

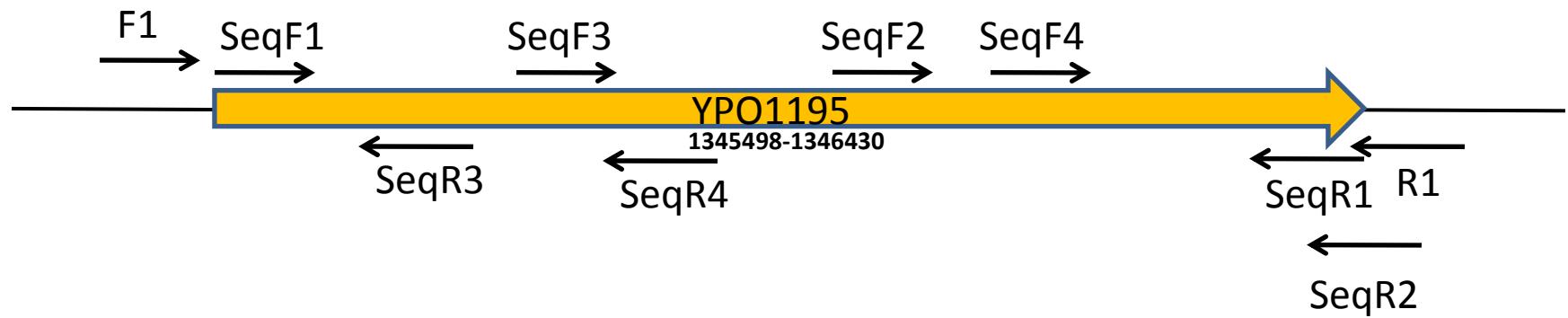
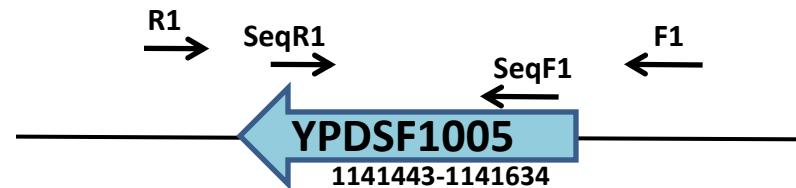
Sequence Validation:

50 ng of CO92 genomic DNA was used as template for amplification of target PCR products. Phusion High Fidelity DNA Polymerase (New England BioLabs, Inc.) with the Phusion HF Reaction Buffer using gene specific primers in Table 1 at a final concentration of 500 nM were used for targeted amplification. The sample was amplified for 30 cycles of 98C for 30 seconds, 55C for 30 seconds and 72C for 60 seconds. The amplified products were purified over a QIAQuick column per manufacturer's recommendation (Qiagen). Purified products were sequenced on the Applied BioSystems 3730/XL instrument using manufacturer's standard operating procedures. Amplified and purified PCR products for YPDSF1005 and YPO1195 were sequenced on an Applied BioSystems 3730/XL per manufacturer's recommendation. Gene YPDSF1005 was sequenced in 4 independent lanes using primers YPDSF1005F1, YPDSF1005R1, YPDSF1005RSEQ2 and YPDSF1005FSEQ2. Gene YPO1195 was amplified using primers YPO1195F1 and YPO1195R1. The amplified material was sequenced by all primers in Table 1 to increase sequence resolution and coverage over the amplicon.

TABLE 1. PCR AND SEQUENCING PRIMERS

PRIMER NAME	SEQUENCE (5'-3')	GENE TARGET	COORDINATES (5'-3')
YPDSF1005F1	AGCAGATAAGGGCTACATAGG	YPDSF1005	1142260
YPDSF1005R1	CGATTGACCGAACTTGGCTAT	YPDSF1005	1141287
YPDSF1005FSEQ2	GCTATCAGAAACAACGATT	YPDSF1005	1141671
YPDSF1005RSEQ2	TTCCTTGAGCATATCGTTCC	YPDSF1005	1141834
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YPO1195F1	TAGCCCGTAGAACCAACCG	YPO1195	1345419
YPO1195R1	CCATGAAACGAATAGCACCA	YPO1195	1346505
YPO1195FSEQ1	ATGGCACTGTTCTAACTC	YPO1195	1345498
YPO1195RSEQ1	TTAGTTTTAATAATCCGTTATC	YPO1195	1346430
YPO1195FSEQ2	GCTCCAATCATCCGTGAAGT	YPO1195	1346245
YPO1195RSEQ2	AAGCTTATAGTGCCACACTGGTC	YPO1195	1346475
YPO1195FSEQ3	ATTGGTATGGCTCTCTCC	YPO1195	1345860
YPO1195RSEQ3	CAATCTACCGGCAGTAATCG	YPO1195	1345738
YPO1195FSEQ4	TGAAAGCTCACCCGAACATC	YPO1195	1346270
YPO1195RSEQ4	CATTGATCCACTAACCTAAATC	YPO1195	1345975

Primer Maps



YPDSF_1005

Query: Sanger sequence of sample

Subject: Y. pestis Pestoides F genome sequence

Query	1	ATGAACAAATATAGCGAACGAGAAAATGCCATTACTTATCCCCGGCTGGCTTGAT 	60
Sbjct	1142086	ATGAACAAATATAGCGAACGAGAAAATGCCATTACTTATCCCCGGCTGGCTTGAT	1142027
Query	61	GCCAAACTAACATGACAGAGAAAAACATAGAGCATACTGAAGCCTTATGGCATCAGGC 	120
Sbjct	1142026	GCCAAACTAACATGACAGAGAAAAACATAGAGCATACTGAAGCCTTATGGCATCAGGC	1141967
Query	121	GTGAAAGAATTCTCTTGCCTGGATTGTAACCTCCTCACGGGTATCGTCTACTTGTTC 	180
Sbjct	1141966	GTGAAAGAATTCTCTTGCCTGGATTGTAACCTCCTCACGGGTATCGTCTACTTGTTC	1141907
Query	181	GTAAACGGGGAGCATTACCGCCTTGTACAGACGCAGCTATACCGGAAACAGTATATGCG 	240
Sbjct	1141906	GTAAACGGGGAGCATTACCGCCTTGTACAGACGCAGCTATACCGGAAACAGTATATGCG	1141847
Query	241	GTGAAACTGGCTTCCTTGAGCATATCGTTCTACACGTAAAACATGTACTCAGATTTA 	300
Sbjct	1141846	GTGAAACTGGCTTCCTTGAGCATATCGTTCTACACGTAAAACATGTACTCAGATTTA	1141787
Query	301	GTCTGGCGTACTGTTCAACCACAGCATGATAGTGCAGTCATGGTTGCCTCAGGAGTT 	360
Sbjct	1141786	GTCTGGCGTACTGTTCAACCACAGCATGATAGTGCAGTCATGGTTGCCTCAGGAGTT	1141727
Query	361	TTTCGCCACTTCCTGACTCAGTACTCACTCAGTACTCAATCGTTGTTCTGATAGCGAAC 	420
Sbjct	1141726	TTTCGCCACTTCCTGACTCAGTACTCACTCAGTACTCAATCGTTGTTCTGATAGCGAAC	1141667
Query	421	AGACTAATGAAGGTCGTCGTTCTGGGAGCGAATGATTGCATGGCTATTCAAGGCTGAAG 	480
Sbjct	1141666	AGACTAATGAAGGTCGTCGTTCTGGGAGCGAATGATTGCATGGCTATTCAAGGCTGAAG	1141607
Query	481	GGCATTATGTATATGTGCGAATGGCTCAGAGGAAGATCGCCCCTGACTTTATGACTA 	540
Sbjct	1141606	GGCATTATGTATATGTGCGAATGGCTCAGAGGAAGATCGCCCCTGACTTTATGACTA	1141547
Query	541	ACTGGGATGATTTTATGGACATGGGCTGATTTTGTGGGGTGATGACAGAGATTGCC 	600
Sbjct	1141546	ACTGGGATGATTTTATGGACATGGGCTGATTTTGTGGGGTGATGACAGAGATTGCC	1141487
Query	601	ATTGCCATAGACTATCGTGATCAGTACAGAACATTGCACTAA 644 	
Sbjct	1141486	ATTGCCATAGACTATCGTGATCAGTACAGAACATTGCACTAA 1141443	

YPO1195

Query: Sanger sequence of sample

Subject: Y. pestis CO92 genome sequence

Query	1	GTGGCTGCTATCATGCTGCTGCATCGAGTGGTATATCGCACGCCAGATAACGTCCGT	60
Sbjct	1345534	GTGGCTGCTATCATGCTGCTGCATCGAGTGGTATATCGCACGCCAGATAACGTCCGT	1345593
Query	61	GTAGGCTCTAAAATTGATACCGAAGGCTCTTGCTGGCAATTATTGTGCAGGTTTG	120
Sbjct	1345594	GTAGGCTCTAAAATTGATACCGAAGGCTCTTGCTGGCAATTATTGTGCAGGTTTG	1345653
Query	121	GAGGCTAACGGGATTAAAACCACCAATAATCCAACTGGGAGCAACCAAAGTCGTACGT	180
Sbjct	1345654	GAGGCTAACGGGATTAAAACCACCAATAATCCAACTGGGAGCAACCAAAGTCGTACGT	1345713
Query	181	GGGGCGATTACTGCCGGTGAGATTGATATTATCCTGAATATACGGGAATGGGGCTTC	240
Sbjct	1345714	GGGGCGATTACTGCCGGTGAGATTGATATTATCCTGAATATACGGGAATGGGGCTTC	1345773
Query	241	TTCTTTCTGATGAACAAGATCCTGCCTGGAAGAGCGCCAAGCGGGCTATGAGAAAGTA	300
Sbjct	1345774	TTCTTTCTGATGAACAAGATCCTGCCTGGAAGAGCGCCAAGCGGGCTATGAGAAAGTA	1345833
Query	301	AAAGCACTGGATTACGaaaaaaaaaaTAAATTGGTATGGCTCTCTCCCGCGCAGCGAACAT	360
Sbjct	1345834	AAAGCACTGGATTACGaaaaaaaaaaTAAATTGGTATGGCTCTCTCCCGCGCAGCGAACAT	1345893
Query	361	ACCTGGACCATTGCTGTACGAAAAAGACTAGCCACGCCAATAATTGCGAACGCTGGAT	420
Sbjct	1345894	ACCTGGACCATTGCTGTACGAAAAGACTAGCCACGCCAATAATTGCGAACGCTGGAT	1345953
Query	421	GATTTAGGTTAGTGGATCAATGGCGGAGGTCAAGTTAAATTAGCCGCCCTAGCCGAGTT	480
Sbjct	1345954	GATTTAGGTTAGTGGATCAATGGCGGAGGTCAAGTTAAATTAGCCGCCCTAGCCGAGTT	1346013
Query	481	ATCGAGCGGCTGATGCGTTACCGCTTCCAACAGGGGTATGGTTTACGCTGAATCAA	540
Sbjct	1346014	ATCGAGCGGCTGATGCGTTACCGCTTCCAACAGGGGTATGGTTTACGCTGAATCAA	1346073
Query	541	GATCAACTACTGTCCTGGCTGGGGAGATACCGCTGTGACGATTAAGCGGCAGCAGA-	599
Sbjct	1346074	GATCAACTACTGTCCTGGCTGGGGAGATACCGCTGTGACGATTAAGCGGCAGCAGAA	1346133
Query	600	CAGATCTCAGGAGTGAATGCCGCCATGGCTTATGGTACCGATGGTCTGTAGCGGCACTG	659
Sbjct	1346134	CAGATCTCAGGAGTGAATGCCGCCATGGCTTATGGTACCGATGGTCTGTAGCGGCACTG	1346193
Query	660	GGTTTACAGACTCTGGAAGATACTAAAGGCCTGCAGCCGATTTATGCGCCAGCTCAATC	719
Sbjct	1346194	GGTTTACAGACTCTGGAAGATACTAAAGGCCTGCAGCCGATTTATGCGCCAGCTCAATC	1346253
Query	720	ATCCGTGAAGTTACTCTGAAAGCTCACCGAACATCCCGGTTGCTTAATCCGGTATT	779
Sbjct	1346254	ATCCGTGAAGTTACTCTGAAAGCTCACCGAACATCCCGGTTGCTTAATCCGGTATT	1346313
Query	780	GCCACACTGGATGGCCCAACGTTACAAAGCTGAATGCACGTATTGCGGTGGA	832
Sbjct	1346314	GCCACACTGGATGGCCCAACGTTACAAAGCTGAATGCACGTATTGCGGTGGA	1346366