Improving the thermostability and activity of a thermophilic subtilase by incorporating structural elements of its psychrophilic counterpart

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Running Head: Further stabilization of thermophilic WF146 protease

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TABLE S1 Oligonucleotide primers used in this study

Primer	Oligonucleotide sequence*
PB-F	5'-AATTCGGATCCGATGGATGAGAGCAAGAGC-3'
PX-R	5'-GTGCTCGAGTTGGTCGCCCGCTTGGACGGT-3'
SA-F	5'-AGCGGCACGGCCATGGCGACGCCG-3'
SA-R	5'-CGTCGCCATGGCCGTGCCGCTGAT-3'
A1-F	5'-CGCATCGAGGCATTGGCTGCTTCCGATCGAACGCCT-3'
A1-R	5'-AGGCGTTCGATCGGAAGCAGCCAATGCCTCGATGCG-3'
A2-F	5'-ACTCCTTCCCAGTCAACGCCTTGGGGG-3'
A2-R	5'-CCAAGGCGTTGACTGGGAAGGAGTCGC-3'
A3-F	5'-CCTTGGGGGATCAAAGCGATTTATCGG-3'
A3-R	5'-CCGATAAATCGCTTTGATCCCCCAAGG-3'
A4-F	5'-GAAGCGATTTATAATAATCCGTCGATC-3'
A4-R	5'-GATCGACGGATTATTATAAATCGCTTC-3'
A5-F	5'-ATTTATCGGAATTCGAACCTCACATCCACCAGCGGCGGC-3'
A5-R	5'-GCCGCCGCTGGTGGATGTGAGGTTCGAATTCCGATAAAT-3'
A7-F	5'-AGCGGCGGCGCGGGGATTCGCGTA-3'
A7-R	5'-GCGAATCCCCGCGCCGCCGCTGGT-3'
A8-F	5'-GGCGGCAAGGGGATTAACATTGCCGTCCTGGATACC-3'
A8-R	5'-GGTATCCAGGACGGCAATGTTAATCCCCTTGCCGCC-3'
A9-F	5'-GATACCGGCGTCAATACCGCGCATCCT-3'
A9-R	5'-ATGCGCGGTATTGACGCCGGTATCCAG-3'
A10-F	5'-GGCACCTATACCAATCATCCTGATCTG-3'
A10-R	5'-CAGATCAGGATGATTGGTATAGGTGCC-3'
A11-F	5'-CCTGATCTGTCCAACAATGTGGAGCAG-3'
A11-R	5'-CTGCTCCACATTGTTGGACAGATCAGG-3'
A12-F	5'-AATGTGGAGCAGTGCAAAGACTTCACCACGAGTTCG-3'
A12-R	5'-CGAACTCGTGGTGAAGTCTTTGCACTGCTCCACATT-3'
A13a-F	5'-GTGGGTACGAACTTTGTGAACGGTTGCGCGGACGGCAAC-3'
A13a-R	5'-AAAGTTCGTACCCACGGTGAAGTTGTAGCACTGCTCCAC-3'
A13b-F	5'-ACCGATAACAGTTGCGCGGACGGCAACGGACATGGG-3'
A13b-R	5'-ACTGTTATCCGGTGACAGGCGAACTCGTGGTGAAGTT-3'
A14+15-F	5'-ACGGACCGCCAGGGACATGGGACCCAC-3'
A14+15-R	5'-CTGGCGGTCCGTGCAACCGTTCACGACAGG-3'
A16-F	5'-GTAGCCGGCTCCGCCCTCGCCAACGGAGGAGGC-3'
A16-R	5'-GTTGGCGAGGGCGGAGCCGGCTACGTGGGTCCC-3'
A18-F	5'-AACGGAGGAACCGGCAGCGGGATT-3'
A18-R	5'-CCCGCTGCCGGTTCCTCCGTTGGC-3'
A19-F	5'-GGCAGCGGGGTTTACGGCGTCGCTCCG-3'
A19-R	5'-AGCGACGCCGTAAACCCCGCTGCCGCC-3'
A21-F	5'-CCGGAAGCCGATCTGTGGTCGTAT-3'
A21-R	5'-CGACCACAGATCGGCTTCCGGAGC-3'
A22-F	5'-AAGCTGTGGGCGTATAAAGTGCTG-3'
A22-R	5'-CACTTTATACGCCCACAGCTTGGC-3'

A23+24-F	5'-AAAGTGCTGGGCGACGATGGCAGCGGGTATGCGGATGAC-3'
A23+24-R	5'-CCCGCTGCCATCGTCGCCCAGCACTTTATACGACCACAG-3'
A26-F	5'-GACATCGCCGAGGCGATCCGCTAC-3'
A26-R	5'-GCGGATCGCCTCGGCGATGTCATC-3'
A27+28-F	5'-GCGATCCGCCACGCCGGTGATCAGGGCGCCAGCAACGGG-3'
A27+28-R	5'-GCCCTGATCACCGGCGTGGCGGATCGCGTAGGCGATGTC-3'
A29-F	5'-GCTGATCAGGCCACCGCCCTCAATACAAAAGTGGTGATTTCC-3'
A29-R	5'-TGTATTGAGGGCGGTGGCCTGATCAGCGGCGTAGCG-3'
A31-F	5'-GTGGTGATTAACATGTCGCTCGGC-3'
A31-R	5'-GAGCGACATGTTAATCACCACTTT-3'
A32-F	5'-GGCGAGAGTTCGTTGATTTCCAATGCC-3'
A32-R	5'-ACTCTCGCCGGAGGAGCCGAGCGACAT-3'
A33-F	5'-TCGTTGATTACCAATGCCGTGACC-3'
A33-R	5'-CACGGCATTGGTAATCAACGAATC-3'
A34-F	5'-AATGCCGTGGACTATGCCCAACAG-3'
A34-R	5'-TTGGGCATAGTCCACGGCATTGGA-3'
A35~37-F	5'-TACGAACAAAGGAGTGCTCATCATAGCGGCTGCCGGCAATTCC-3'
A35~37-R	5'-TATGATGAGCACTCCTTTGTCGTAGGCATAGGTCACGGCATT-3'
A38-F	5'-AAGCCCGGTTCGATCGGCTATCCGGGAGCG-3'
A38-R	5'-CGAACCGGGCTTAGGGCCGGAATTGCCGGC-3'
A39-F	5'-GGAGCGTTGGTGAACGCAGTAGCGGTC-3'
A39-R	5'-CGCTACTGCGTTCACCAACGCTCCCGG-3'
A41-F	5'-CTGGAAAATACAATTCAAAACGGAACGTAC-3'
A41-R	5'-TCCGTTTTGAATTGTATTTTCCAGAGCGGC-3'
A43-F	5'-CACAAGCGAACGGCCGGCGATTATGTG-3'
A43-R	5'-TCGCTTGTGCCCCCGGGAGGAGGAGTC-3'
A44-F	5'-GTGATTCAGAAGGGGGACGTGGAAGTATCCGCG-3'
A44-R	5'-TTCCACGTCCCCCTTCTGAATCACATAATCGCC-3'
A46-F	5'-GACGTGGAAATATCCGCGCCAGGACGG-3'
A46-R	5'-TGGCGCGGATATTTCCACGTCCCGCTC-3'
A47-F	5'-GCGCCAGGAGCGGCGGTCGAATCGACGTGG-3'
A47-R	5'-TTCGACCGCCGCTCCTGGCGCGGATACTTC-3'
A48-F	5'-CGGGCGGTCTATTCGACGTGGAACAACGGC-3'
A48-R	5'-CCACGTCGAATAGACCGCCCGTCCTGGCGC-3'
A49-F	5'-TCGACGTGGTTCGACGGCGGGTACAATTCCATCAGCGGC-3'
A49-R	5'-GTACCCGCCGTCGAACCACGTCGATTCGACCGCCCGTCC-3'
A50-F	5'-GGCGGGTACGCTACCATCAGCGGCACGTCCATGGCGACG-3'
A50-R	5'-GCCGCTGATGGTAGCGTACCCGCCGTTGTTCCACGTCGA-3'
A51-F	5'-TCCATGGCGTCGCCGCATATCTCCGGG-3'
A51-R	5'-GATATGCGGCGACGCCATGGACGTGCC-3'
A52-F	5'-ACGCCGCATGCCGCCGGGTTGGCAGCC-3'
A52-R	5'-TGCCAACCCGGCGGCATGCGGCGTCGC-3'
A53+54-F	5'-CAGTCTCCGGCAGCGAGCAATAACGATGTCCGTACCGAGCTGCAA-3'
A53+54-R	5'-CGCTGCCGGAGACTGTGCCCAGATTTTGGCTGCCAACCCGGAGAT-3'

A55-F	5'-TGGAGCAATGTCGATGTCCGTACCGAG-3'
A55-R	5'-ACGGACATCGACATTGCTCCATGTCGG-3'
A56-F	5'-GATGTCCGTGGCGAGCTGCAAAACCGC-3'
A56-R	5'-TTGCAGCTCGCCACGGACATCGTTATT-3'
A57-F	5'-GAGCTGCAAACCCGCGCGAAAGCG-3'
A57-R	5'-TTTCGCGCGGGTTTGCAGCTCGGT-3'
A58-F	5'-AACCGCGCGTCAGTGAATGACATCCTGGGAGGCATC-3'
A58-R	5'-GATGTCATTCACTGACGCGCGGTTTTGCAGCTCGGT-3'
A59+60-F	5'-GACATCCTGTCAGGCAACAGCGCTGCGGGAGGCGATGAT-3'
A59+60-R	5'-CGCAGCGCTGTTGCCTGACAGGATGTCATTCGCTTTCGC-3'
A61-F	5'-ATCAGCGCTGGGTCAGGCGATGATATCGCTTCCGGC-3'
A61-R	5'-ATCATCGCCTGACCCAGCGCTGATGCCTCCCAGGAT-3'
A63-F	5'-TTCGGATTCGCGAAAGTCCAAGCGGGCGACCAAGTCGAC-3'
A63-R	5'-CGCTTGGACTTTCGCGAATCCGAAGCCGGAAGCGATATC-3'
A64-F	5'-TTCCCGACCGTCCAACTCGAG*CACCACCACCACCACCAC*-3'
A64-R	5'-*GTGGTGGTGGTGGTGGTG*CTCGAGTTGGACGGTCGGGAA-3'
B1+2-F	5'-CGTGCTTCCCAACAAATCCCTTGGGGGATCGAAGCGATTTATCGG-3'
B1+2-R	5'-GATTTTGTTGGGAAGCACGCAATGCCTCGATGCGCCCCTCCTCCTC-3'
B4+5-F	5'-AACAATGATACGTTAACATCCACCAGCGGCGGCAAGGGGATT-3'
B4+5-R	5'-TGTTAACGTATCATTGTTATAAATCGCTTCGATCCCCCAAGG-3'
B6-F	5'-CAATCCACCACCGGCGGCAAGGGG-3'
B6-R	5'-CTTGCCGCCGGTGGTGGATTGGAT-3'
B7-F	5'-AGCGGCGGCTCGGGGATTCGCGTA-3'
B7-R	5'-GCGAATCCCCGAGCCGCCGCTGGT-3'
B9+10-F	5'-GTCAATACCTCGCATCCTGATCTGTCCGCCAAT-3'
B9+10-R	5'-CGAGGTATTGACGCCGGTATCCAGGACGGCTAC-3'
B11-F	5'-CCTGATCTGGTCAACAATGTGGAGCAGTGC-3'
B11-R	5'-CTCCACATTGTTGACCAGATCAGGATGCGC-3'
B13a -F	5'-AACTTCACCGGAGCTACGACTCCTGTCGTGAACGGTTGCGCG-3'
B13a -R	5'-CACGACAGGAGTCGTAGCTCCGGTGAAGTTGTAGCACTGCTC-3'
B13b'-F	5'-AGTTCGCCTATCAACAACGGTTGCGCGGAC-3'
B13b'-R	5'-GCAACCGTTGTTGATAGGCGAACTCGTGGT-3'
B13b''-F	5'-GTCGTGAACAGTTGCGCGGACGGC-3'
B13b''-R	5'-GTCCGCGCAACTTGTTCACGACAGG-3'
B14-F	5'-AACGGTTGCACGGACGGCAACGGA-3'
B14-R	5'-GTTGCCGTCCGTGCAACCGTTCAC-3'
B15-F	5'-TGCGCGGACCGCAACGGACATGGG-3'
B15-R	5'-ATGTCCGTTGCGGTCCGCGCAACC-3'
B16-F	5'-GCCGGCACCGCCCTCGCCAACGGA-3'
B16-R	5'-GTTGGCGAGGGCGGTGCCGGCTAC-3'
B17-F	5'-ATCCTCGCCGACGGAGGAGGCGGC-3'
B17-R	5'-GCCTCCTCCGTCGGCGAGGATGGT-3'
B18-F	5'-AGCGACCAAGCCGGGATTTGGGGCGTCGCTCCG-3'
B18-R	5'-GGCTTGGTCGCTTCCTCCGTTGGCGAGGATGGT-3'

B19-F	5'-AGCGGGATTTACGGCGTCGCTCCG-3'
B19-R	5'-AGCGACGCCGTAAATCCCGCTGCC-3'
B20-F	5'-GTCGCTCCGGATGCCAAGCTGTGG-3'
B20-R	5'-CAGCTTGGCATCCGGAGCGACGCC-3'
B23+24-F	5'-AAAGTGCTGTTAGACAGTGGCAGCGGGTATGCGGATGAC-3'
B23+24-R	5'-CCCGCTGCCACTGTCTAACAGCACTTTATACGACCACAG-3'
B25-F	5'-AGCGGGTATTCGGATGACATCGCC-3'
B25-R	5'-GATGTCATCCGAATACCCGCTGCC-3'
B26-F	5'-GACATCGCCGCCGCGATCCGCTAC-3'
B26-R	5'-GCGGATCGCGGCGGCGATGTCATC-3'
B27-F	5'-GCGATCCGCCACGCCGCTGATCAG-3'
B27-R	5'-ATCAGCGGCGTGGCGGATCGCGTA-3'
B29a -F	5'-GCCAACCGCCACCGGGGTAAAAGTGGTGATTTCCATG-3'
B29a -R	5'-GGTGGCGGTGGCCTGATCAGCGGCGTAGCGGATCGC-3'
B29b+30-F	5'-ACAAAAACGATTATTTCCATGTCGCTCGGCTCCTCC-3'
B29b+30-R	5'-AATCGTTTTTGTCCCGTTGCTGGCGCCCTGATCAGC-3'
B32-F	5'-GGCTCCTCCGCCAATAATTCGTTGATTTCCAATGCCGTG-3'
B32-R	5'-AATCAACGAATTATTGGCGGAGGAGCCGAGCGACATGGA-3'
B33-F	5'-TTGATTTCCAGTGCCGTGACCTAT-3'
B33-R	5'-GGTCACGGCACTGGAAATCAACGA-3'
B34-F	5'-AATGCCGTGAACTATGCCCAACAG-3'
B34-R	5'-TTGGGCATAGTTCACGGCATTGGA-3'
B35-F	5'-ACCTATGCCTATTCGAAAGGAGCGCTCGTGGTAGCGGCT-3'
B35-R	5'-GAGCGCTCCTTTCGAATAGGCATAGGTCACGGCATTGGA-3'
B36+37-F	5'-CAGCGAGGAGTGCTCATCGTAGCGGCTGCCGGCAATTCC-3'
B36+37-R	5'-AGCCGCTACGATGAGCACTCCTCGCTGTTGGGCATAGGT-3'
B38-F	5'-AATTCCGGCTATTCGCAGGGTACGATCGGCTATCCGGGAGCGTTG-3'
B38-R	5'-GCCGATCGTACCCTGCGAATAGCCGGAATTGCCGGCAGCCGCTAC-3'
B39+40-F	5'-CCGAACGCAATTGCGGTCGCCGCTCTGGAAAATATA-3'
B39+40-R	5'-AATTGCGTTCGGCAACGCTCCCGGATAGCCGATCGT-3'
B41-F	5'-CTGGAAAATGTACAGCAAAACGGA-3'
B41-R	5'-GTTTTGCTGTACATTTTCCAGAGC-3'
B42-F	5'-GTGGCTGACTACTCCTCCCGGGGG-3'
B42-R	5'-CCGGGAGGAGTAGTCAGCCACACG-3'
B43-F	5'-TCCCGGGGGTACATCTCAACGGCCGGCGATTATGTG-3'
B43-R	5'-GCCGGCCGTTGAGATGTACCCCCGGGAGGAGGAGTC-3'
B44~46-F	5'-GGGGACATCGAAATTTCCGCGCCAGGACGGGCGGTCGAA-3'
B44~46-R	5'-AATTTCGATGTCCCCCTCCTGAATCACATAATCGCCGGC-3'
B47+48-F	5'-TCGTCGGTCTATTCGACGTGGAACAACGGCGGGTAC-3'
B47+48-R	5'-ATAGACCGACGATCCTGGCGCGGATACTTCCACGTC-3'
B49-F	5'-TCGACGTGGTACAACGGCGGGTAC-3'
B49-R	5'-CCCGCCGTTGTACCACGTCGATTC-3'
B50-F	5'-GGGTACAATACCATCAGCGGCACG-3'
B50-R	5'-GCCGCTGATGGTATTGTACCCGCC-3'

B52-F	5'-ACGCCGCATGTCTCCGGGTTGGCA-3'
B52-R	5'-CAACCCGGAGACATGCGGCGTCGC-3'
B53+54-F	5'-GAGAATCCGTCATTGAGCAATAACGATGTCCGTACCGAG-3'
B53+54-R	5'-CAATGACGGATTCTCTGCCCAGATTTTGGC
B55-F	5'-TGGAGCAATACCCAACTCCGTACCGAGCTGCAAAACCGC-3'
B55-R	5'-CTCGGTACGGAGTTGGGGTATTGCTCCATGTCGGATGCGA-3'
B56-F	5'-GATGTCCGTTCCAACCTGCAAAACCGCGCG-3'
B56-R	5'-GTTTTGCAGGTTGGAACGGACATCGTTATT-3'
B57-F	5'-GAGCTGCAAGAGCGCGCGAAAGCG-3'
B57-R	5'-TTTCGCGCGCTCTTGCAGCTCGGT-3'
B58-F	5'-CGCGCGAAATCGGTTGACATCCTGGGAGGC-3'
B58-R	5'-CAGGATGTCAACCGATTTCGCGCGGTTTTG-3'
B59-F	5'-AATGACATCAAGGGAGGCATCAGC-3'
B59-R	5'-GATGCCTCCCTTGATGTCATTCGC-3'
B60-F	5'-CTGGGAGGCTACGGCGCTGCGGGAGGCGAT-3'
B60-R	5'-TCCCGCAGCGCCGTAGCCTCCCAGGATGTC-3'
B61-F	5'-AGCGCTGCGATTGGCGATGATATC-3'
B61-R	5'-ATCATCGCCAATCGCAGCGCTGAT-3'
B62-F	5'-GGCGATGATTACGCTTCCGGCTTC-3'
B62-R	5'-GCCGGAAGCGTAATCATCGCCTCC-3'
B63-F	5'-TTCGGATTCGCGCGCGTCCAAGCGGGCGAC-3'
B63-R	5'-CGCTTGGACGCGCGCGAATCCGAAGCCGGA-3'

* Underlined sequences indicate the restriction enzyme sites used in this study. Boxes indicate the mutated nucleotides. Italicized sections indicate the His_6-tag-encoding DNA sequences.

TABLE S2 Primer pairs, template, PCR methods and restriction enzyme sites used in plasmid construction.

Plasmid	Primer pair	Template	Method	Restriction site
pWT	PB-F/PX-R	Genomic DNA		*Bam*HI/*Xho* I
pWA1	PB-F/A1-R (5'-end) A1-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA2	A2-F/A2-R	pWT	Quickchange	–
pWA3	A3-F/A3-R	pWT	Quickchange	–
pWA4	A4-F/A4-R	pWT	Quickchange	–
pWA5	PB-F/A5-R (5'-end) A5-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA7	PB-F/A7-R (5'-end) A7-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA8	A8-F/A8-R	pWT	Quickchange	–
pWA9	PB-F/A9-R (5'-end) A9-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA10	A10-F/A10-R	pWT	Quickchange	–
pWA11	A11-F/A11-R	pWT	Quickchange	–
pWA12	A12-F/A12-R	pWT	Quickchange	–
pWA13a	PB-F/A13a-R (5'-end) A13a-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA13b	PB-F/A13b-R (5'-end) A13b-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA14+15	PB-F/A14+15-R (5'-end) A14+15-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA16	PB-F/A16-R (5'-end) A16-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA18	A18-F/A18-R	pWT	Quickchange	–
pWA19	A19-F/A19-R	pWT	Quickchange	–
pWA21	A21-F/A21-R	pWT	Quickchange	–
pWA22	A22-F/A22-R	pWT	Quickchange	–
pWA23+24	PB-F/A23+24-R (5'-end) A23+24-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA26	A26-F/A26-R	pWT	Quickchange	–
pWA27+28	PB-F/A27+28-R (5'-end) A27+28-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA29	PB-F/A29-R (5'-end) A29-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA31	A31-F/A31-R	pWT	Quickchange	–
pWA32	PB-F/A32-R (5'-end) A32-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA33	A33-F/A33-R	pWT	Quickchange	–
pWA34	PB-F/A34-R (5'-end) A34-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I

pWA35~37	PB-F/A35~37-R (5'-end) A35~37-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA38	PB-F/A38-R (5'-end) A38-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA39	A39-F/A39-R	pWT	Quickchange	–
pWA41	A41-F/A41-R	pWT	Quickchange	–
pWA43	PB-F/A43-R (5'-end) A43-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA44	A44-F/A44-R	pWT	Quickchange	–
pWA46	A46-F/A46-R	pWT	Quickchange	–
pWA47	A47-F/A47-R	pWT	Quickchange	–
pWA48	A48-F/A48-R	pWT	Quickchange	–
pWA49	PB-F/A49-R (5'-end) A49-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA50	PB-F/A50-R (5'-end) A50-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA51	A51-F/A51-R	pWT	Quickchange	–
pWA52	A52-F/A52-R	pWT	Quickchange	–
pWA53+54	PB-F/A53+54-R (5'-end) A53+54-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWA55	A55-F/A55-R	pWT	Quickchange	–
pWA56	A56-F/A56-R	pWT	Quickchange	–
pWA57	A57-F/A57-R	pWT	Quickchange	–
pWA58	A58-F/A58-R	pWT	Quickchange	–
pWA59+60	A59+60-F/A59+60-R	pWT	Quickchange	–
pWA61	A61-F/A61-R	pWT	Quickchange	–
pWA63	A63-F/A63-R	pWT	Quickchange	–
pWA64	A64-F/A64-R	pWT	Quickchange	–
pWB1+2	PB-F/B1+2-R (5'-end) B1+2-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB4+5	PB-F/B4+5-R (5'-end) B4+5-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB6	B6-F/B6-R	pWT	Quickchange	–
pWB7	B7-F/B7-R	pWT	Quickchange	–
pWB9+10	PB-F/B9+10-R (5'-end) B9+10-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB11	B11-F/B11-R	pWT	Quickchange	–
pWB13a	B13a-F/B13a-R	pWT	Quickchange	–
pWB13b'	B13b'-F/B13b'-R	pWT	Quickchange	–
pWB13b"	B13b"-F/B13b"-R	pWT	Quickchange	–
pWB14	B14-F/B14-R	pWT	Quickchange	–
pWB15	B15-F/B15-R	pWT	Quickchange	–
pWB16	B16-F/B16-R	pWT	Quickchange	–
pWB17	B17-F/B17-R	pWT	Quickchange	–

pWB18	PB-F/B18-R (5'-end) B18-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB19	B19-F/B19-R	pWT	Quickchange	–
pWB20	B20-F/B20-R	pWT	Quickchange	–
pWB23+24	PB-F/B23+24-R (5'-end) B23+24-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB25	B25-F/B25-R	pWT	Quickchange	–
pWB26	B26-F/B26-R	pWT	Quickchange	–
pWB27	B27-F/B27-R	pWT	Quickchange	–
pWB29a	PB-F/B29a-R (5'-end) B29a-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB29b+30	PB-F/B29b+30-R (5'-end) B29b+30-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB32	B32-F/B32-R	pWT	Quickchange	–
pWB33	B33-F/B33-R	pWT	Quickchange	–
pWB34	B34-F/B34-R	pWT	Quickchange	–
pWB35	B35-F/B35-R	pWT	Quickchange	–
pWB36+37	PB-F/B36+37-R (5'-end) B36+37-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB38	PB-F/B38-R (5'-end) B38-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB39+40	PB-F/B39+40-R (5'-end) B39+40-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB41	B41-F/B41-R	pWT	Quickchange	–
pWB42	B42-F/B42-R	pWT	Quickchange	–
pWB43	PB-F/B43-R (5'-end) B43-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB47+48	PB-F/B47+48-R (5'-end) B47+48-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB49	B49-F/B49-R	pWT	Quickchange	–
pWB50	B50-F/B50-R	pWT	Quickchange	–
pWB52	B52-F/B52-R	pWT	Quickchange	–
pWB53+54	PB-F/B53+54-R (5'-end) B53+54-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB55	B55-F/B55-R	pWT	Quickchange	–
pWB56	B56-F/B56-R	pWT	Quickchange	–
pWB57	B57-F/B57-R	pWT	Quickchange	–
pWB58	B58-F/B58-R	pWT	Quickchange	–
pWB59	B59-F/B59-R	pWT	Quickchange	–
pWB60	PB-F/B60-R (5'-end) B60-F/PX-R (3'-end)	pWT	Overlap	*Bam*HI/*Xho* I
pWB61	B61-F/B61-R	pWT	Quickchange	–
pWB62	B62-F/B62-R	pWT	Quickchange	–
pWB63	PB-F/B63-R (5'-end)	pWT	Overlap	*Bam*HI/*Xho* I

	B63-F/PX-R (3'-end)			
pWA23+24+31	A31-F/A31-R	pWA23+24	Quickchange	–
pWA31+47	A31-F/A31-R	pWA47	Quickchange	–
pWA23+24+47	A47-F/A47-R	pWA23+24	Quickchange	–
pWA47+WB16	B16-F/B16-R	pWA47	Quickchange	–
pWA31+WB16	B16-F/B16-R	pWA31	Quickchange	–
pWA31+WB50	B50-F/B50-R	pWA31	Quickchange	–
pSatb	B50-F/B50-R	pWA31+47	Quickchange	–
pSatg	PB-F/A23+24-R (5'-end) A23+24-F/PX-R (3'-end)	pSatb	Overlap	*Bam*HI/*Xho* I
pSatf	B16-F/B16-R	pSatb	Quickchange	–
pSath	PB-F/A23+24-R (5'-end) A23+24-F/PX-R (3'-end)	pSatf	Overlap	*Bam*HI/*Xho* I
pWA31+49	A31-F/A31-R	pWA49	Quickchange	–
pWA22+56	A22-F/A22-R	pWA56	Quickchange	–
pWA22+58	A22-F/A22-R	pWA58	Quickchange	–
pWA31+56	A31-F/A31-R	pWA56	Quickchange	–
pWA31+58	A31-F/A31-R	pWA58	Quickchange	–
pWA49+56	A56-F/A56-R	pWA49	Quickchange	–
pWA49+56	A58-F/A58-R	pWA49	Quickchange	–
pWA56+58	A58-F/A58-R	pWA56	Quickchange	–
pWA22+31	A22-F/A22-R	pWA31	Quickchange	–
pWA22+49	A22-F/A22-R	pWA49	Quickchange	–
pWA22+31+49	A31-F/A31-R	pWA22+49	Quickchange	–
pPBL5X	PB-F/A22-R (5'-end) A22-F/PX-R (3'-end) PB-F/A49-R (5'-end) A49-F/PX-R (3'-end) A56-F/A56-R	pSatf	Overlap + Quickchange	*Bam*HI/*Xho* I
pPBL5X+WA58	A58-F/A58-R	pPBL5X	Quickchange	–
pS/A	SA-F/SA-R	pWT	Quickchange	–
pP-S/A	SA-F/SA-R	pPBL5X	Quickchange	–

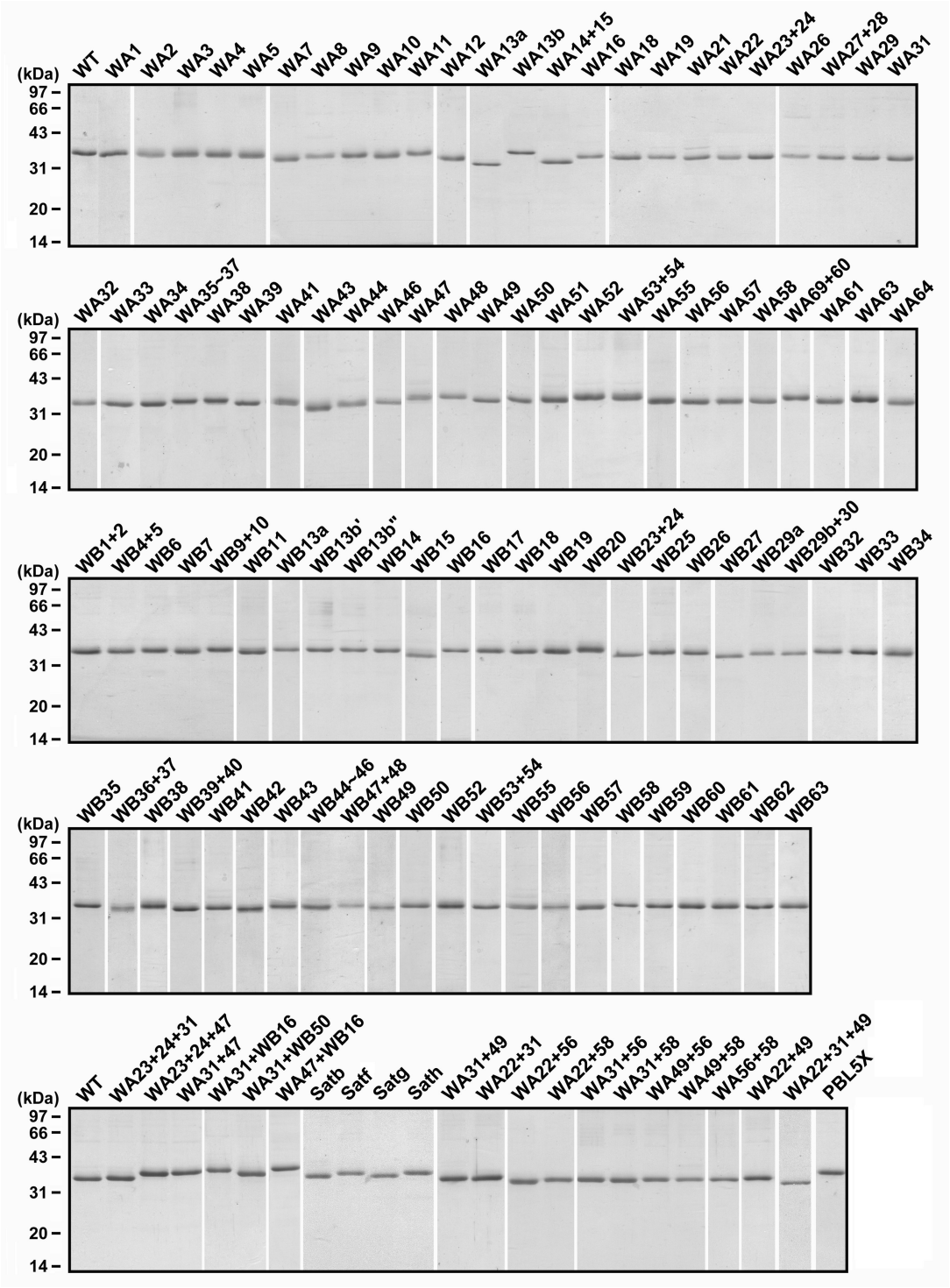

Figure S1. SDS-PAGE analysis of the purified samples of the mature enzymes. The mature forms of wild-type WF146 protease (WT) and its variants were purified and subjected to SDS-PAGE analysis as described under "Materials and Methods".