCAGCGCAG
                                                                                                  BspEI (894)
 801 CAATTTGGTCTTTCCACTCACAACGGCTTTTGTGCTTTCTGGCATCGAACCACATGCACTGGTTTATGGGGTCTGAGACCAAAGGAGACCATTCCGGAAG
 901 GGCATAGGTGTCCTGCCTTCTGCTTTTAACAAGGCTCTCCGGGACAGTGCAAAACAGGTGAACCCCAACTTTGCCATAAGTACGATTATCTGCCACAAAC
1001 CAGCCAGGGGTCCTACCACCTCTTCAATTGATTTTGGGCTGACACCCAAGGCTTGATGTAGGATTCCTCAGCTGCCAAGCTGGCAGCTGACCCCCATTGA
1101 GAGAGAATGCAGTTTCAGAATTGTTTGCAGCCCTGAGCCGGAGATCATTAGCATCTGAACGCCCCAATTAGAATACGAAAGCGCAAATCACCAGACTTTC
1201 TTGTTGGCGGTTCCTTAAGTGATTCTTTGGGTAGGGAGGTCAGGTGTCCTCATAGATGTGCAGAATTTGAGGACCATGCTTAGTGGGGTCTGCATTATGG
1301 TACTGCAATACATTCCATGATCACCACACATAGTAGCTATACTTCAATTTTCACACAATAGCGCTGATGGCCCCAGAACCCTATAAGTCAGATACCTGAG
1401 ATAGCTCTTGAGAACGTTTTATCAAGGACTAGCATGAACCCCTGGCCTCATGAAGCCCATGACTATGGGATCATAGATGGTGATAGGGGAGAAAACTCAA
1501 TCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCGCCGTGCGGCAACCCTGGTCTTTACACACAAAGGATTCTTTGCAGAGAGTAAG
1601 CCGACCTTGTTGATTTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAATGCATGTGTCCTGCCCCCCTAGTGGCATTTGTGACCCCCCTGAGGTACT
1701 GAACTTGGGTGACCCACAGCATTGCCATTTGTGAATTCCAATACCAGGGGTGGGGGGGCTCTTTAGGTTTGGCGCAATCGATTTTGCTGCCACCACGCGG
1801 CAACTCCCGCGTATAAACCCCACAACTGATTCAGCAGACGTCCAAGAATAGGGCATGGTCTCCTTGCAGAATTCTTATGCCTCCTGGATGCTCGTGTGAA
1901 TGTAGCATGTTCCTTTGAGTCCTGGGTGTAAGTGCCCTGCACGCTCATCCCCCGGACATGAAAATAGAATCTCTATTTTCTCACCAACCTTTTTCTTTCC
                                                  AvrII (2043)     XhoI (2057)     NcoI (2070)
                                        SdaI (2039)         BamHI (2050)    SmaI (2066)
2001 TTGTGGTGATTCGGGAACTGTAGGCAATGGCTCCTGCAGGTCCTAGGTGGATCCTCTCGAGTCCCGGGCCATGGACCCTGTTGTGCTGCAAAGGAGAGAC
                                                                          1 MetAspProValValLeuGlnArgArgAsp
2101 TGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCCACCCTCCCTTTGCCTCTTGGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCCAGCC
  11 TrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerG
2201 AGCAGCTCAGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCCTGCCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCCAGAGGCAGT
  44 lnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaVa
2301 TCCCAAGAAGAAGAGGAAAGTTGAGGCTGACACTGTTGTGGTGCCAAGCAACTGGCAGATGCATGGCTATGATGCCCCCATCTACACCAATGTCACCTAC
  77 lProLysLysLysArgLysValGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAlaProI leTyrThrAsnValThrTyr
2401 CCCATCACTGTGAACCCCCCTTTTGTGCCCACTGAGAACCCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGA
 111 ProI leThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnT
2501 CCAGGATCATCTTTGATGGAGTCAACTCTGCCTTCCACCTCTGGTGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGA
 144 hrArgI leI lePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAs
2601 CCTCTCTGCCTTCCTCAGAGCTGGAGAGAACAGGCTGGCTGTCATGGTGCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGTGGAGGATG
 177 pLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMet
2701 TCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCCCACCACCCAGATTTCTGACTTCCATGTTGCCACCAGGTTCAATGATGACTTCAGCAGAGCTG
 211 SerGlyI lePheArgAspValSerLeuLeuHisLysProThrThrGlnI leSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaV
2801 TGCTGGAGGCTGAGGTGCAGATGTGTGGAGAACTCAGAGACTACCTGAGAGTCACAGTGAGCCTCTGGCAAGGTGAGACCCAGGTGGCCTCTGGCACAGC
 244 alLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAl
2901 CCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCTATGCTGACAGAGTCACCCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATC
 277 aProPheGlyGlyGlul lel leAspGluArgGlyGlyTyrAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGlul le
3001 CCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGCTGATGGCACCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTCAGAGAAGTCAGGATTGAGA
 311 ProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeul leGluAlaGluAlaCysAspValGlyPheArgGluValArgI leGluA
3101 ATGGCCTGCTGCTGCTCAATGGCAAGCCTCTGCTCATCAGGGGAGTCAACAGGCATGAGCACCACCCTCTGCATGGACAAGTGATGGATGAACAGACAAT
 344 snGlyLeuLeuLeuLeuAsnGlyLysProLeuLeul leArgGlyValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMe
                EcoRV (3211)          XmnI (3233)
3201 GGTGCAAGATATCCTGCTAATGAAGCAGAACAACTTCAATGCTGTCAGGTGCTCTCACTACCCCAACCACCCTCTCTGGTACACCCTGTGTGACAGGTAT
 377 tValGlnAspI leLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyr
3301 GGCCTGTATGTTGTTGATGAAGCCAACATTGAGACACATGGCATGGTGCCCATGAACAGGCTCACAGATGACCCCAGGTGGCTGCCTGCCATGTCTGAGA
 411 GlyLeuTyrValValAspGluAlaAsnI leGluThrHisGlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluA
3401 GAGTGACCAGGATGGTGCAGAGAGACAGGAACCACCCCTCTGTGATCATCTGGTCTCTGGGCAATGAGTCTGGACATGGAGCCAACCATGATGCTCTCTA
 444 rgValThrArgMetValGlnArgAspArgAsnHisProSerVal I lel leTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTy
```

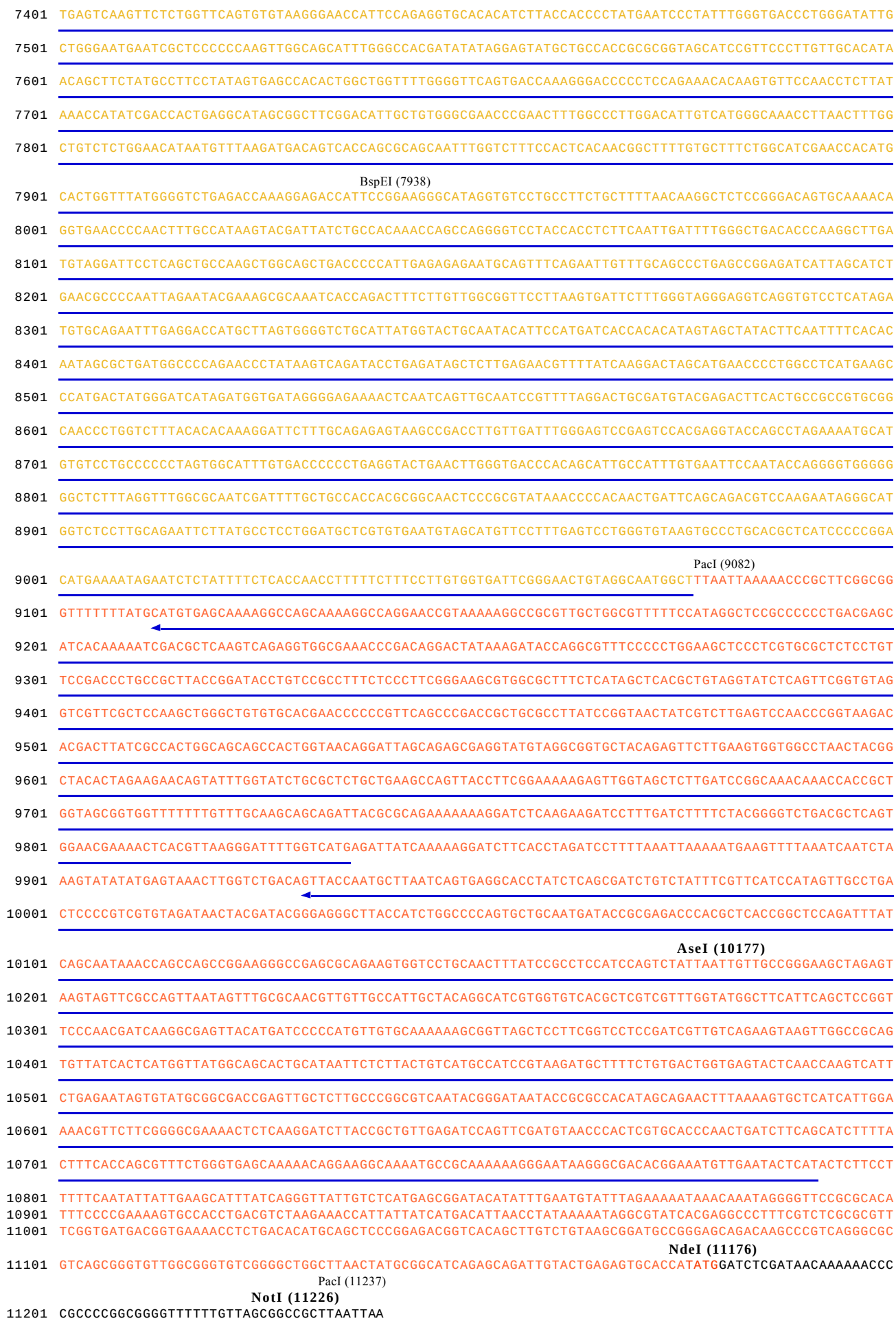

NheI (5165)
HpaI (5563)
BspEI (6643)


7201 GAAGCCCATGACTATGGGATCATAGATGGTGATAGGGGAGAAAACTCAATCAGTTGCAATCCGTTTTAGGACTGCGATGTACGAGACTTCACTGCCGCCG
7301 TGCGGCAACCCTGGTCTTTACACACAAAGGATTCTTTGCAGAGAGTAAGCCGACCTTGTTGATTTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAA
7401 TGCATGTGTCCTGCCCCCCTAGTGGCATTTGTGACCCCCCTGAGGTACTGAACTTGGGTGACCCACAGCATTGCCATTTGTGAATTCCAATACCAGGGGT
7501 GGGGGGGCTCTTTAGGTTTGGCGCAATCGATTTTGCTGCCACCACGCGGCAACTCCCGCGTATAAACCCCACAACTGATTCAGCAGACGTCCAAGAATAG
7601 GGCATGGTCTCCTTGCAGAATTCTTATGCCTCCTGGATGCTCGTGTGAATGTAGCATGTTCCTTTGAGTCCTGGGTGTAAGTGCCCTGCACGCTCATCCC
PacI (7787)
7701 CCGGACATGAAAATAGAATCTCTATTTTCTCACCAACCTTTTTCTTTCCTTGTGGTGATTCGGGAACTGTAGGCAATGGCTTTAATTAAAAACCCGCTTC
7801 GGCGGGTTTTTTTATGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGA
7901 CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT
8001 CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGG
8101 TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT
8201 AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
8301 TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA
8401 CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC
8501 TCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCA
8601 ATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG
8701 CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGA
AseI (8882)
8801 TTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
8901 AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCT
9001 CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGC
9101 CGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAG
9201 TCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA
XmnI (9309)
9301 TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATC
9401 TTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTC
9501 TTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC
9601 GCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGC
9701 GCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAG
NdeI (9881)
9801 GGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGATCTCGATAACAAAA
PacI (9942)
NotI (9931)
9901 AACCCCGCCCCGGCGGGGTTTTTTGTTAGCGGCCGCTTAATTAA

pWHERE-rEF1 sequence




EcoRV (4506)
NheI (6460)
HpaI (6858)


BspEI (7938)
PacI (9082)
AseI (10177)
NdeI (11176)
PacI (11237)
NotI (11226)