

Primers used for sequencing phage display binding peptide

PIII leader sequence **Kpn I**

5'...TTA TTC GCA ATT CCT TTA GTG GTA CCT TTC TAT TCT CAC TCT
3'...AAT AAG CGT TAA GGA AAT CAC CAT GGA AAG ATA AGA GTG AGA
...*Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser His Ser*

↓ Start of mature heptapeptide-gIII fusion **Eag I**

NNK NNK NNK NNK NNK NNK NNK GGT GGA GGT TCG GCC GAA ACT GTT GAA
NNM NNM NNM NNM NNM NNM NNM CCA CCT CCA AGC CGG CTT TGA CAA CTT
Xxx Xxx Xxx Xxx Xxx Xxx Xxx Gly Gly Gly Ser Ala Glu Thr Val Glu

AGT TGT TTA GCA AAA TCC CAT ACA GAA AAT TCA TTT ACT AAC GTC TGG
TCA ACA AAT CGT TTT AGG GTA TGT CTT TTA AGT AAA TGA TTG CAG ACC
Ser Cys Leu Ala Lys Ser His Thr Glu Asn Ser Phe Thr Asn Val Trp
← -28 gIII Primer

AAA GAC GAC AAA ACT TTA GAT CGT TAC GCT AAC TAT GAG GGC...-3'
TTT CTG CTG TTT TGA AAT CTA GCA ATG CGA TTG ATA CTC CCG...-5'
Cys Asp Asp Lys Thr Leu Asp Arg Tyr Ala Asn Tyr Glu Gly...
← -96 gIII Primer

K-G or T;M-A or C

Figure 3. The N-terminal sequence of seven random peptide-gIII fusion protein

Sequencing HSA-3 binder Result

CTTTCGGACGTTAGTAAATGAATTTTCTGTATGGGATTTTGCTAAACAAC
TTTCAACAGTTTCGGCCGAACCTCCACCATTAAGCTTCTAACTCTCCGCA
GAGTGAGAATAGAAAGGTACCACTAAAGGAATTGCGAATAATAATTTTT
CACGTTGAAAATCTCCAAAAAAGGCTCCAAAAGGAGCCTTTAATTGTA
TCGGTTTATCAGCTTGCTTTCGAGGTGAATTTCTTAAACAGCTTGATACC
GATAGTTGCGCCGACAATGACAACAACCATCGCCACGCATAACCGATAT
ATTCGGTCGCTGAGGCTTGCAGGGAGTTAAAGGCCGCTTTTGCGGGATCG
TCACCCTCAGCAGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTAC
AGAGGCTTTGAGGACTAAAGACTTTTTTCATGAGGAAGTTTCCATTAACG
GGTAAAATACGTAATGCCACTACGAAGGCACCAACCTAAAACGAAAGAGG
CAAAGAATACACTAAACACTCATCTTTGACCCCCAGCGATTATACCAA
GCGCGAAACAAAGTACAACGGAGATTTGTATCATCGCCTGATAAATTGTG
TCGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGCGCAGAC
GGTCAATCATAAGGGAACCGAACTGACCAACTTTGAAAGAGGACAGATGA
ACGGTGTACAGACCAGGCGCATAGGCTGGCTGACCTTCATCAAGAGTAAT
CTTGACAAGAACCGGATATTCATTACCCAATCAACGTAACAAAGCTGCT
CATTCAAGTGAATAAGGCTTGC

GenBank Submissions # 2410423

-96 gIII Primer sequencing: 5'-ATTAAGCTTCTAACTCTCCGC-3'

HSA-3 DNA: 5'-GCG GAG AGT TAG AAG CTT AAT-3'

HSA-3 peptide: Ala-Glu-Ser-Gln-Lys-Leu-Asn (AESQKLN)

Sequencing HSA-4 binder Result

CAGACGTTAGTAAATGAATTTTCTGTATGGGATTTTGCTAAACAAC
TTTCAACAGTTTCGGCCGAA**ACCTCCACCACGCAACGGAAGCGGC**GTAAGA
GAGTGAGAATAGAAAGGTACCACTAAAGGAATTGCGAATAATAATTTTTT
CACGTTGAAAATCTCCAAAAAAAAGGCTCCAAAAGGAGCCTTTAATTGTA
TCGGTTTATCAGCTTGCTTCGAGGTGAATTTCTTAAACAGCTTGATACC
GATAGTTGCGCCGACAATGACAACAACCATCGCCACGCATAACCGATAT
ATTCGGTCGCTGAGGCTTGCAGGGAGTTAAAGGCCGCTTTTGCGGGATCG
TCACCCTCAGCAGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTAC
AGAGGCTTTGAGGACTAAAGACTTTTTTCATGAGGAAGTTTCCATTAAACG
GGTAAAATACGTAATGCCACTACGAAGGCACCAACCTAAAACGAAAGAGG
CAAAGAATACACTAAAACACTCATCTTTGACCCCCAGCGATTATACCAA
GCGCGAAACAAAGTACAACGGAGATTTGTATCATCGCCTGATAAATTGTG
TCGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGCGCAGAC
GGTCAATCATAAGGGAACCGAACTGACCAACTTTGAAAGAGGACAGATGA
ACGGTGTACAGACCAGGCGCATAGGCTGGCTGACCTTCATCAAGAGTAAT
CTTGACAAGAACCGGATATTCATTACCCAAATCAACGTAACAAAGCTGCT
CATTCAAGTGAATAAGGCTTGC

Genebank Submission # 2411052

-96 gIII Primer sequencing: 5' **ACGCAACGGAAGCGGC**GTAAG 3'

HSA-4 DNA: 5'-**CTT ACG CCG CTT CCG TTG CGT**-3'

HSA-4 peptide: Leu-Thr-Pro-Leu-Pro-Leu-Arg (ITPLPLR)