Amino Acid Sequence #1

MDFPQQLEACVKQANQALSRFIAPLPFQNTPVVETMQYGALLGGKRLRPFLVYATGHMFGVSTNTLDAPAAAVECIHAYSLIHDDLPAMDDDDLRRGLPTCHVKFGEANAILAGDALQTLAFSILSDANMPEVSDRDRISMISELASASGIAGMCGGQALDLDAEGKHVPLDALERIHRHKTGALIRAAVRLGALSAGDKGRRALPVLDKYAESIGLAFQVQDDILDVVGDTATLGKRQGADQQLGKSTYPALLGLEQARKKARDLIDDARQSLKQLAEQSLDTSALEALADYIIQRNK

Link: <https://www.ncbi.nlm.nih.gov/nuccore/CP033717>

Strain: FDAARGOS\_579 (1644556.. 1645476)

Amino Acid Sequence #2 (original one found while blastp)

MSDTHAPVDFKQQLAAHQQRVNQALLDFIAPLPFGNSNLIEAMRYGAVIGGKRLRPYLVYATGQMFGLSFANLDAPAAAIECIHAYSLIHDDLPAMDDDDLRRGQPTCHVKFGEAHAILAGDALQTLAFSILAEAPMPDVADKDRLSMIIELAQASGAAGMCAGQALDLEAETRPVLLGDLEQIHRHKTGALIRAAVRLGALAAGEAGRKTLLSLDRYAEAIGLAFQVQDDILDVIGDTA

TIGKRQGSDQQLGKSTYPALLGLDCAQTKAMDLYQEALEALDELAKQSYNTDSLQALARFIIERNN

Link: <https://www.ncbi.nlm.nih.gov/protein/WP_012304510.1?report=genbank&log$=protalign&blast_rank=1&RID=2HTJZCMY016>

PCR yPispA-F/yPispA-R on Y. pseudotuberculosis gDNA (977bp, pcrpdt)

***Potentially need to clone out BsmBI site…***

PCR o1/o2 on pLYC73S (14896 bp, back)

assemble pcrpdt, back (BsaI, pLYC73S-yPispA)

transform pLYC73S-yPispA (Mach1, Amp)

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> yPispA-F Cloning of yPispA

ccataGGTCTCaTAACttaccccagatactgagtagcc

> yPispA-R Cloning of yPispA

tatggGGTCTCaTTGTttaattattacgctcaataatgaaacgagc

> o1 Forward BsaI for pLYC73S template

ccataGGTCTCaACAATAAGTATTAATAGGCCCCTG

> o2 Reverse BsaI for pLYC73S template

ccataGGTCTCaGTTAGAGAGGGCTGCTTGAACCCA

> yPispA rbs.CDS of Y. pseudotuberculosis ispA

ttaccccagatactgagtagccttatgtcggatacccatgcacctgttgattttaaacaacaacttgccgctcatcagcagcgagtcaatcaggctctgctggatttcattgctcccctgccgtttggcaatagcaatttaatcgaggccatgcgctatggtgcggtaatcgggggtaagcgtctccgcccctatctggtctatgccacagggcaaatgtttggtttgtcattcgccaacttagacgccccggcggcggcaattgaatgtattcatgcctattcattaatacatgatgatctgcccgccatggacgatgatgatttacgccgtgggcagccgacttgccacgttaagtttggcgaggcccacgctattttggccggtgatgcactgcaaacgctggccttttctattttggctgaagcccccatgcccgatgtcgctgataaagatcgcctatcgatgattatcgagttggctcaagccagtggggctgcggggatgtgtgcgggtcaggcgctagatctggaagcagaaactcgccctgttttattaggtgaccttgagcaaatccaccgtcataaaaccggtgcgttgattcgtgcggcagttcgccttggcgccctggcagccggtgaagctggccgtaaaacattattgtcactcgatcgttacgccgaggccatcggtttagcctttcaagtccaggacgatattttagacgtcattggcgacacagctacaattggtaaacgtcaaggctcagaccaacaactaggcaaaagtacttatccggcgctacttggtctggattgtgcccaaacaaaagcgatggatctttatcaggaggcactagaggccttggatgaattggcaaaacagtcctataacacggattcattgcaggcattagctcgtttcattattgagcgtaataattaa