**NOTE: I highly doubt this actually exists in S. pneumoniae… The sequences obtained are very similar to the degree that I can use the same primers for both S. flexneri and S. pneumoniae…**

Amino Acid Sequence #1

MDFPQQLEACVKQANQALSRFIAPLPFQNTPVVETMQYGALLGGKRLRPFLVYATGHMFGVSTNTLDAPAAAVECIHAYSLIHDDLPAMDDDDLRRGLPTCHVKFGEANAILAGDALQTLAFSILSDANMPEVSDRDRISMISELASASGIAGMCGGQALDLDAEGKHVPLDALERIHRHKTGALIRAAVRLGALSAGDKGRRALPVLDKYAESIGLAFQVQDDILDVVGDTATLGKRQGADQQLGKSTYPALLGLEQARKKARDLIDDARQSLKQLAEQSLDTSALEALADYIIQRNK

Link: <https://www.ncbi.nlm.nih.gov/nuccore/CABEEG010000004>

Strain: NCTC7978 (1222532..1223431)

PCR sFispA-F/sFispA-R on S. pneumoniae gDNA (956bp, pcrpdt)

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

assemble pcrpdt, back (BsaI, pLYC73S-sPispA)

transform pLYC73S-sPispA (Mach1, Amp)

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> sFispA-F Cloning of sPispA

ccataGGTCTCaTAACcccttttacaccggacaatgag

> sFispA-R Cloning of sPispA

tatggGGTCTCaTTGTttatttattacgctggatgatgtagt

> o1 Forward BsaI for pLYC73S template

ccataGGTCTCaACAATAAGTATTAATAGGCCCCTG

> o2 Reverse BsaI for pLYC73S template

ccataGGTCTCaGTTAGAGAGGGCTGCTTGAACCCA

> sFispA rbs.CDS of S. pneumoniae ispA

cccttttacaccggacaatgagtaatggactttccgcagcaactcgaagcctgcgttaagcaggccaaccaggcgctgagccgttttatcgccccactgccctttcagaacactcccgtggtcgaaaccatgcagtatggcgcattattaggtggtaagcgcctgcgaccttttctggtttatgccaccggtcatatgttcggcgttagcacaaacacgctggacgcacccgctgccgccgtagagtgtatccacgcttactcattaattcatgatgatttaccggcgatggatgatgacgatctgcgtcgcggtttgccgacctgccatgtgaagtttggcgaagcaaacgcgattctcgctggcgacgctttacaaacgctggcgttctcgattctaagcgatgccaatatgccggaagtgtcggatcgcgacagaatttcgatgatttctgaactggcgagcgccagcggtattgccggaatgtgcggtggtcaggcattagatttagacgcggaaggcaaacacgtacctctggacgcgcttgagcgtattcatcgtcataaaaccggcgcattgattcgcgccgccgttcgccttggtgcattaagcgccggagataaagggcgtcgtgctctgccagtactcgacaagtacgcagagagcatcggccttgccttccaggttcaagatgacatcctggatgtggtaggagatactgcaacgttgggaaaacgccagggtgccgatcagcaacttggtaaaagtacctaccctgcacttctgggtcttgagcaagcccggaagaaagcccgggatctgatcgacgatgcccgccagtcgctgaaacaactggctgaacaatcactcgatacctcggcactggaagcgctagcggactacatcatccagcgtaataaataa