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[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

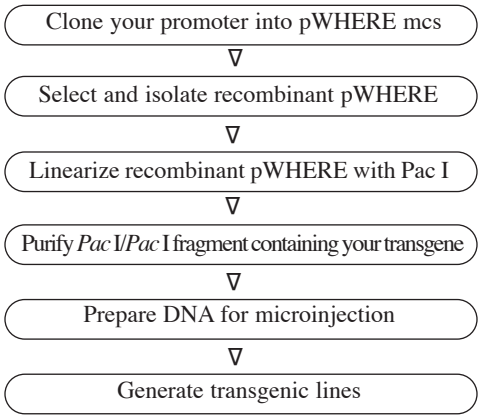
• **pMB1 ori:** a minimal *E. coli* origin of replication to limit vector size but with the same activity as the longer Ori.

• **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



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.

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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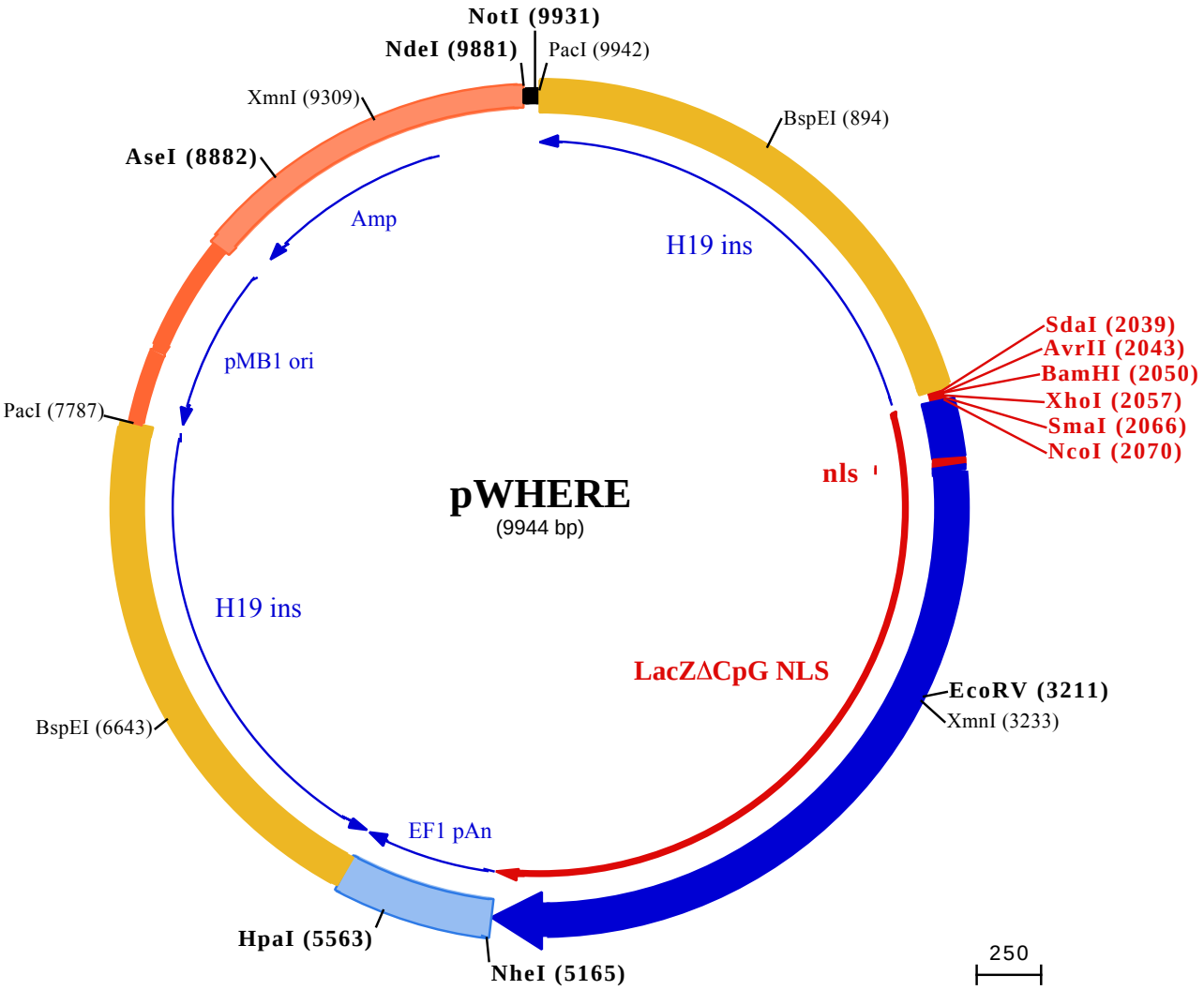
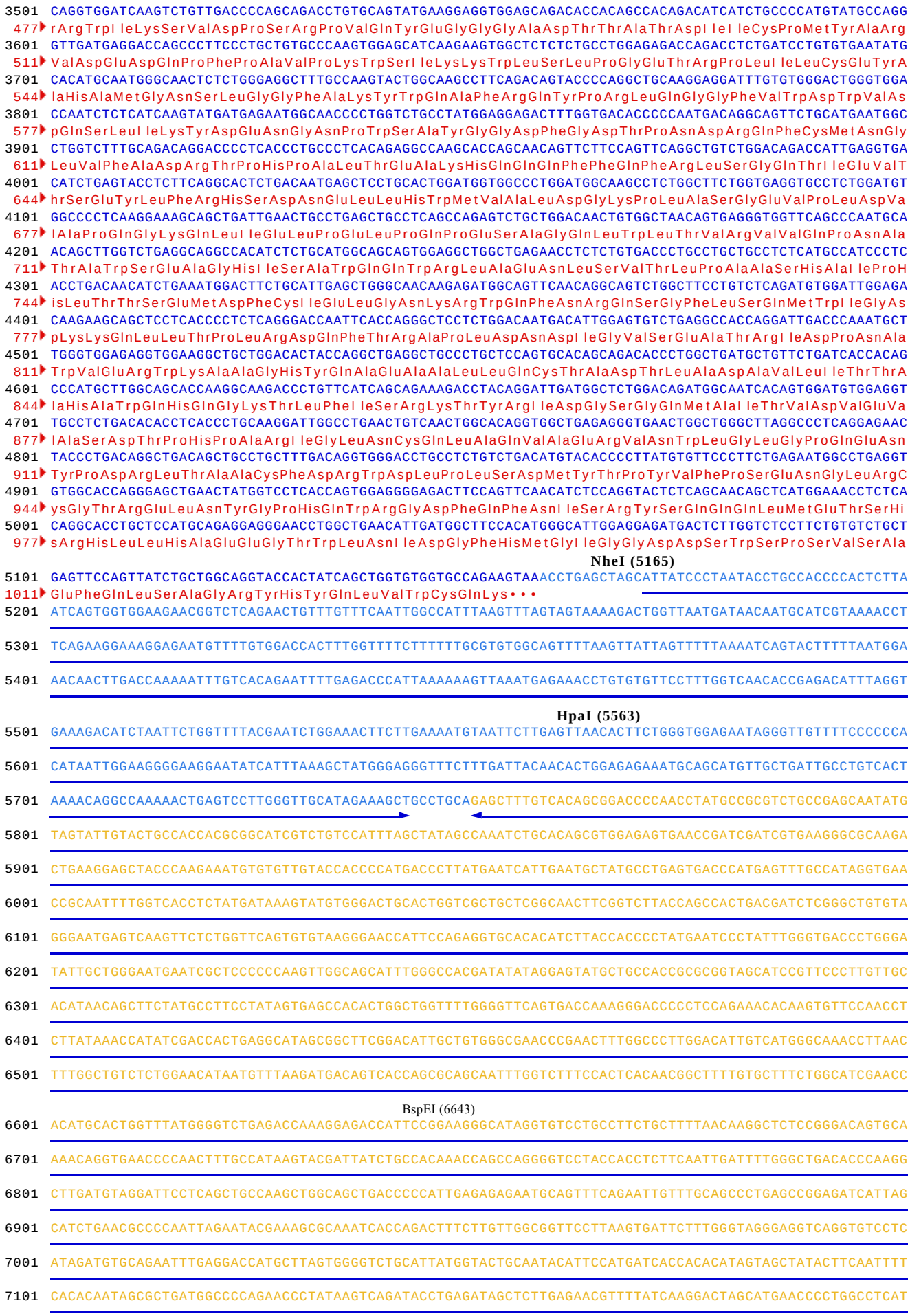
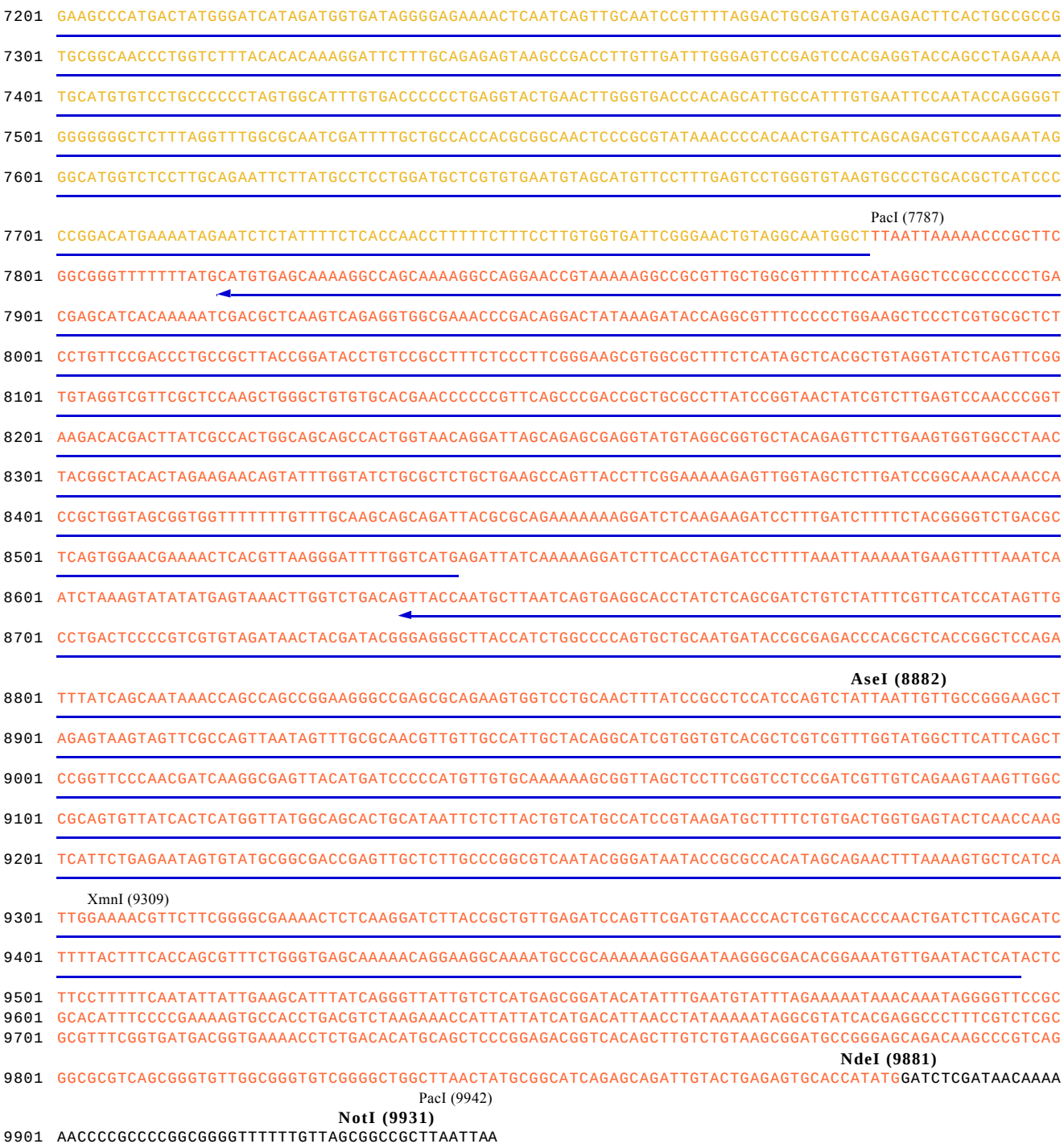

NotI (9931)
NdeI (9881)
PacI (9942)
XmnI (9309)
BspEI (894)
AseI (8882)
Amp
H19 ins
pMB1 ori
SdaI (2039)
AvrII (2043)
BamHI (2050)
XhoI (2057)
SmaI (2066)
NcoI (2070)
PacI (7787)
nls
pWHERE
(9944 bp)
H19 ins
LacZΔCpG NLS
EcoRV (3211)
XmnI (3233)
BspEI (6643)
EF1 pAn
HpaI (5563)
NheI (5165)
250

InvivoGen


BspEI (894)
AvrII (2043)
XhoI (2057)
NcoI (2070)
SdaI (2039)
BamHI (2050)
SmaI (2066)
EcoRV (3211)
XmnI (3233)


NheI (5165)
HpaI (5563)
BspEI (6643)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7301 TGCGGCAACCCTGGTCTTTACACACAAAGGATTCTTTGCAGAGAGTAAGCCGACCTTGTTGATTTGGGAGTCCGAGTCCACGAGGTACCAGCCTAGAAAA
7401 TGCATGTGTCCTGCCCCCCTAGTGGCATTTGTGACCCCCCTGAGGTACTGAACTTGGGTGACCCACAGCATTGCCATTTGTGAATTCCAATACCAGGGGT
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7601 GGCATGGTCTCCTTGCAGAATTCTTATGCCTCCTGGATGCTCGTGTGAATGTAGCATGTTCCTTTGAGTCCTGGGTGTAAGTGCCCTGCACGCTCATCCC
PacI (7787)
7701 CCGGACATGAAAATAGAATCTCTATTTTCTCACCAACCTTTTTCTTTCCTTGTGGTGATTCGGGAACTGTAGGCAATGGCTTTAATTAAAAACCCGCTTC
7801 GGCGGGTTTTTTTATGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGA
7901 CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT
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8101 TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT
8201 AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
8301 TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA
8401 CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC
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8601 ATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG
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AseI (8882)
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9201 TCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA
XmnI (9309)
9301 TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATC
9401 TTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTC
9501 TTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC
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NdeI (9881)
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PacI (9942)
NotI (9931)
9901 AACCCCGCCCCGGCGGGGTTTTTTGTTAGCGGCCGCTTAATTAA