

FIGURE 1

SEQ ID NO. 14

M-VIP ELP1-120 (M added to N terminus of VIP)

MHSDAVFTDNYTRLRKOMAVKKYLSILN

VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
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 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGWP (SEQ ID NO: 14)

ELP1-120 = (VPGXG)₁₂₀ where X = V₅G₃A₂

FIGURE 2

SEQ ID NO. 15

MAA-VIP ELP1-120 (before processing, MAA added to N terminus of VIP)

MAAHSDAVFTDNYTRLRKOMAVKKYLSILN

VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
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 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGVG VPGVG VPGGG VPGAG VPGVG VPGVG VPGVG VPGGG VPGAG VPGGG
 VPGWP (SEQ ID NO: 15)

ELP1-120 = (VPGXG)₁₂₀ where X = V₅G₃A₂

FIGURE 3

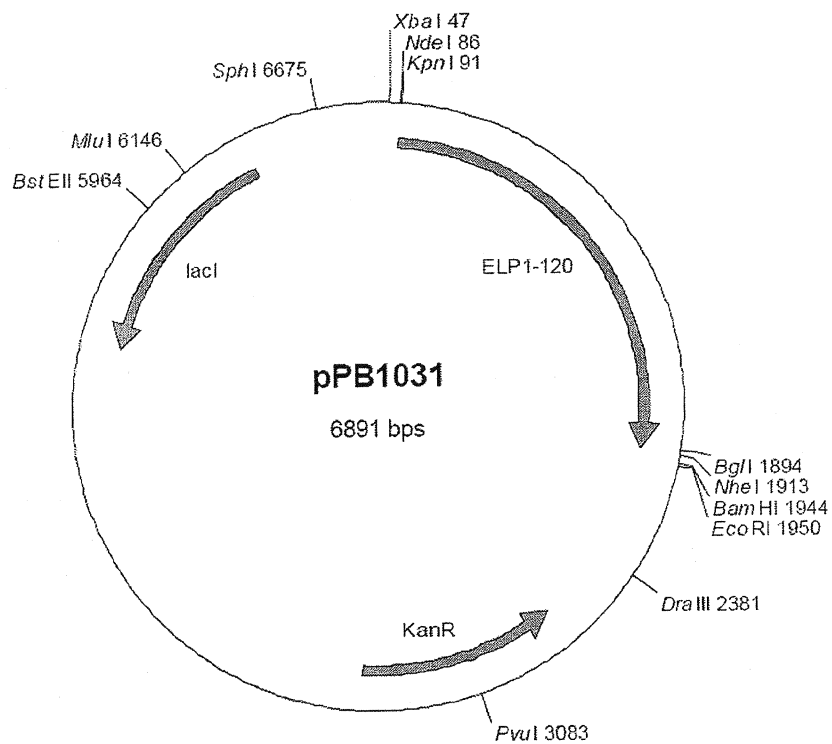
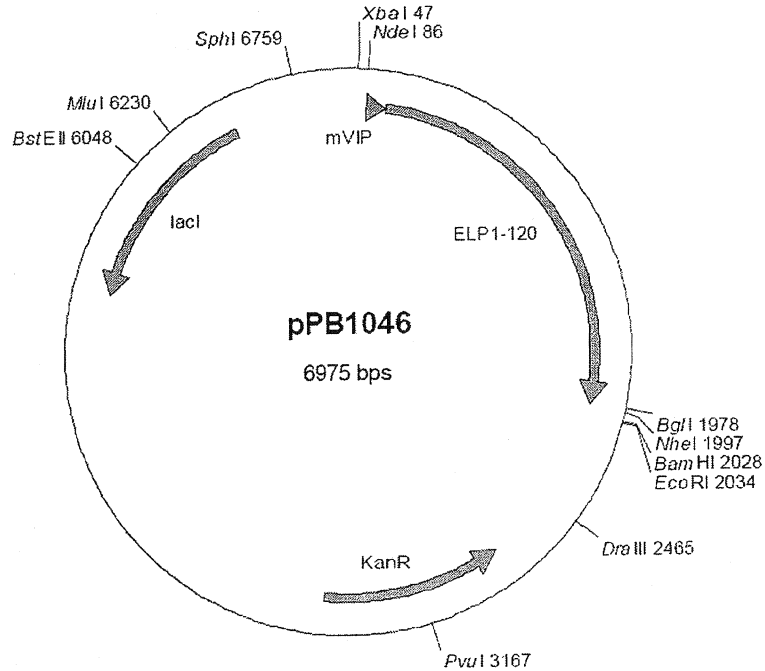


FIGURE 4



XbaI
+-----
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attatgctga gtgatatccc ctttaacaactc gctattgtt aaggggagat ctttattaaa
GAGAT CTTTATTAAA
< P0048

NdeI
+-----

P0064
CTGTTTTCA CTGACAACTA

P0045 >
TGTTTAACTT TAAGAAGGAG ATATACATAT GCACTCTGAC G
61 tgtttaactt taagaaggag atatacatat gcaactctgac gctgttttca ctgacaacta
acaaattgaa attcttccctc tatatgtata cgtgagactg cgacaaaagt gactgttgat
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P0048

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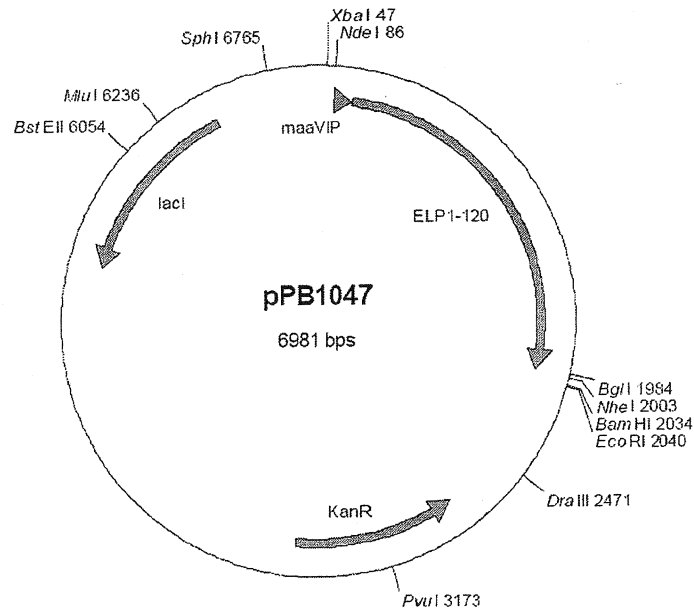
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gtgagcagac gcatttgtct accgacaatt ttcatggac ttgagatagg acttgcatgg
G
P0048

TGAGCAGAC GCATTGTCT ACCGACAATT TTTCATGGAC TTGAGATAGG ACTTG
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>.....mVIP.....>>>>..
y t r l r k q m a v k k y l n s i l n v

181 gggcgtgggt gttccgggag tgggtgttcc ggggtggcgtt ytgccggggg caggtgttcc
ccgcaccca caaggccgc acccacaagg ccacccgcca cccggccgc gtcacaagg
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p g v g v p g v g v p g g g v p g a g v

FIGURE 5



```

+-----
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1 taatacgact cactataggg gaattgtgag cggataacaa ttccctctta gaaataattt
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NdeI
---+-----

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m a a h s d a v f t

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CTTGATG
P0067
TGA GCAGACGCAT TTGTCTACCG ACAATTTTTC ATGGACTTGA GATAGGACTT
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d n y t r l r k q m a v k k y l n s i l

P0065
G
P0064 >
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gcattggccg caccacaaag gcccgcaacc acaaggccca cggccacacg gcccggtcc
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FIGURE 6

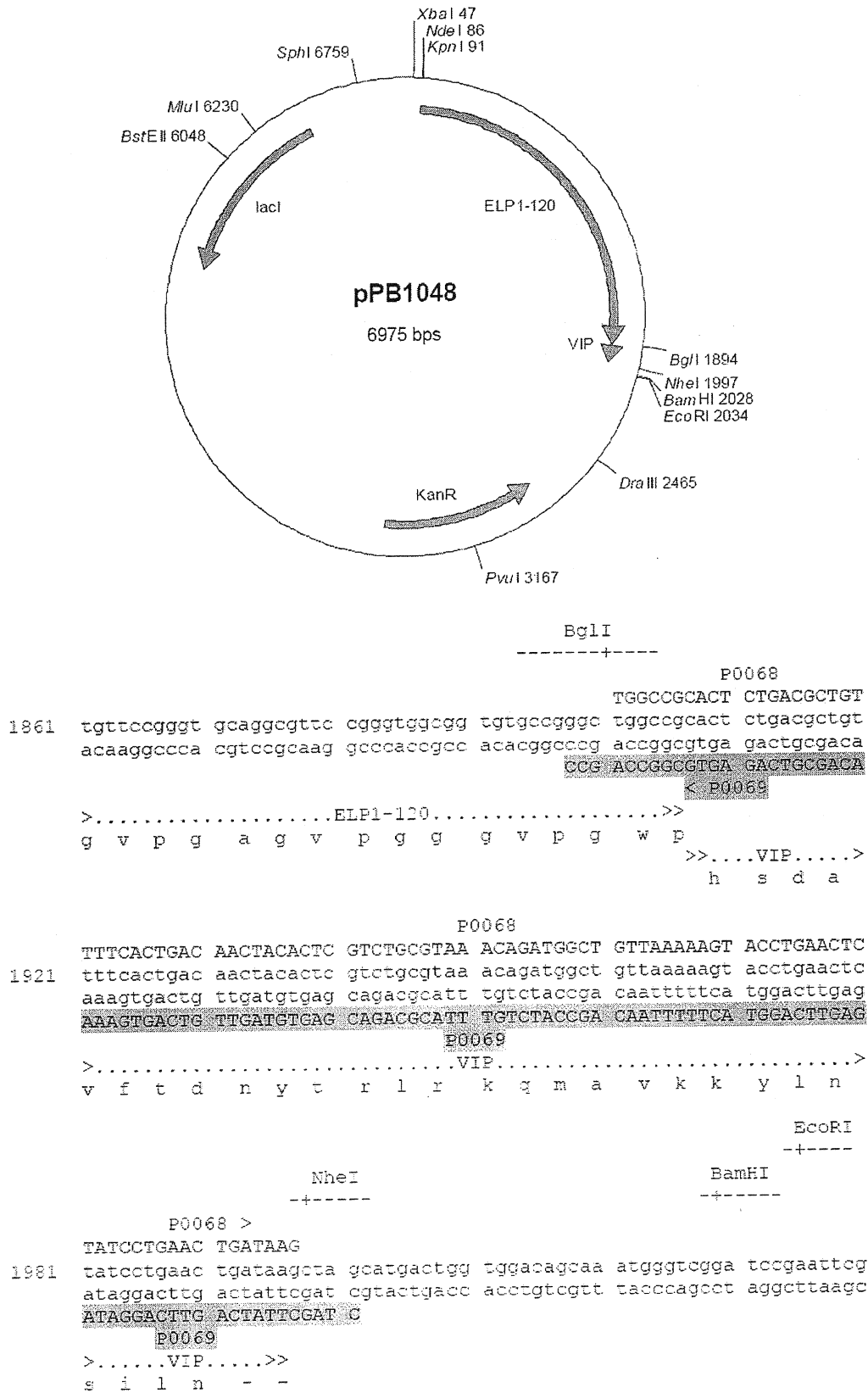
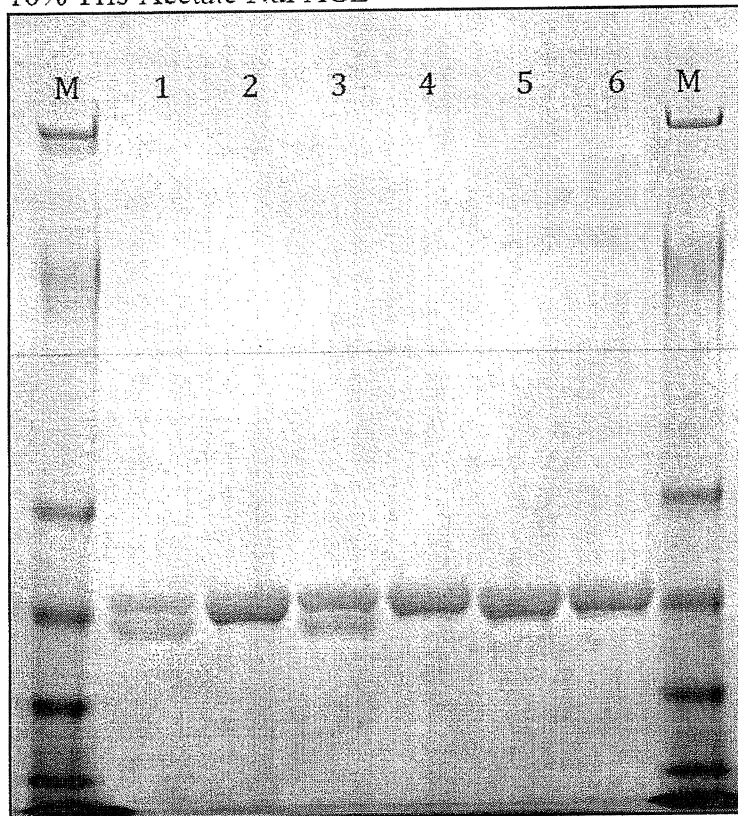


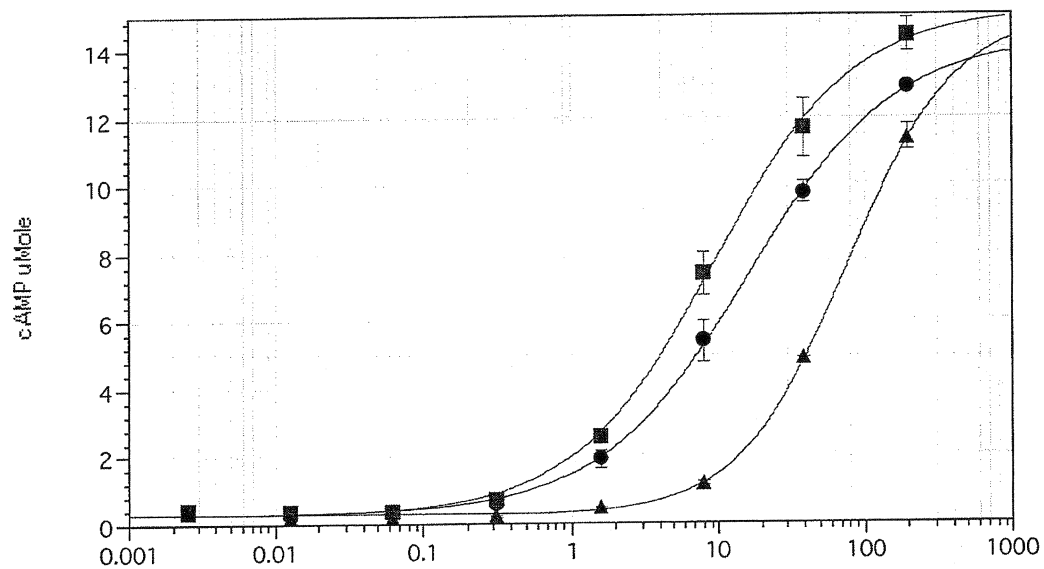
FIGURE 7

10% Tris-Acetate NuPAGE



M. Mol. wt. markers
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2. PB1046 heated*
3. PB1047
4. PB1047 heated*
5. PB1048
6. PB1048 heated*
M. Mol. wt. markers

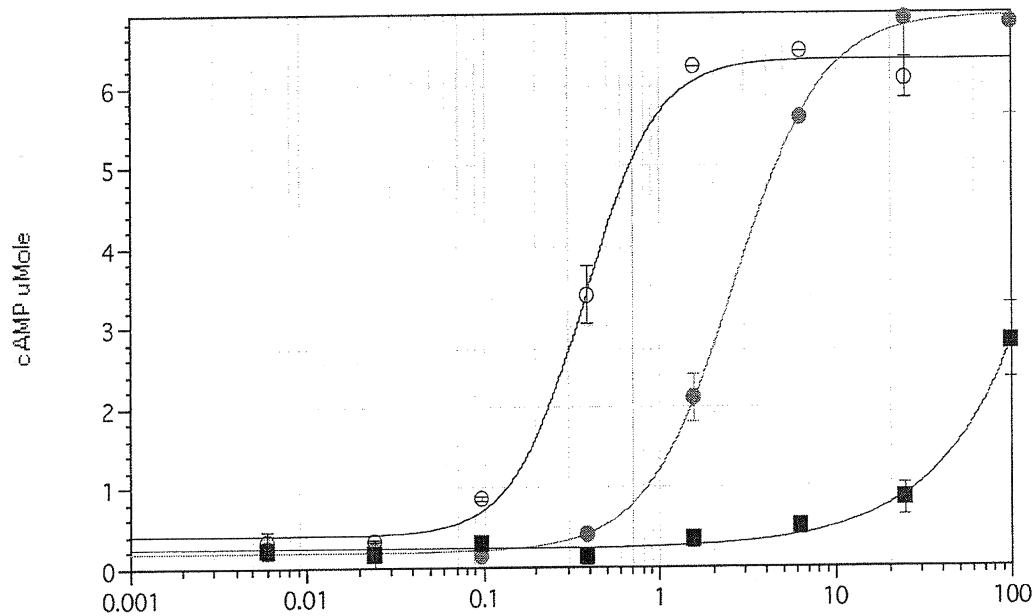
FIGURE 8



4-P Fit: $y = (A - D) / (1 + (x/C)^B) + D$:

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R²</u>
● VIP-Peptide (VIP2: concentration vs MeanRe...	0.269	0.859	16.2	14.3	0.999
■ PB1046 (A2: concentration vs MeanResult)	0.248	0.909	9.53	15.1	0.999
▲ PB1047 (C2: concentration vs MeanResult)	0.237	1.19	77.3	15	1

FIGURE 9



4-P Fit: $y = (A - D) / (1 + (x/C)^B) + D$:

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R²</u>
● PB1047 (B: concentration vs MeanResult)	0.182	1.73	2.66	6.87	1
■ PB1046 (C: concentration vs MeanResult)	0.224	0.96	1.88e+08	2.72e+06	0.993
○ VIP Peptide (VIP: concentration vs MeanRes...)	0.386	2.15	0.379	6.32	0.997

FIGURE 10

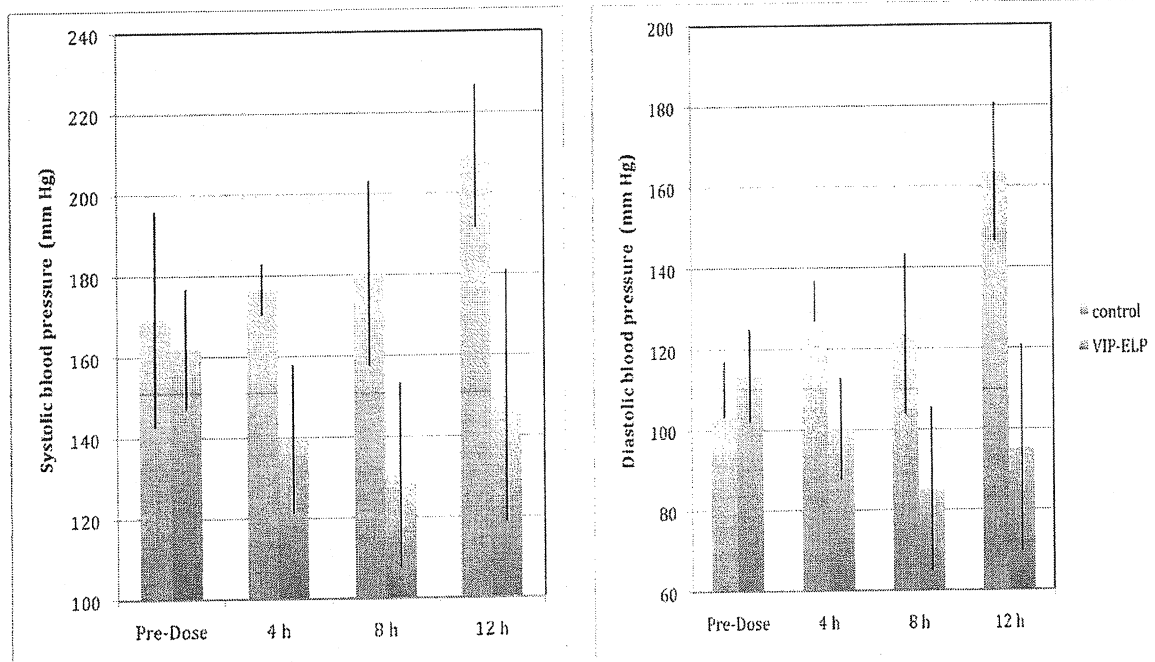


FIGURE 11

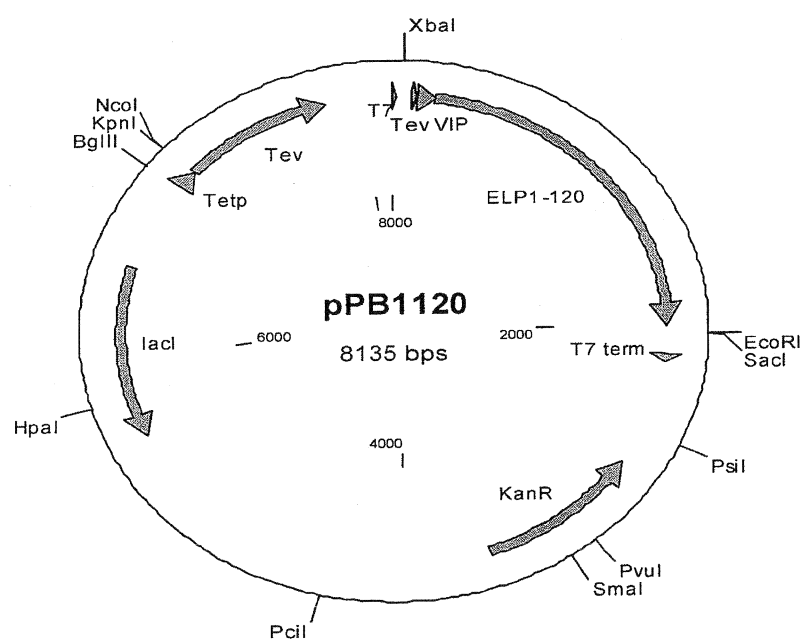


FIGURE 12

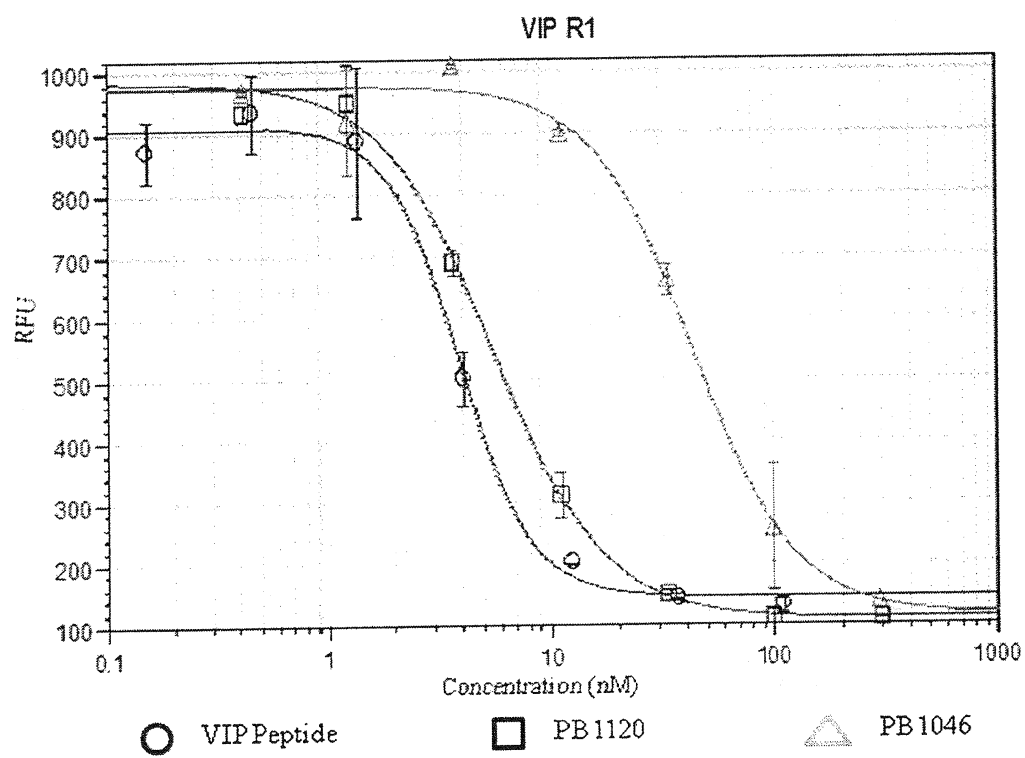


FIGURE 13

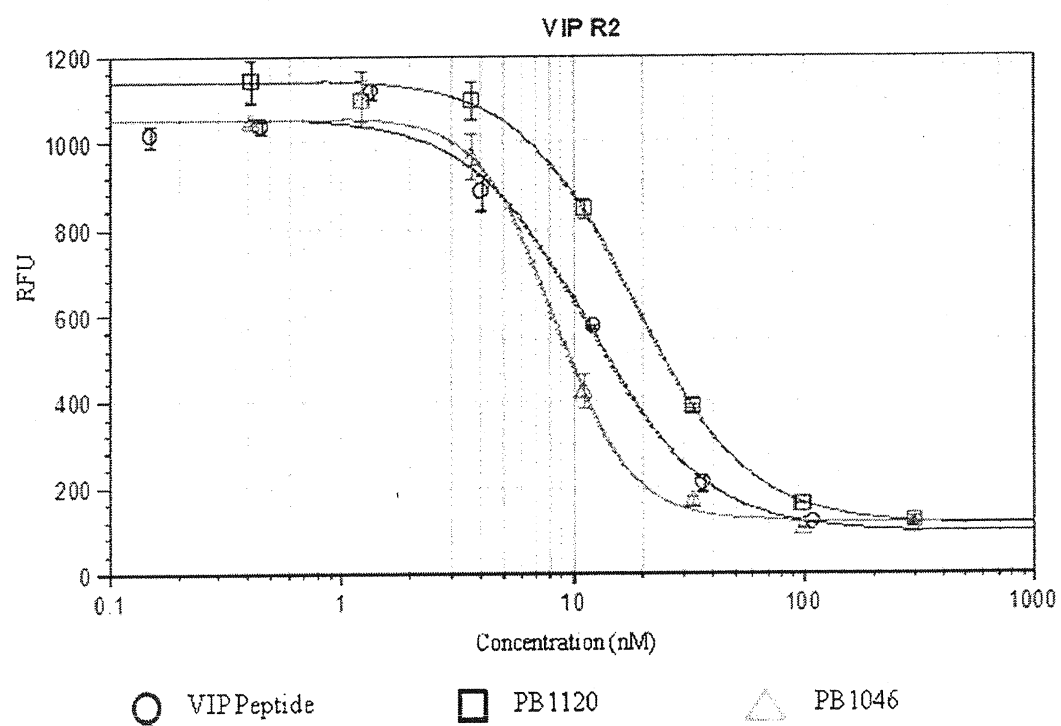
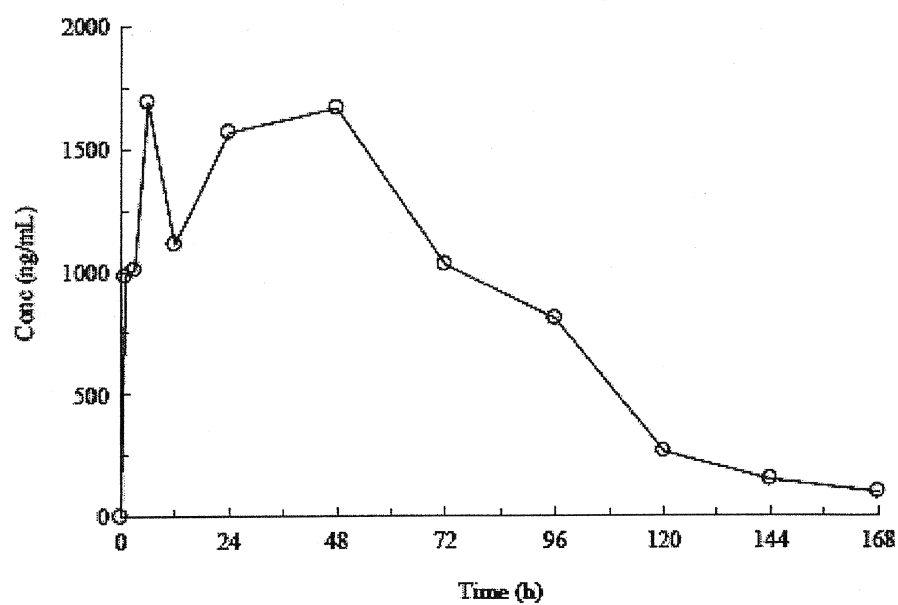


FIGURE 14

A



B

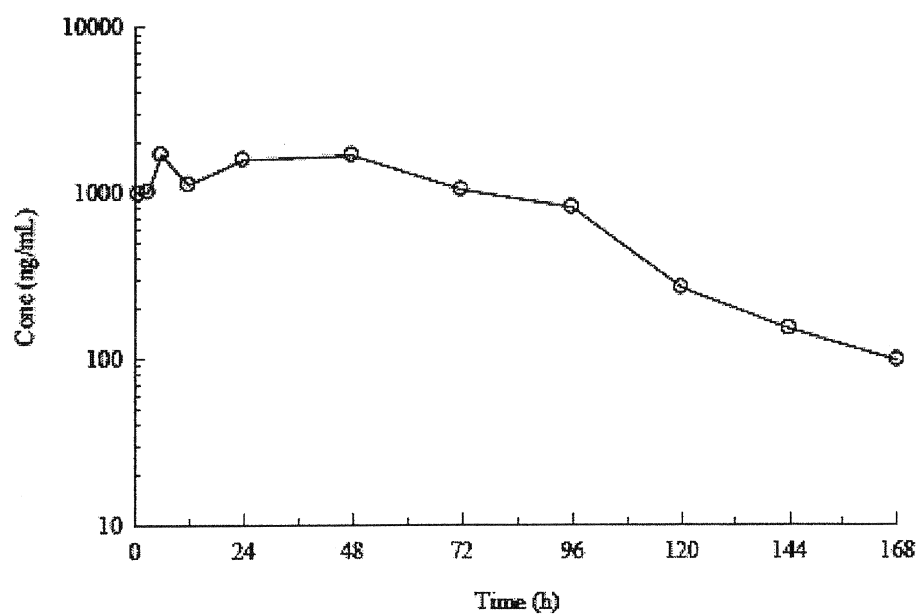


FIGURE 15A

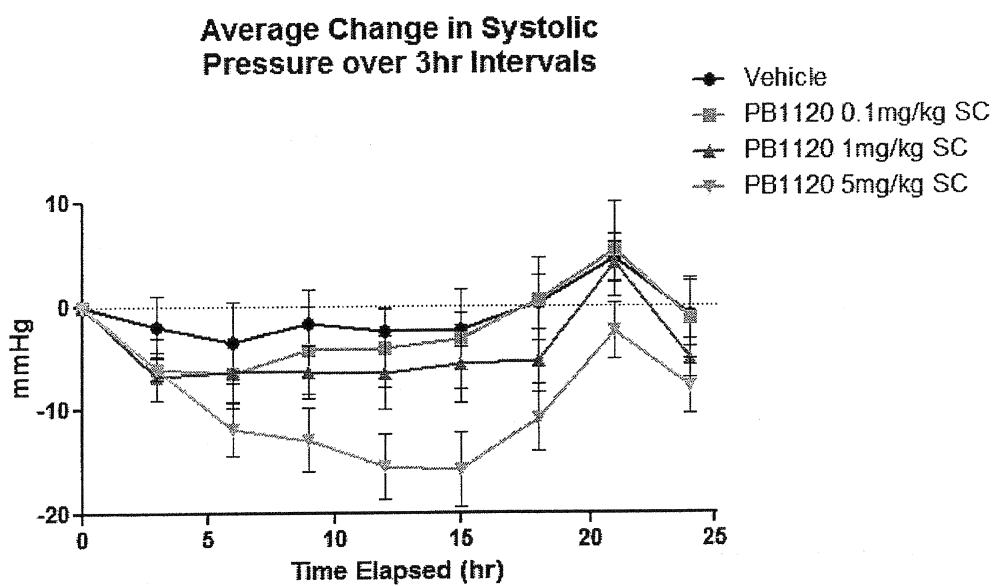


FIGURE 15B

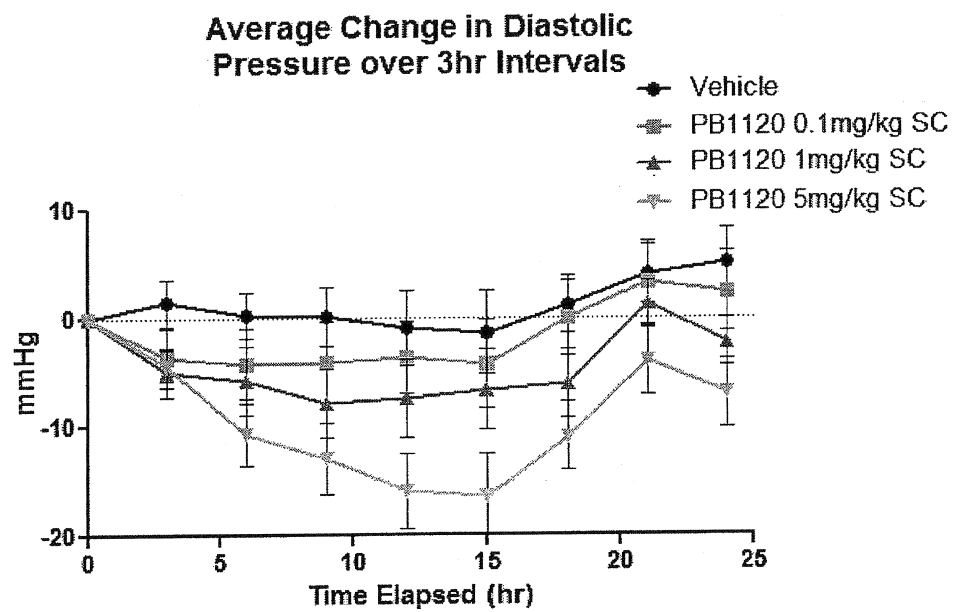


FIGURE 15C

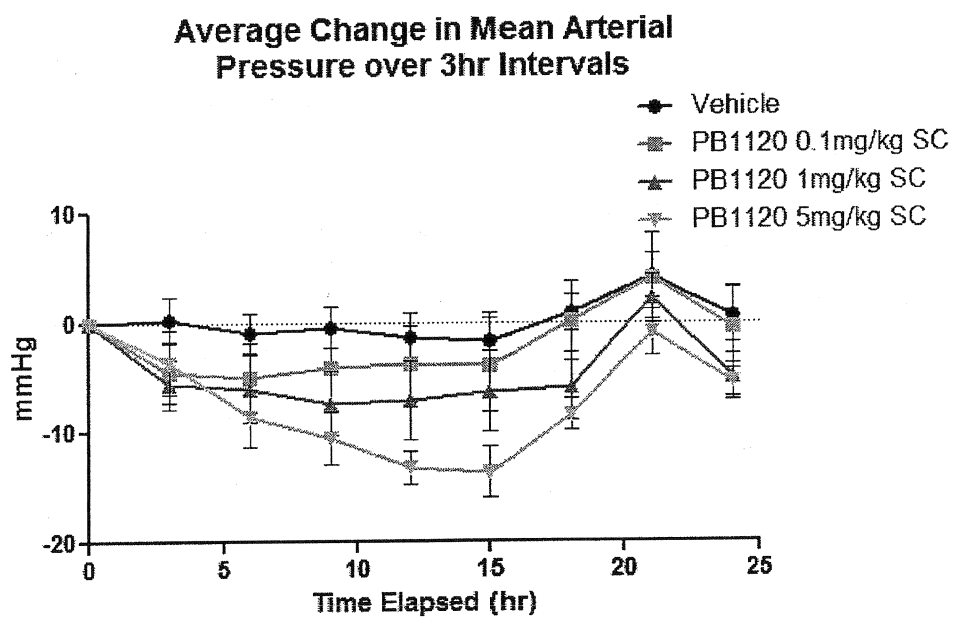
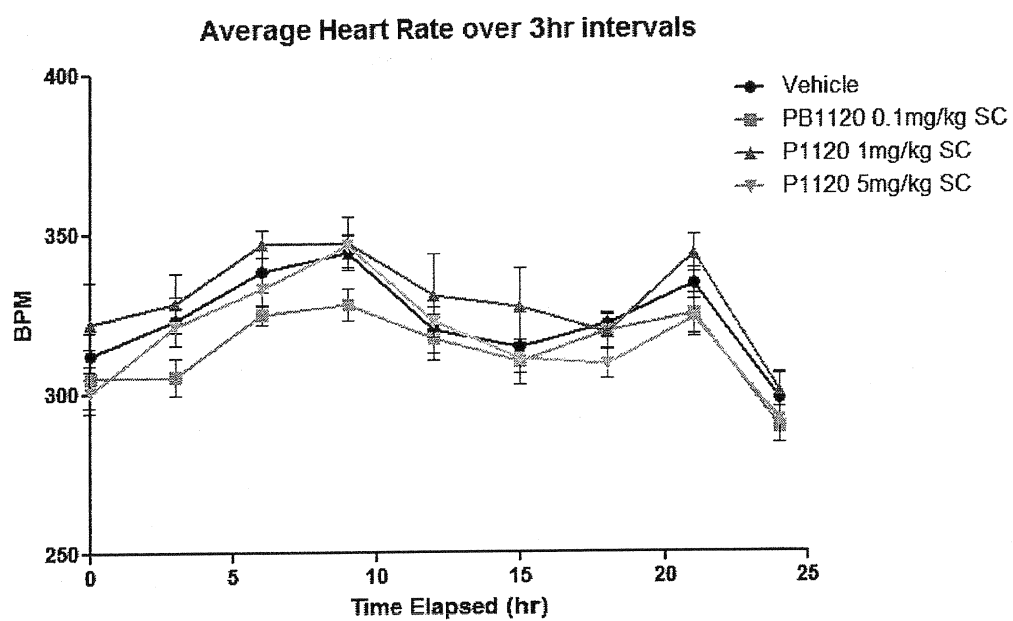


FIGURE 15D



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<130> SG3177A

<150> US 61/234,151

<151> 2009-08-14

<160> 50

<170> PatentIn version 3.5

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20 25 30

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580 585 590

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	115	120 125
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130	135	140
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Gly Asp Gly
50

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Leu Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Asn
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<223> VIP : MODIFIED PROTEIN

<400> 22

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<223> Xaa may be Ser or Gly

<400> 24

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Arg Arg Gln Leu Ala Val Arg Arg Tyr Leu Asn Ser Ile Leu Asn
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<223> Xaa may be Ser or Gly

<400> 27

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1 5 10 15

Arg Arg Gln Leu Ala Val Arg Arg Tyr Leu Ala Ala Ile Leu Asn
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His Ser Asp Gly Ile Phe Thr Asp Ser Tyr Ser Arg Tyr Arg Lys Gln
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu
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<210> 29
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<212> PRT
<213> UNKNOWN

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<223> PACAP-38 : NATIVE PROTEIN

<400> 29

His Ser Asp Gly Ile Phe Thr Asp Ser Tyr Ser Arg Tyr Arg Lys Gln
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Met Ala Val Lys Lys Tyr Leu Ala Ala Val Leu Gly Lys Arg Tyr Lys
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Gln Arg Val Lys Asn Lys
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<212> PRT

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<223> PACAP : MODIFIED PROTEIN

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Arg Arg Gln Leu Ala Val Arg Arg Tyr Leu Ala Ala Val Leu Gly Val
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Pro Gly Glu Gly Val Pro Gly Asp Gly
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aaaattatttt ctagag 76

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ttaaacaaaa ttattt 76

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<223> P0069 : PRIMER

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<223> mVIP-ELP 1-120 fusion sequence

<400> 40

Met His Ser Asp Ala Val Phe Thr Asp Asn Tyr Thr Arg Leu Arg Lys
1 5 10 15

Gln Met Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Asn Val Pro Gly
20 25 30

Val Gly Val Pro Gly Val Gly Val Pro Gly Gly Gly Val Pro Gly Ala
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Gly Val
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<223> mVIP-ELP 1-120 fusion sequence

<400> 42

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Arg Lys Gln Met Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Val Pro
20 25 30

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Gly Gly Val Pro Gly
35 40 45

Ala

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<211> 180

<212> DNA

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<223> pPB1048 ELP 1-120-VIP fusion sequence

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tatcctgaac tgataagcta gcatgactgg tggacagcaa atgggtcgga tccgaattcg 180

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<223> ELP 1-120-VIP fusion sequence

<400> 44

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Trp Pro
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His Ser Asp Ala Val Phe Thr Asp Asn Tyr Thr Arg Leu Arg Lys Gln
20 25 30

Met Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Asn
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<210> 45

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<223> Modified VIP N-terminus

<400> 45

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<223> Modified VIP N-terminus

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Gly Phe Leu Gly
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<212> DNA

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 <213> Artificial Sequence

<220>
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			20					25					30		

Gly	Val	Pro	Gly	Val	Gly	Val	Pro	Gly	Gly	Gly	Val	Pro	Gly	Ala	Gly
		35					40					45			

Val	Pro	Gly	Val	Gly	Val	Pro	Gly	Val	Gly	Val	Pro	Gly	Val	Gly	Val
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

50		55		60										
Pro 65	Gly	Gly	Gly	Val 70	Pro 75	Gly	Ala	Gly	Val 75	Pro 80	Gly	Gly	Gly	Val 80
Gly	Val	Gly	Val	Pro 85	Gly	Val	Gly	Val	Pro 90	Gly	Gly	Gly	Val	Pro 95
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Val	Pro 290	Gly	Gly	Gly	Val	Pro 295	Gly	Ala	Gly	Val	Pro 300	Gly	Val	Gly
Pro 305	Gly	Val	Gly	Val	Pro 310	Gly	Val	Gly	Val	Pro 315	Gly	Gly	Gly	Val
Gly	Ala	Gly	Val	Pro 325	Gly	Gly	Gly	Val	Pro 330	Gly	Val	Gly	Val	Pro 335

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Pro Gly Gly Gly Val Pro Gly Trp Pro
625 630