Table S3: *Primers for the Ec 1ALigN (multiple) mutants and degree of reversibility upon thermal unfolding.*

Name[(1)]	Mutated residues[(2)]	Template	Forward primer	Analyzed[(3)]	Rev. / %[(4)]
Ex1D	41	*WT*	5'- ACGCTTCGCCATCATGATTATCTTTATCATGTGAT -3'	***Yes***	***100***
Ex2D	24,27	*WT*	5'-GCAGGCTTCATGGATTCAATCGATCAACAACTGACAGAACT-3'	***Yes***	***100***
Ex3D	63,66,68	*WT*	5'-TGATGCGCGATCTGCGCGATCTGGATACCAAACATCC-3'	***Yes***	***100***
Ex4D	41,63,66,68	*Ex1D*	5'- TGATGCGCGATCTGCGCGATCTGGATACCAAACATCC -3'	***Yes***	***100***
Ex5D	24,27,63,66,68	*Ex2D*	5'- TGATGCGCGATCTGCGCGATCTGGATACCAAACATCC -3'	***Yes***	***100***
Dx2E	54,58	*WT*	5'- AAATTCCCGAAGCTGAATACGAAAGGCTGATGCGCGAACT -3'	***Yes***	***100***
Dx3E	54,58,78	*Dx2E*	5'- AGAACTGATTACGCCTGAATCGCCTACCCAACGTGTA -3'	***Yes***	***100***
Dx4E	48,54,58,78	*Dx3E*	5'- CTTTATCATGTGATGGAAGCGCCGGAAATTCCCGA -3'	***Yes***	***100***
Ex2Q	24,27	*WT*	5'- GCAGGCTTCATGCAATCAATCCAACAACAACTGACAGAACT -3'	***Yes***	***100***
Qx2E	28,29	*WT*	5'- ATGGAATCAATCGAAGAAGAACTGACAGAACTGCGAACGA -3'	***Yes***	***100***

(1) XY*x*nWZ involves *n* substitutions from X or Y to W or Z respectively. The quotation mark indicates that an alternative cumulative pathway has been used. (2) From the sequence: MSYYHHHHHHLESTSLYKKAGFMESIEQQLTELRTTLRHHEYLYHVMDAPEIPDAEYDRLMRELRELETKHPELITPDSPTQRVGAAPLAAF (3) *Yes* = Protein expressed, purified and tested over KCl. *No* = DNA used as an intermediate for cloning. (4) Reversibility upon unfolding. The value corresponds to the lower value found for all the salt concentrations. Values in red indicate that a change in the T_m is observed after varying the scanning rate in the experiment and these values have not been used in the study.