Supplemental Information for Cahoon et al.

Supplemental Figures

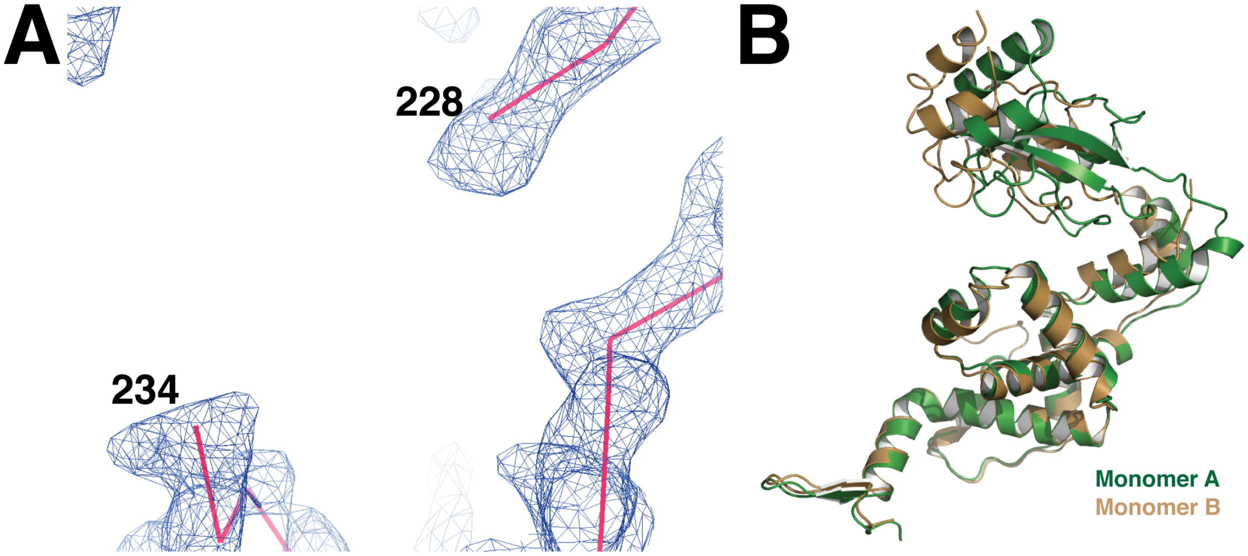

Figure S1: PrsA1 monomers exhibit different conformations of the PPIase (A) Electron density of residues 228 to 234 in monomer B contoured at 1 rmsd or 0.19 $e/\text{Å}^3$. (B) Monomer A (green) and monomer B (gold) were aligned in Coot relative to the Foldase domain (residues 22-125 and 238-282) with an RMS of 1.021 Å^2. The PPIase of monomer B is shifted by approximately 8 Å compared to monomer A.

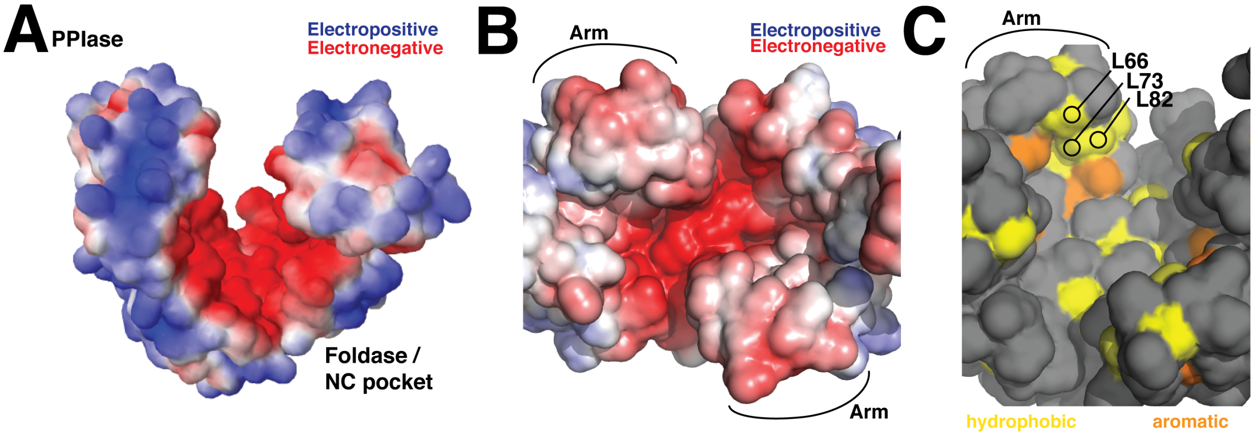

Figure S2: Molecular surface comparison of *B. subtilis* PrsA and *Lm* PrsA1 (A) The dimeric PrsA structure (PDB accession code 4WO7) was used to calculate surface charge by APBS. (B) Electrostatic surface potential of the *Lm* PrsA1 foldase domain pocket generated by APBS. The contour is -5 to 5 kT/e. The ‘Arm’ regions of the Foldase domain are also outlined. (C) The hydrophobic character of *B. subtilis* NC or foldase domain. The hydrophobic patch on the arm domain is highlighted. Hydrophobic residues (A, G, I, L, M, F, V) are colored in yellow, aromatic residues (Y, W, but not F) in orange.

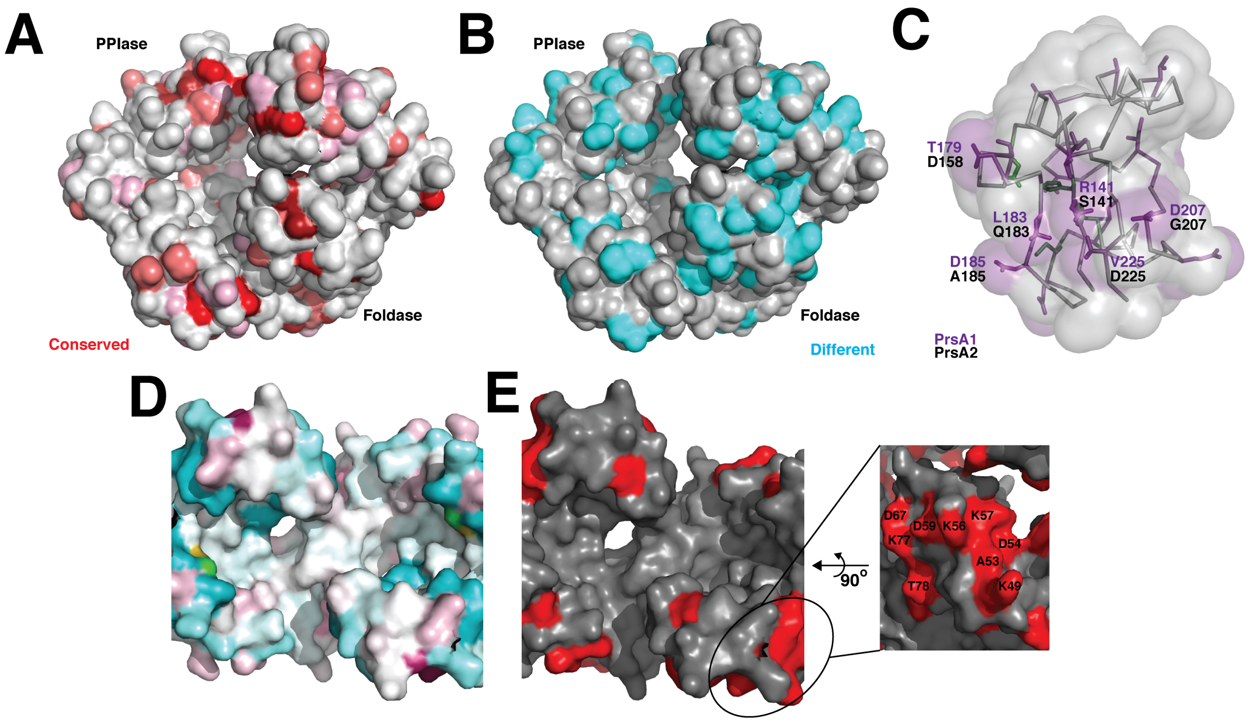

Figure S3: Surface residue conservation of the Lm PrsA1 and PrsA2 molecular chaperones. (A) Conserved surface residues of the PrsA chaperone family mapped to the *Lm* PrsA1 structure as determined by Consurf. Homologus residues are pink and dark pink and strictly conserved in red. (B) Surface residues that differ between *Lm* PrsA1 and *Lm* PrsA2 are shown in blue (C) A 90° rotation from Figure 3A is shown to display the surface opposite the PPIase active site. A patch of residue difference between PrsA1 (purple) and PrsA2 (black) is shown. (D) Consurf server output of the Lm PrsA1 foldase pocket showing conserved residues from 89 PrsA sequences. Blue represents no convervation (random residue), white neutral, and pink to dark red increasing conservation with dark red as absolution conservation. (E) Non-homologous sequence differences between *Lm* PrsA1 and *Lm* PrsA2 foldase are highlighted in red. Left panel is looking down into the Foldase domain from the PPIase domain(s). The right panel is a 90° rotation and zoom of the outer face of the ‘Arm’ region displaying a highly charged patch of residues that differ between *Lm* PrsA1 and *Lm* PrsA2. The PrsA1 residues are listed.

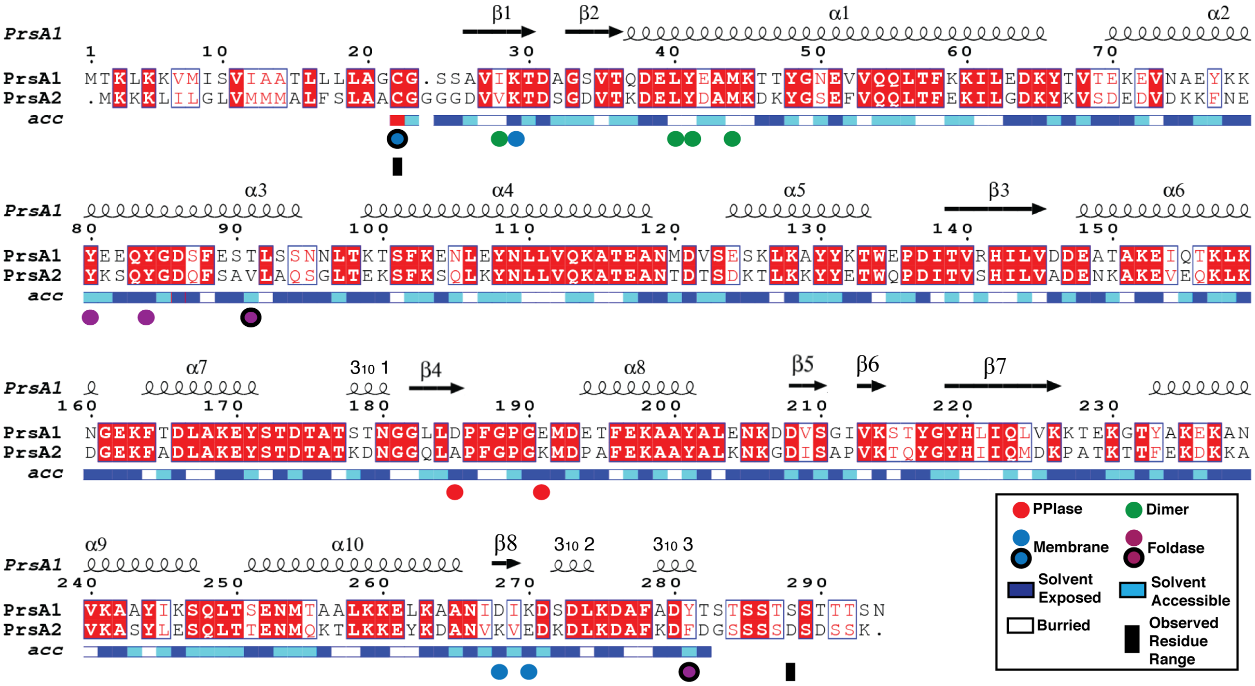

Figure S4: Annotated sequence alignment of *Lm* PrsA1 and *Lm* PrsA2 The alignment was generated using ClustalW and annotated with secondary structural elements using Espript3 (http://espript.ibcp.fr/ESPript/ESPript/). Areas of sequence identity are outlined by red boxes and sequence homology by red letters. Secondary structure elements are represented by spirals (α-helix) and arrows (β-sheet). Solvent accessible and buried residues are indicated by blue (solvent exposed), cyan (solvent accessible), and white (buried) boxes. Residues that were mutated are indicated by colored circle with a black outline showing residues that were studied individually.

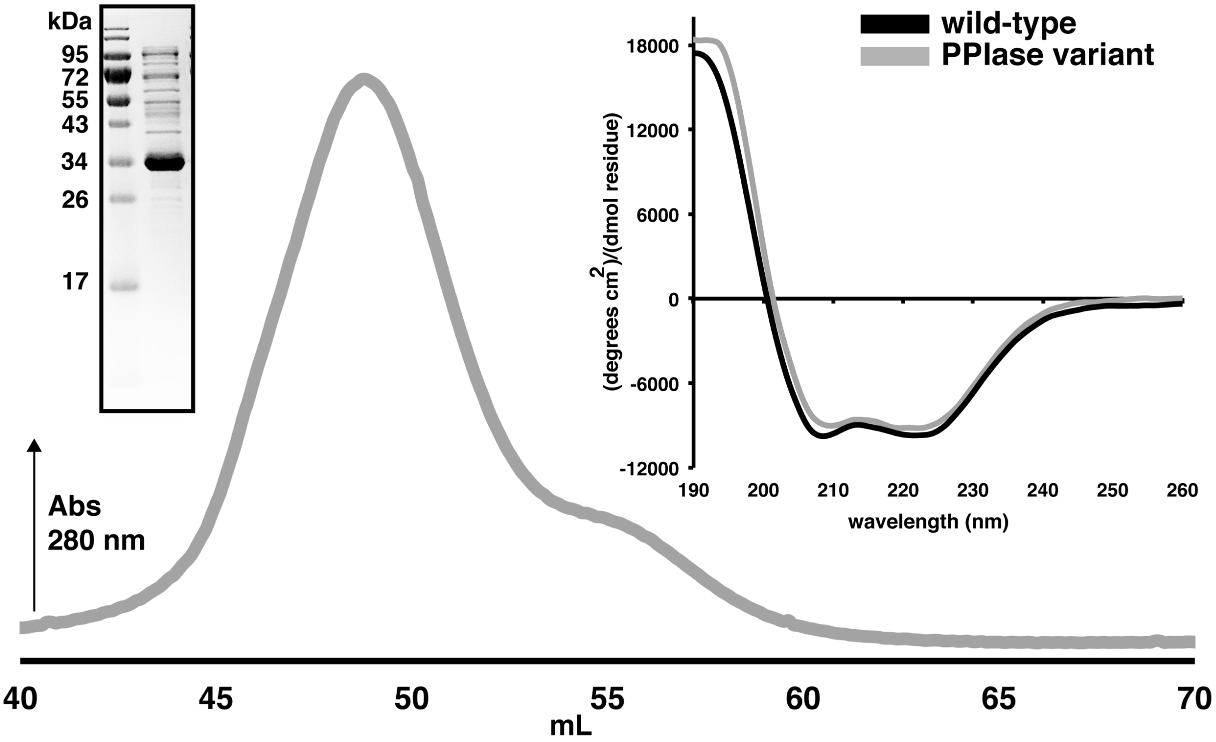

Figure S5: PPIase variant control assay Gel-filtration profile of the PrsA1 PPIase variant (D185A/E191K). Dimerized PrsA1 elutes at ~ 49 mL and monomeric PrsA1 at ~ 55 mL. The left inset shows the purified variant PrsA1 separated by SDS-PAGE gel visualized by Coomassie stain. The right inset shows the circular dichroism spectra of PrsA1 wild-type and the PPIase variant plotted as mean residue ellipticity.

Table S1. Strains and Designations

Strain or Plasmid	Description	Designation	Reference
TOP10	*E. coli* propagation strain		Invitrogen
S17-1	*E. coli* conjugation strain		
SM10	*E. coli* conjugation strain		
BL21 (DE3)*	*E. coli* protein expression strain		Invitrogen
DH5α I/q	*E. coli* protein expression strain		NEB
NF-L100	*L. monocytogenes* 10403S parent strain	wt	(Bishop & Hinrichs, 1987)
NF-L1651	10403S with Δ*prsA2*::*erm*	Δ*prsA2*	(Alonzo *et al.*, 2009)
NF-L1631	NF-L1438 (Δ*prsA1*) transduced with Δ*prsA2*::*erm*	Δ*prsA2*/Δ*prsA1*	(Alonzo & Freitag, 2010)
NF-L3790	NF-L1651 with integrated pPL2-*prsA2* wild-type	*prsA2* wt complement	This work
NF-L3792	NF-L1651 with integrated pPL2-*prsA2* A185D/K191E	PPIase A185D/K191E	This work
NF-L3794	NF-L1651 with integrated pPL2-*prsA2* K268A/K29A	Membrane K268A/K29A	This work
NF-L3796	NF-L1651 with integrated pPL2-*prsA2* K268A/K29A/C21S	Membrane K268A/K29A/C21S	This work
NF-L3798	NF-L1651 with integrated pPL2-*prsA2* C21S	Membrane C21S	This work
NF-L3800	NF-L1651 with integrated pPL2-*prsA2* F281A	Foldase F281A	This work
NF-L3802	NF-L1651 with integrated pPL2-*prsA2* F281A/Y80A/Y84A	Foldase F281A/Y80A/Y84A	This work
NF-L3804	NF-L1651 with integrated pPL2-*prsA2* V90T	Foldase V90T	This work
NF-L3806	NF-L1651 with integrated pPL2-*prsA2* V28A/L40A/Y41A/M44A	Dimer V28A/L40A/Y41A/M44A	This work
NF-L3808	NF-L1651 with integrated pPL2-*prsA2* V28A/L40A/Y41A	Dimer V28A/L40A/Y41A	This work
NF-L3810	NF-L1651 with integrated pPL2-*prsA2* V28A/L40A	Dimer V28A/L40A	This work
NF-L3812	NF-L1651 with integrated	Dimer V28A	This work

	pPL2-*prsA2* V28A		
NF-L3814	NF-L1631 with integrated pPL2-*prsA1* wild-type	*prsA1* wt complement	This work
NF-L3816	NF-L1631 with integrated pPL2-*prsA1* D185A/E191K	PPIase D185A/E191K	This work
NF-L3818	NF-L1631 with integrated pPL2-*prsA1* K270E/K29A	Membrane K270E/K29A	This work
NF-L3820	NF-L1631 with integrated pPL2-*prsA1* K270E/K29A/C21S	Membrane K270E/K29A/C21S	This work
NF-L3822	NF-L1631 with integrated pPL2-*prsA1* C21S	Membrane C21S	This work
NF-L3823	NF-L1631 with integrated pPL2-*prsA1* Y281A	Foldase Y281A	This work
NF-L3824	NF-L1631 with integrated pPL2-*prsA1* Y281A/Y80A/Y84A	Foldase Y281A/Y80A/Y84A	This work
NF-L3826	NF-L1631 with integrated pPL2-*prsA1* T91V	Foldase T91V	This work
NF-L3828	NF-L1631 with integrated pPL2-*prsA1* V28A/L40A/Y41A/M44A	Dimer V28A/L40A/Y41A/M44A	This work
NF-L3830	NF-L1631 with integrated pPL2-*prsA1* V28A/L40A/Y41A	Dimer I28A/L40A/Y41A	This work
NF-L3832	NF-L1631 with integrated pPL2-*prsA1* V28A/L40A	Dimer I28A/L40A	This work
NF-L3834	NF-L1631 with integrated pPL2-*prsA1* V28A	Dimer I28A	This work
pQE60	C-terminal His-tagged expression strain		Qiagen
pNF-1965	pQE60-*prsA1*		(Alonzo *et al.*, 2011)
pNF-1764	pQE60-*prsA2*	wild-type	(Alonzo et al., 2011)
pNF-4037	pQE60-*prsA1* V28A/L40A/Y41A/M44A	dimer variant	This work
pPL2	Integrative vector for single copy gene complementation in *Lm*		(Lauer *et al.*, 2002)
pNF-3838	pPL2-*prsA2* (containing the open reading from of *prsA2* including 580 bp upstream and 68 bp downstream region)		This work
pNF-3840	pPL2-*prsA2* A185D/K191E		This work
pNF-3842	pPL2-*prsA2* K268A/K29A		This work
pNF-3844	pPL2-*prsA2*		This work

	K268A/K29A/C21S		
pNF-3846	pPL2-*prsA2* C21S		This work
pNF-3848	pPL2-*prsA2* F281A		This work
pNF-3850	pPL2-*prsA2* F281A/Y80A/Y84A		This work
pNF-3852	pPL2-*prsA2* V90T		This work
pNF-3854	pPL2-*prsA2* V28A/L40A/Y41A/M44A		This work
pNF-3855	pPL2-*prsA2* V28A/L40A/Y41A		This work
pNF-3856	pPL2-*prsA2* V28A/L40A		This work
pNF-3858	pPL2-*prsA2* V28A		This work
pNF-3860	pPL2-*prsA1* (containing the open reading from of *prsA1* including 95 bp upstream and 66 bp downstream region)		This work
pNF-3862	pPL2-*prsA1* D185A/E191K		This work
pNF-3864	pPL2-*prsA1* K270E/K29A		This work
pNF-3866	pPL2-*prsA1* K270E/K29A/C21S		This work
pNF-3867	pPL2-*prsA1* C21S		This work
pNF-3868	pPL2-*prsA1* Y281A		This work
pNF-3870	pPL2-*prsA1* Y281A/Y80A/Y84A		This work
pNF-3872	pPL2-*prsA1* T91V		This work
pNF-3874	pPL2-*prsA1* V28A/L40A/Y41A/M44A		This work
pNF-3876	pPL2-*prsA1* V28A/L40A/Y41A		This work
pNF-3878	pPL2-*prsA1* V28A/L40A		This work
pNF-3880	pPL2-*prsA1* V28A		This work

Table S2. Oligonucleotides used in this study.

Oligonucleotide	**Sequence**	**Use**
prsA2UPSacF1	5’AGTAGAGCTCggctagctgccgtatgtatt	Forward primer for amplification of and complementation with of *prsA2*
prsA2XmaR1	5'GCGCCCGGGTTCGTTTTATAATC	Reverse primer for amplification of and complementation with of *prsA2*
prsA1UPSacF1	5’AGTAGAGCTCGACGGGCACAAATTCGAAGT	Forward primer for amplification of and complementation with of *prsA1*
prsA1XmaR2	5’AGTACCCGGGGCGTGTGTTTTTGACGGGAA	Reverse primer for amplification of and complementation with of *prsA1*

LmPrsA2F1	5’ATCGAAAGTGCAGAACGTGG	Sequencing of *prsA2*
LmPrsA2F1RC	5’CCACGTTCTGCACTTTCGAT	Sequencing of *prsA2*
LmPrsA2F2	5’CTGACACTAGCGACAAAACAC	Sequencing of *prsA2*
LmPrsA2F2RC	5’GTGTTTTGTCGCTAGTGTCAG	Sequencing of *prsA2*
LmPrsA1F1	5’CCAGATATTACTGTTCGCC	Sequencing of *prsA1*
LmPrsA1F1RC	5’GGCGAACAGTAATATCTGG	Sequencing of *prsA1*
prsA1-D185A	5’CAAAAAAATCTTAGAAGcTAAATACACCGTAACAG	Site directed mutagenesis
prsA1-D185Arc	5’CTGTTACGGTGTATTTAgCTTCTAAGATTTTTTTG	Site directed mutagenesis
prsA1-E191K	5’TAAATACACCGTAACAaAAAAAGAAGTAAATGC	Site directed mutagenesis
prsA1-E191Krc	5’GCATTTACTTCTTTTTtTGTTACGGTGTATTTA	Site directed mutagenesis
prsA1-I28A	5’GGAAGTAGCGCTGTTgcAAAAACAGATGCAGG	Site directed mutagenesis
prsA1-I28Arc	5’CCTGCATCTGTTTTTgcAACAGCGCTACTTCC	Site directed mutagenesis
prsA1-L40A	5’GTCACACAAGATGAGgcTTACGAAGCAATGAAAACG	Site directed mutagenesis
prsA1-L40Arc	5’CGTTTTCATTGCTTCGTAAgcCTCATCTTGTGTGAC	Site directed mutagenesis
prsA1-Y41A	5’CACACAAGATGAGgcTgcCGAAGCAATGAAAACGAC	Site directed mutagenesis , contains mutation L40A in addition to Y41A
prsA1-Y41Arc	5’GTCGTTTTCATTGCTTCGgcAgcCTCATCTTGTGTG	Site directed mutagenesis, contains mutation L40A in addition to Y41A
prsA1-M44A	5’GAGgcTgcCGAAGCAgcGAAAACGACTTATGGAAATG	Site directed mutagenesis, contains mutations L40A and Y41A in addition to M44A
prsA1-M44Arc	5’CATTTCCATAAGTCGTTTTCgcTGCTTCGgcAgcCTC	Site directed mutagenesis, contains mutations L40A and Y41A in addition to M44A
prsA1-K270E	5’GCTAATATTGATAATTgAAGATAGTGATTT	Site directed mutagenesis
prsA1-K270Erc	5’AAATCACTATCTTcAATTATCAATATTAGC	Site directed mutagenesis
prsA1-K29A	5’GTAGCGCTGTTATAgcAACAGATGCAGG	Site directed mutagenesis
prsA1-K29Arc	5’CCTGCATCTGTTgcTATAACAGCGCTAC	Site directed mutagenesis
prsA1-C21S	5’CTGCTCGCTGGTTcTGGAAGTAGCGC	Site directed mutagenesis
prsA1-C21Src	5’GCGCTACTTCCAgAACCAGCGAGCAG	Site directed mutagenesis
prsA1-Y281A	5’GATGCATTTGCAGATgcCACAAGTACATCCTCAAC	Site directed mutagenesis
prsA1-Y281YArc	5’GTTGAGGATGTACTTGTGgcATCTGCAAATGCATC	Site directed mutagenesis
prsA1-Y80A	5’CAGAATATAAGAAAgcTGAAGAACAATACG	Site directed mutagenesis
prsA1-Y80Arc	5’CGTATTGTTCTTCAgcTTTCTTATATTCTG	Site directed mutagenesis
prsA1-Y84A	5’GAAAgcTGAAGAACAAgcCGGTGACTCATTTG	Site directed mutagenesis, contains mutation Y80A in addition to Y84A
prsA1-Y84Arc	5’CAAATGAGTCACCGgcTTGTTCTTCAgcTTTC	Site directed mutagenesis, contains mutation Y80A in addition to Y84A
prsA1-T90V	5’GTGACTCATTTGAATCAgtGTTATCCTCTAATAATT	Site directed mutagenesis
prsA1-T90Vrc	5’AATTATTAGAGGATAACacTGATTCAAATGAGTCAC	Site directed mutagenesis
prsA2-A185D	5’GTGGACAATTAGaTCCATTCGGTCC	Site directed mutagenesis

prsA2-A185Drc	5’GGACCGAATGGAtCTAATTGTCCAC	Site directed mutagenesis
prsA2-K191E	5’CATTCGGTCCTGGTgAAATGGATCCAGC	Site directed mutagenesis
prsA2-K191Erc	5’GCTGGATCCATTTcACCAGGACCGAATG	Site directed mutagenesis
prsA2-V28A	5’GTGGCGGCGGAGATGTCGcTAAGACAGACTCTGGCG	Site directed mutagenesis
prsA2-V28Arc	5’CGCCAGAGTCTGTCTTAgCGACATCTCCGCCGCCAC	Site directed mutagenesis
prsA2-L40A	5’GTAACAAAAGACGAAgcTTATGACGCAATGAAAG	Site directed mutagenesis
prsA2-L40Arc	5’CTTTCATTGCGTCATAAgcTTCGTCTTTTGTTAC	Site directed mutagenesis
prsA2-Y41A	5’CAAAAGACGAAgcTgcTGACGCAATGAAAG	Site directed mutagenesis, contains mutation L40A in addition to Y41A
prsA2-Y41Arc	5’CTTTCATTGCGTCAgcAgcTTCGTCTTTTG	Site directed mutagenesis, contains mutation L40A in addition to Y41A
prsA2-M44A	5’GAAgcTgcTGACGCAgcGAAAGATAAATATG	Site directed mutagenesis, contains mutations L40A and Y41A in addition to M44A
prsA2-M44Arc	5’CATATTTATCTTTCgcTGCGTCAgcAgcTTC	Site directed mutagenesis, contains mutations L40A and Y41A in addition to M44A
prsA2-K268A	5’CAAAGATGCTAACGTGgcAGTGGAAGACAAAGAC	Site directed mutagenesis
prsA2-K268Arc	5’GTCTTTGTCTTCCACTgcCACGTTAGCATCTTTG	Site directed mutagenesis
prsA2-K29A	5’GGAGATGTCGTTgcGACAGACTCTGG	Site directed mutagenesis
prsA2-K29Arc	5’CCAGAGTCTGTCgcAACGACATCTCC	Site directed mutagenesis
prsA2-C21S	5’GGCATTGTTCAGTCTAGCAGCGTcCGGTGGCGGCGGAGATGTCG	Site directed mutagenesis
prsA2-C21Src	5’CGACATCTCCGCCGCCACCGgACGCTGCTAGACTGAACAATGCC	Site directed mutagenesis
prsA2-F281A	5’GATGCTTTTAAAGATgcTGATGGTTCTTCATCCAG	Site directed mutagenesis
prsA2-F281Arc	5’CTGGATGAAGAACCATCAgcATCTTTAAAAGCATC	Site directed mutagenesis
prsA2-Y80A	5’CAAGAAATTCAACGAGgcTAAATCACAATACGGAG	Site directed mutagenesis
prsA2-Y80Arc	5’CTCCGTATTGTGATTTAgcCTCGTTGAATTTCTTG	Site directed mutagenesis
prsA2-84YtoA	5’CGAGgcTAAATCACAAgcCGGAGATCAATTCTCTGC	Site directed mutagenesis, contains mutation Y80A in addition to Y84A
prsA2-Y84Arc	5’GCAGAGAATTGATCTCCGgcTTGTGATTTAgcCTCG	Site directed mutagenesis, contains mutation Y80A in addition to Y84A
prsA2-V90T	5’GAGATCAATTCTCTGCAacTTTAGCTCAAAGTGGC	Site directed mutagenesis
prsA2-V90Trc	5’GCCACTTTGAGCTAAAgtTGCAGAGAATTGATCTC	Site directed mutagenesis

References for Tables

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