

Synthesis, Cloning, and Sequencing of a Codon Optimized Variant of Proteinase Inhibitor II Designed for Expression in *Escherichia coli*

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SUPPLEMENTAL MATERIAL

S. TABLE 1. Strains and plasmids used in this study.

Name	Strain	Plasmids		
	DH5α	pET30b(+)	pET32a(+)	TOPO-TA
Description	Bacterial host for plasmid storage and expression	Expression vector with kanamycin resistance	Expression vector with thioredoxin tag and ampicillin resistance	Storage Vector with LacZ multiple cloning site, and ampicillin and kanamycin resistance
Source	UBC	Novagen	Novagen	Invitrogen

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PI2 DNA Keil  ATGGATGTTACAAGGAAGTTAATTCGTTGCTTACCTACTAATTGTTCTTGGTAAGATTTTCCTTTACTCCTTT
PI2 cDNA Keil  ATGGATGTTACAAGGAAGTTAATTCGTTGCTTACCTACTAATTGTTCTTGG
PI2 DNA JEMI  ATGGATGTTACAAGGAAGTTAATTCGTTGCTTACCTACTAATTGTTCTTGGTAAGATTTTCCTTTACTCCTTT

PI2 DNA Keil  TTTTTTTTTTTTAAAAAAATTCCTGGTTTATACATATATATATATATACACAAGTAGTTTTATATTTTCCTTT
PI2 cDNA Keil
PI2 DNA JEMI  TTTTTTTTTTTTAAAAAAATTCCTGGTTTATACATATATATATATATACACAAGTAGTTTTATATTTTCCTTT

PI2 DNA Keil  ATATTATATTTGTTTGTAGGATTATTGGTACTTGTAAGCGCGATGGAGCATGTTGATGCGAAGGCTTGACCTTTA
PI2 cDNA Keil  ATATTGTTACTTGTAAGCGCGATGGAGCATGTTGATGCGAAGGCTTGACCTTTA
PI2 DNA JEMI  ATATTATATTTGTTTGTAGGATTATTGGTACTTGTAAGCGCGATGGAGCATGTTGATGCGAAGGCTTGACCTTTA

PI2 DNA Keil  GAATGTGGTAATCTTGGGTTTGGGATATGCCACGTTCAGAAGGAAGTCCGGAAAAATCGCATATGCACCAACTGT
PI2 cDNA Keil  GAATGTGGTAATCTTGGGTTTGGGATATGCCACGTTCAGAAGGAAGTCCGGAAAAATCGCATATGCACCAACTGT
PI2 DNA JEMI  GAATGTGGTAATCTTGGGTTTGGGATATGCCACGTTCAGAAGGAAGTCCGGAAAAATCGCATATGCACCAACTGT

PI2 DNA Keil  TGTGCAGGTTATAAAGGTTGCAATTATTATAGTGCAAAATGGGGCTTTCATTTGTGAAGGACAATCTGACCCAAAA
PI2 cDNA Keil  TGTGCAGGTTATAAAGGTTGCAATTATTATAGTGCAAAATGGGGCTTTCATTTGTGAAGGACAATCTGACCCAAAA
PI2 DNA JEMI  TGTGCAGGTTATAAAGGTTGCAATTATTATAGTGCAAAATGGGGCTTTCATTTGTGAAGGACAATCTGACCCAAAA

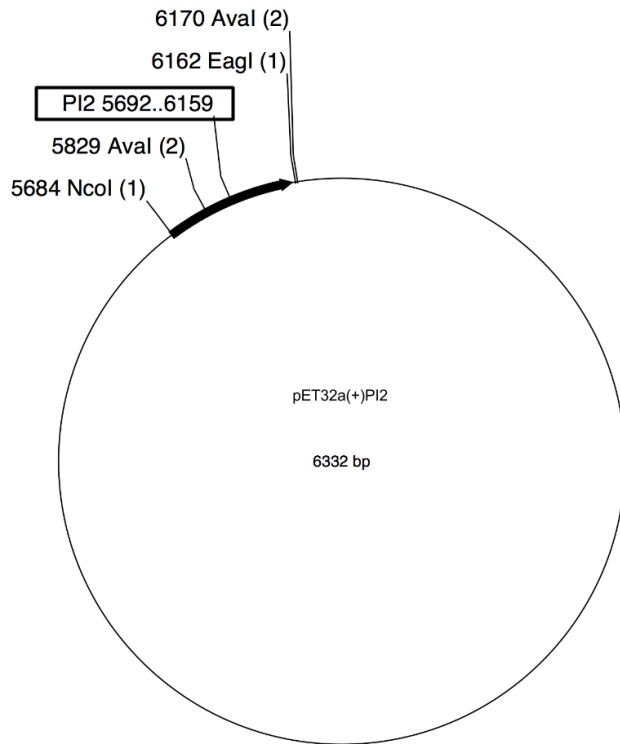
PI2 DNA Keil  AAACCAAAAGCATGCCCCCTAAATTGCGATCCACATATTGCCTACTCAAAGTGCCCGTTCAGAAGGAAAAATCG
PI2 cDNA Keil  AAACCAAAAGCATGCCCCCTAAATTGCGATCCACATATTGCCTACTCAAAGTGCCCGTTCAGAAGGAAAAATCG
PI2 DNA JEMI  AAACCAAAAGCATGCCCCCTAAATTGCGATCCACATATTGCCTACTCAAAGTGCCCGTTCAGAAGGAAAAATCG

PI2 DNA Keil  CTAATTTATCCCACCGGATGTACCACATGCTGCACAGGGTACAAGGGTTGCTACTATTTCCGGTAAAAATGGCAAG
PI2 cDNA Keil  CTAATTTATCCCACCGGATGTACCACATGCTGCACAGGGTACAAGGGTTGCTACTATTTCCGGTAAAAATGGCAAG
PI2 DNA JEMI  CTAATTTATCCCACCGGATGTACCACATGCTGCACAGGGTACAAGGGTTGCTACTATTTCCGGTAAAAATGGCAAG

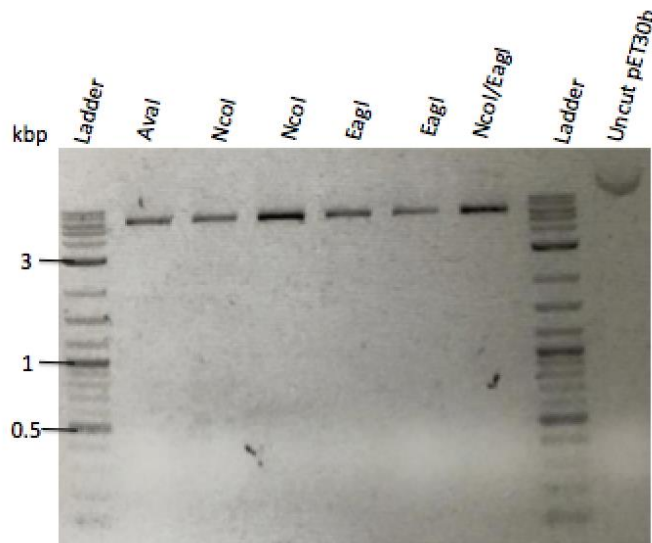
PI2 DNA Keil  TTTGTATGTGAAGGAGAGAGTGATGAGCCCAAGGCAAAATATGTACCCTGCAATGTGA
PI2 cDNA Keil  TTTGTATGTGAAGGAGAGAGTGATGAGCCCAAGGCAAAATATGTACCCTGCAATGTGA
PI2 DNA JEMI  TTTGTATGTGAAGGAGAGAGANNNGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
    
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S. FIG. 1.

Comparing Keil et al.'s PI2 primary DNA and cDNA sequence with JEMI-PI2. * indicates the location of the intron, + indicates nucleotide base changes relative to Keil et al.'s primary DNA sequence, N represents either an A, T, G, or C nucleotide.



SUPPLEMENTARY FIG. 2. Plasmid map of pET32a(+)/PI2. Plasmid maps designed in APE program.



S. FIG. 3. Confirming competent DH5 α and ensuring functional *Nco*I and *Eag*I. Single and double digests of pET30b visualized via gel electrophoresis in 1.5% agarose gel with SYBR Safe. 2-log ladder from NEB.

AJAE PI2 C T T G C C G G C C G C A T T A T T A C A T C G C C G G G T A C A T G T T C G C T T T C G G T T C G T C A G A T T C A C C T T C G C A A A C G A A T T
Sequenced TOPO PI2 C T T G C C G G C C G C A T T A T T A C A T C G C C G G G T A C A T G T T C G C T T T C G G T T C G T C A G A T T C A C C T T C G C A A A C G A A T T
AJAE PI2 T A C C G T T T T A C C G A A G T A G T A G C A A C C T T T G T A A C C G G T G C A G C A G G T G G T G C A A C C G G T C G G G T A G A T C A G A G
Sequenced TOPO PI2 T A C C G T T T T A C C G A A G T A G T A G C A A C C T T T G T A A C C G G T G C A G C A G G T G G T G C A A C C G G T C G G G T A G A T C A G A G
AJAE PI2 A T T T A C C T T C A G A A C G C G G G C A T T T A G A G T A C G C G A T G T G C G G G T C G C A G T T C A G C G G G C A C G C T T T C G G T T T T
Sequenced TOPO PI2 G G T T C T C C G G A A A A C C G T A T C T G C A C C A A C T G C T G C G C G G G T T A C A A A G G T T G C A A C T A C T A C T C T G C G A A C G G T
AJAE PI2 T C G G G T C A G A C T G A C C T T C G C A G A T G A A C G C A C C G T T C G C A G A G T A G T A G T T G C A A C C T T T G T A A C C C G C G C A G C
Sequenced TOPO PI2 T C G G G T C A G A C T G A C C T T C G C A G A T G A A C G C A C C G T T C G C A G A G T A G T A G T T G C A A C C T T T G T A A C C C G C G C A G C
AJAE PI2 A G T T G G T G C A G A T A C G G T T T T C C G G A G A A C C T T C A G A T C T C G G G C A G A T A C C G A A A C C C A G G T T A C C G C A T T C C A
Sequenced TOPO PI2 A G T T G G T G C A G A T A C G G T T T T C C G G A G A A C C T T C A G A T C T C G G G C A G A T A C C G A A A C C C A G G T T A C C G C A T T C C A
AJAE PI2 G G G T G C A C G C T T T C G C G T C A A C G T G T T C C A T C G C A G A A A C C A G A A C C A G C A G A C C C A G A A C G A T C A G C A G G T A C G
Sequenced TOPO PI2 G G G T G C A C G C T T T C G C G T C A A C G T G T T C C A T C G C A G A A A C C A G A A C C A G C A G A C C C A G A A C G A T C A G C A G G T A C G
AJAE PI2 C A A C G A A G T T A A C T T C T T T G T G A A C G T C C A T A G C C A T G G A T G
Sequenced TOPO PI2 C A A C G A A G T T A A C T T C T T T G T G A A C G T C C A T A G C C A T G G A T G

S. FIG. 4. Sequenced PI2 gene in pCR2.1-TOPO.