

Table S2: Primers for the *Hv 1ALigN* (multiple) mutants and degree of reversibility upon thermal unfolding.

Name ⁽¹⁾	Mutated residues ⁽²⁾	Template	Forward primer	Analyzed ⁽³⁾	Rev. / % ⁽⁴⁾
Ex2D	104,107	<i>WT</i>	5'-ACGCGCTGGATACGGTCGATCACGTCTAGG-3'	Yes	98
Ex4D	47,51,104,107	<i>Ex2D</i>	5'-CACTCCTCCGCGATGCCGTCCGTGATCACGACCAC-3'	<i>No</i>	-
Ex5D	31,47,51,104,107	<i>Ex4D</i>	5'-AACCCGCCGATTTCGCTCTCGCGCGA-3'	<i>No</i>	-
Ex6D	26,28,47,51,104,107	<i>Ex4D</i>	5'-TTCCCTCCGACCGATTTTCGATCCCGCCGA-3'	Yes	100
Ex7D	26,28,31,47,51,104,107	<i>Ex6D</i>	5'-AACCCGCCGATTTCGCTCTCGCGCGA-3'	<i>No</i>	-
Ex8D	26,28,31,47,51,80,104,107	<i>Ex7D</i>	5'-GACTCGTTGCGCTCGATGACGCGTTC-3'	<i>No</i>	-
Ex9D	26,28,31,47,51,80,98,104,107	<i>Ex8D</i>	5'-TCGGCGGCGATCCCATCGACG-3'	Yes	89
Dx1E	101	<i>WT</i>	5'-GGCGAGCCCATCGAAGCGCTGGAGACGGT-3'	<i>No</i>	-
Dx2E	53,101	<i>Dx1E</i>	5'-GCCGTCCGTGAGCAGCAACACCGCTACTAC G-3'	<i>No</i>	-
Dx3E	84,86,101	<i>Dx1E</i>	5'-CGCTCGAAGACGCGTTTCGAACTCGAAACGACCAACA -3'	Yes	95
Dx4E	53,84,86,101	<i>Dx3E</i>	5'-GCCGTCCGTGAGCAGCAACACCGCTACTACG-3'	Yes	85
Dx4E'	81,84,86,101	<i>Dx3E</i>	5'-GACTCGTCCGCTCGAAGAAGCGTTTCGAACTCGAAACGA -3'	Yes	98
Dx5E	53,81,84,86,101	<i>Dx4Ealt</i>	5'-GCCGTCCGTGAGCAGCAACACCGCTACTACG-3'	<i>No</i>	-
Dx7E	53,66,70,81,84,86,101	<i>Dx5E</i>	5'-GCTCGTCTCCGAAGCGGCATACGAAGCGCTGTTCT -3'	Yes	94
Dx8E	53,61,66,70,81,84,86,101	<i>Dx7E</i>	5'-TCGCAGCCGAACCGCTCGTCTCCGA-3'	Yes	90
NQx1DE	41	<i>WT</i>	5'-CCGAAGGGGAGGCCGCACTCCTCCGCGAGGC-3'	<i>No</i>	-
NQx2DE	89,93	<i>WT</i>	5'-TCGACACGACCGACAGCCCGACGGACCGCGTCGG-3'	<i>No</i>	-
NQx3DE	41,89,93	<i>NQx2DE</i>	5'-CCGAAGGGGAGGCCGCACTCCTCCGCGAGGC-3'	Yes	100
DEX1NQ	12	<i>WT</i>	5'-CATCACCATCACCTTCAATCAACAAGTTTGTACAA-3'	Yes	100
DEX3NQ	26,28,31	<i>WT</i>	5'-CCCTCCGACCCAGTTCCAACCCGCCAGTCGCTCTCG -3'	<i>No</i>	-
DEX4NQ	12,26,28,31	<i>DEX3NQ</i>	5'-CATCACCATCACCTTCAATCAACAAGTTTGTACAA-3'	Yes	100
DEX4NQ'	12,47,51,53	<i>DEX1NQ</i>	5'-CACTCCTCCGCCAGGCCGTCCGTCAGCACAACCACCGCTA-3'	Yes	100
DEX7NQ	12,26,28,31,47,51,53	<i>DEX4NQ</i>	5'-CACTCCTCCGCCAGGCCGTCCGTCAGCACAACCACCGCTA-3'	Yes	92
DEX8NQ	12,26,28,31,98,101,104,107	<i>DEX4NQ</i>	5'-TCGGCGGCCAGCCCATCAACGCGTGCAGACGGTCCAACACG-3'	Yes	100
Rx1K	75	<i>WT</i>	5'-GCTGTTCTCCAAACTCGTCGCGCTCGAA-3'	Yes	98
Rx2K	35,75	<i>Rx1K</i>	5'-GAGTCGCTCTCGAAGGAGGCGGCCGAAG-3'	Yes	97
Rx3K	35,55,75	<i>Rx2K</i>	5'-GAGCACGACCACAAGTACTACGTGCGGG-3'	Yes	99
Rx4K	35,46,50,75	<i>Rx2K</i>	5'-GCACTCCTCAAGGAGGCCGTCAAGGAGCACGACC-3'	Yes	100
Sx2K	13,15	<i>WT</i>	5'-CACCATCACCTTGAAAAACAAGTTGTACAAAAAAGCAGGCTT-3'	Yes	-
Sx3K	13,15,74	<i>Sx2K</i>	5'-ACGACGCGCTGTTCAAACGACTCGTCGCGCTCGAAGACG-3'	Yes	-
Sx4K	13,15,32,34	<i>Sx2K</i>	5'-TCGAACCCGCCGAGAAGCTCAAGCGCGAGGCGGCCGAAG-3'	Yes	-
Sx5K	13,15,32,34,74	<i>Sx4K</i>	5'-ACGACGCGCTGTTCAAACGACTCGTCGCGCTCGAAGACG-3'	Yes	-

(1) XY_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: MSYYHHHHHLESTSLYKKGFPPTFEPAESLSREAAEGQAALLREAVREHDHRYVAADPLVSDAAYDALFSRLVALEDAFDLDTTNSPTNRVGGE

PIDALETVEHV. (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.