

Supplementary data for the article:

Kostić, A. Ž.; Gašić, U. M.; Pešić, M. B.; Stanojević, S. P.; Barać, M. B.;
Mačukanović-Jocić, M. P.; Avramov, S. N.; Tešić, Ž. L. Phytochemical Analysis and Total
Antioxidant Capacity of Rhizome, Above-Ground Vegetative Parts and Flower of Three
Iris Species. *Chemistry and Biodiversity* **2019**, *16* (3), 1–17.

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Protein engineering of cellobiose dehydrogenase from *Phanerochaete chrysosporium* in yeast *Saccharomyces cerevisiae* InvSc1 for increased activity and stability

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Supplementary material

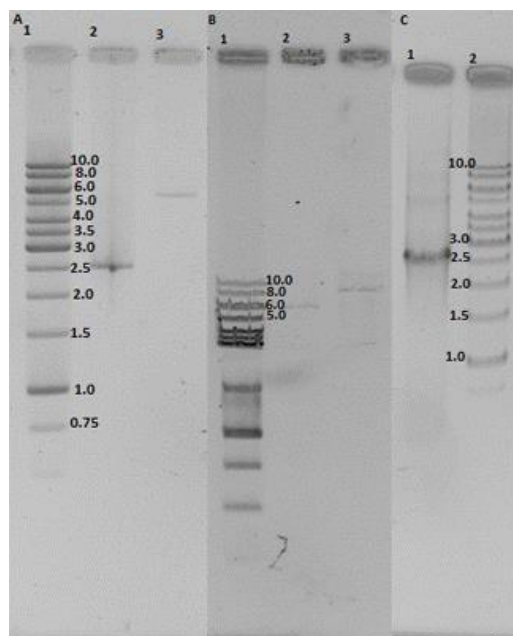


Fig. S1 Agarose gel electrophoresis, A) Double digested DNA: 1) DNA markers (fermentas 0.25-10.0kbp), 2) double digested PCR product CDH for cloning in pYES2, 3) double digested pYES2 vector. Double digestion was done with EcoRI and XbaI enzymes overnight at 37°C and inactivated at 80°C. B) Restricted genes using EcoRI enzyme: 1) DNA markers, 2) restricted empty vector pYES2 and 3) restricted construct CDH-pYES2 obtained from *E.coli* colony miniprep to yield lane at 8.5kbp. C) 1) PCR product of CDH from miniprep of *E.coli* colony with construct CDH-pYES2 (B)3) and 2) DNA markers.

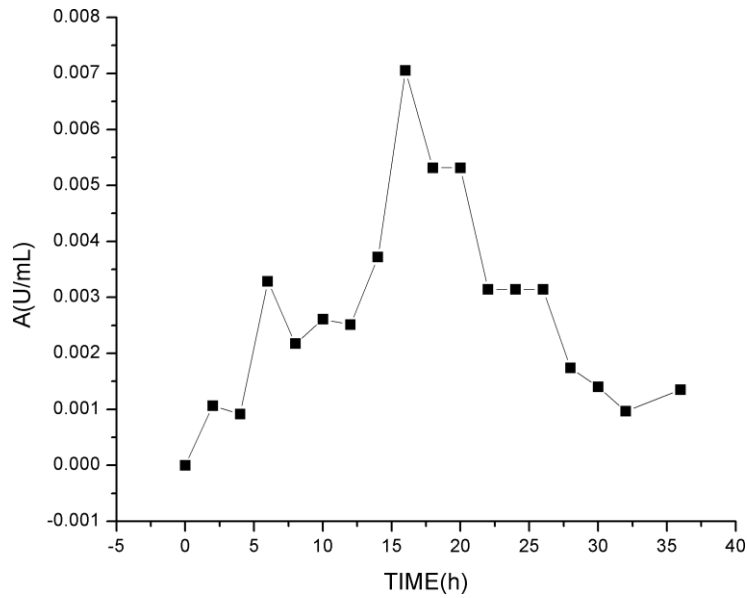


Fig. S2 The activity of wtCDH during fermentation in YNB-CAA Gal induction medium. Activity was measured in 0.1M sodium acetate buffer pH 4.5 with 30 mM lactose and 0.3 mM DCIP.

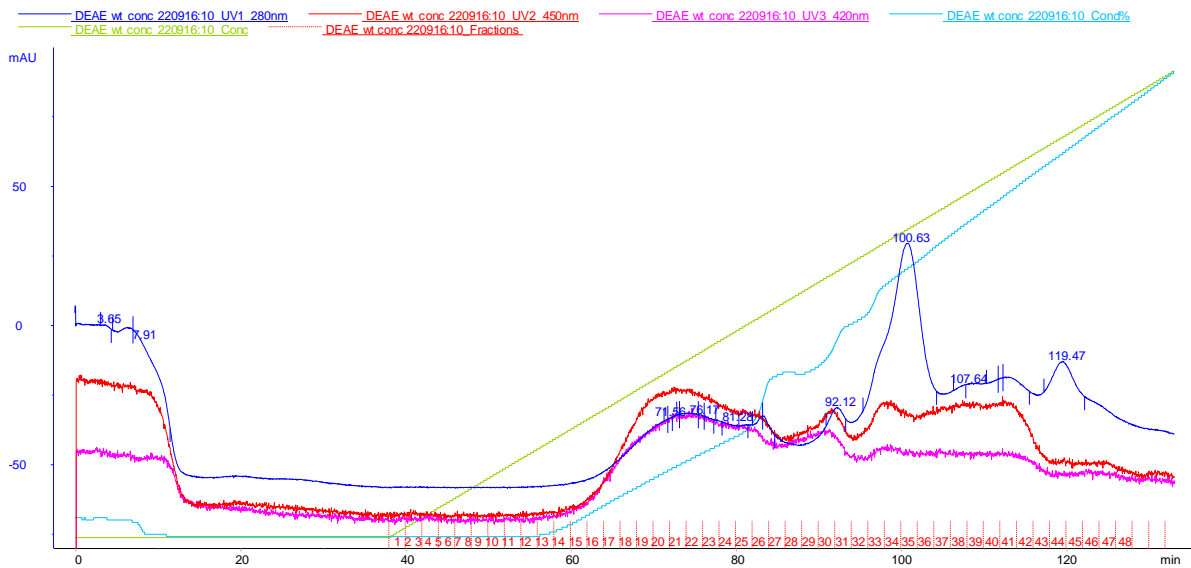


Fig. S3 Ion-exchange chromatography of wtCDH in 10mM Na-phosphate buffer pH6, applied on HiTrap DEAE. Fractions from 23-29 showed activity.

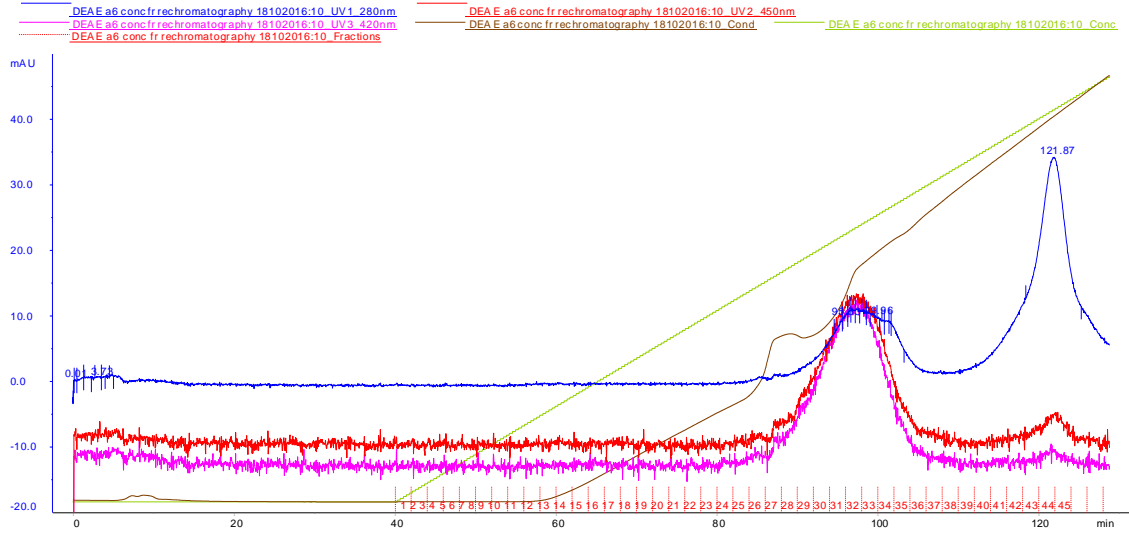


Fig. S4 Ion-exchange chromatography of S137N CDH in 10mM Na-phosphate buffer pH6, applied on HiTrap DEAE. Fractions from 28-31 showed activity and were collected.

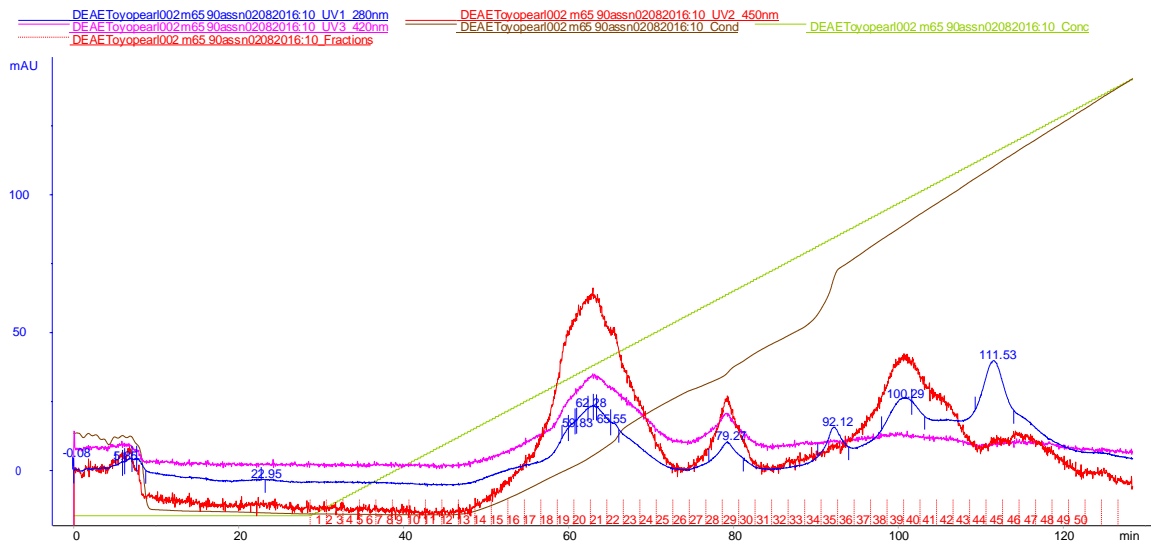


Fig. S5 Ion-exchange chromatography of M65S CDH in 10mM Na-phosphate buffer pH6, applied on HiTrap DEAE. Fractions from 13-20 showed activity and were collected.

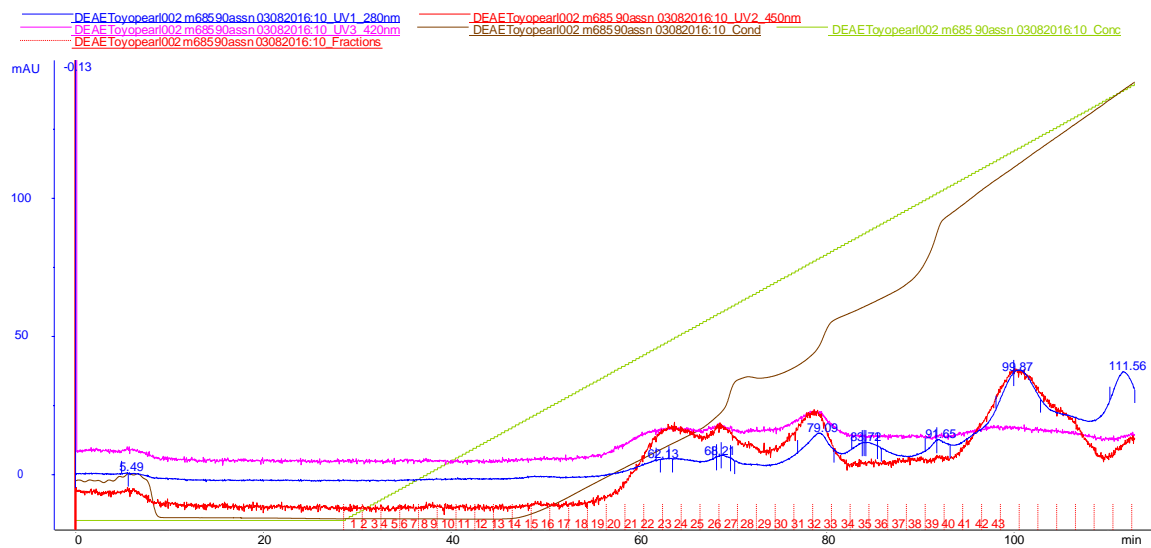


Fig. S6 Ion-exchange chromatography of M685V CDH in 10 mM Na-phosphate buffer pH6, applied on HiTrap DEAE. Fractions from 24-26 showed activity and were collected.



Fig. S7 IEF gel of purified wtCDH-pYES2 stained with CBB. Calculated pI was 4.69.

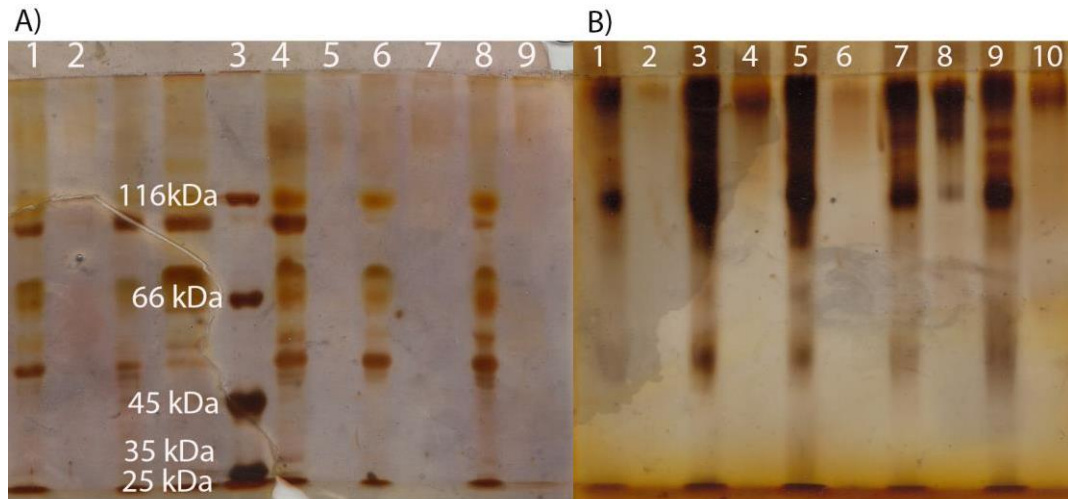


Figure S8 **A) 8% polyacrylamide gel electrophoresis** of (1) crude sample M65S, (2) purified M65S, (3) MW markers (4) crude sample S137N, (5) purified S137N, (6), crude sample M685V, (7) purified M685V, (8) crude samples of wtCDH, (10) purified wtCDH. Protein bands were stained with silver staining. **B) 10% polyacrylamide gel electrophoresis** of (1) crude sample M65S, (2) concentrated purified M65S, (3) crude sample S137N, (4) concentrated purified S137N, (5), crude sample M685V, (6) concentrated purified M685V, (7,8,9) crude samples of wtCDH, (10) concentrated purified wtCDH. Protein bands were stained with silver staining.

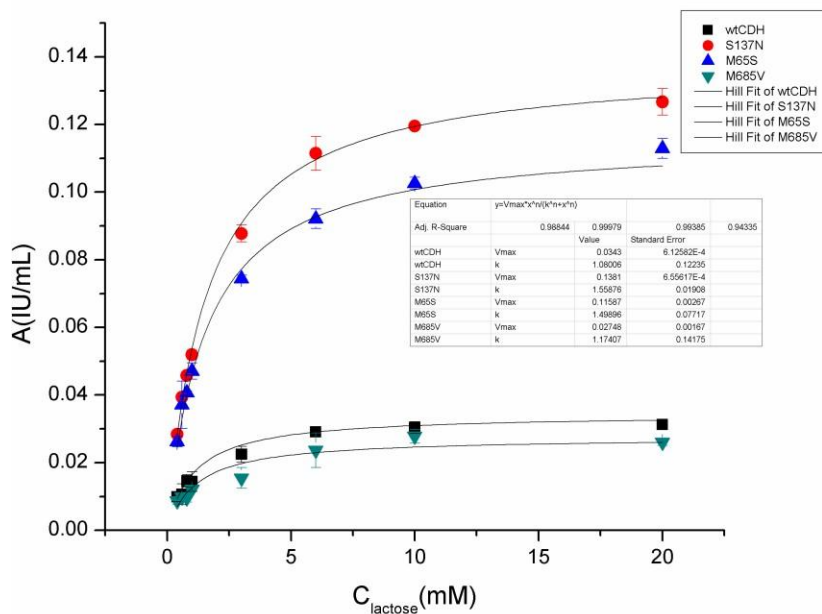


Fig. S9 Kinetic measurements for wt and mutant CDHs for lactose.

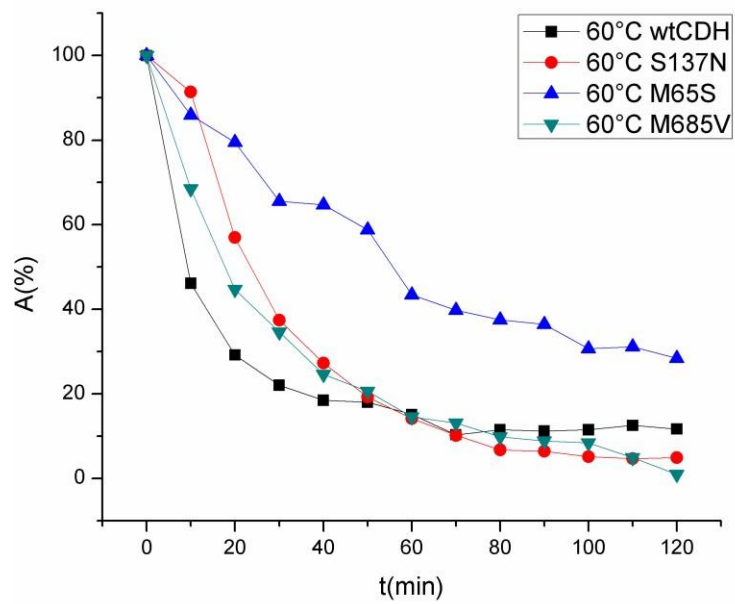


Fig. S10 Thermostability of wt and mutant CDHs on 60°C. Aliquots were taken every 10 minutes and activity was measured with DCIP assay.

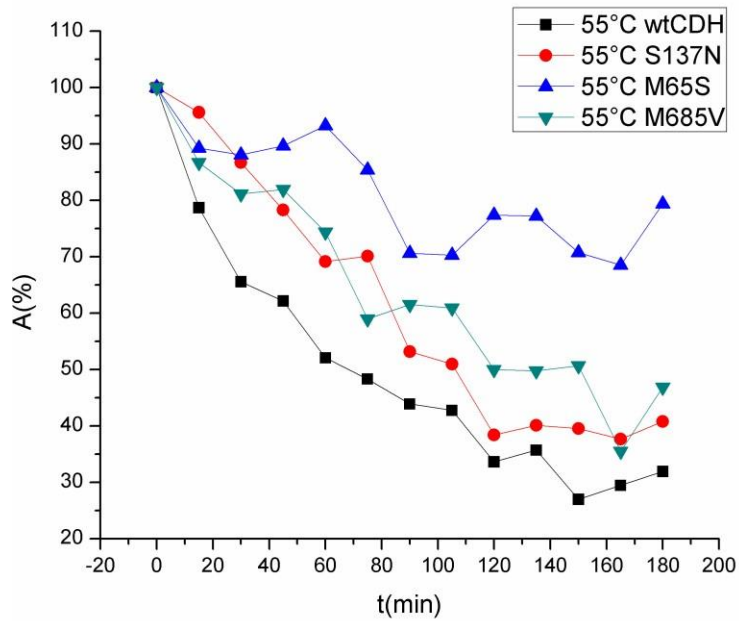


Fig. S11 Thermostability of wt and mutant CDHs on 55°C. Aliquots were taken every 10 minutes and activity was measured with DCIP assay.

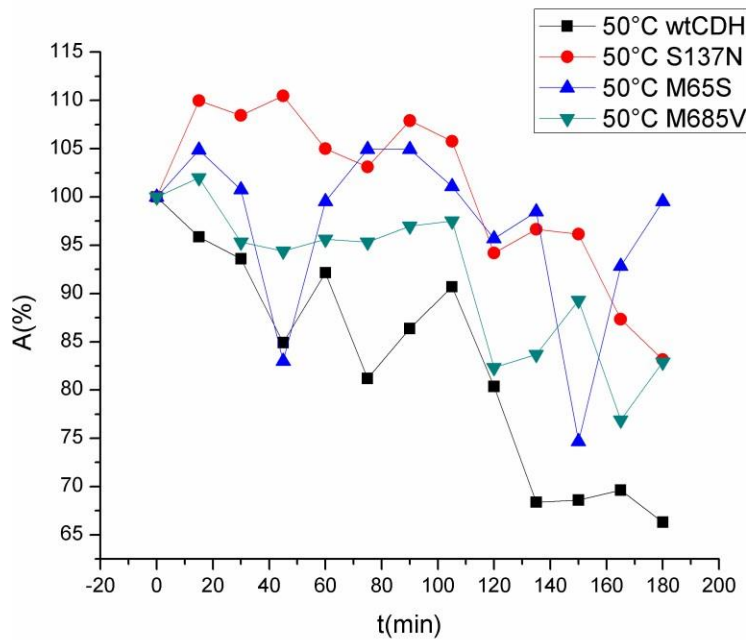


Fig. S12 Thermostability of wt and mutant CDHs on 50°C. Aliquots were taken every 10 minutes and activity was measured with DCIP assay.

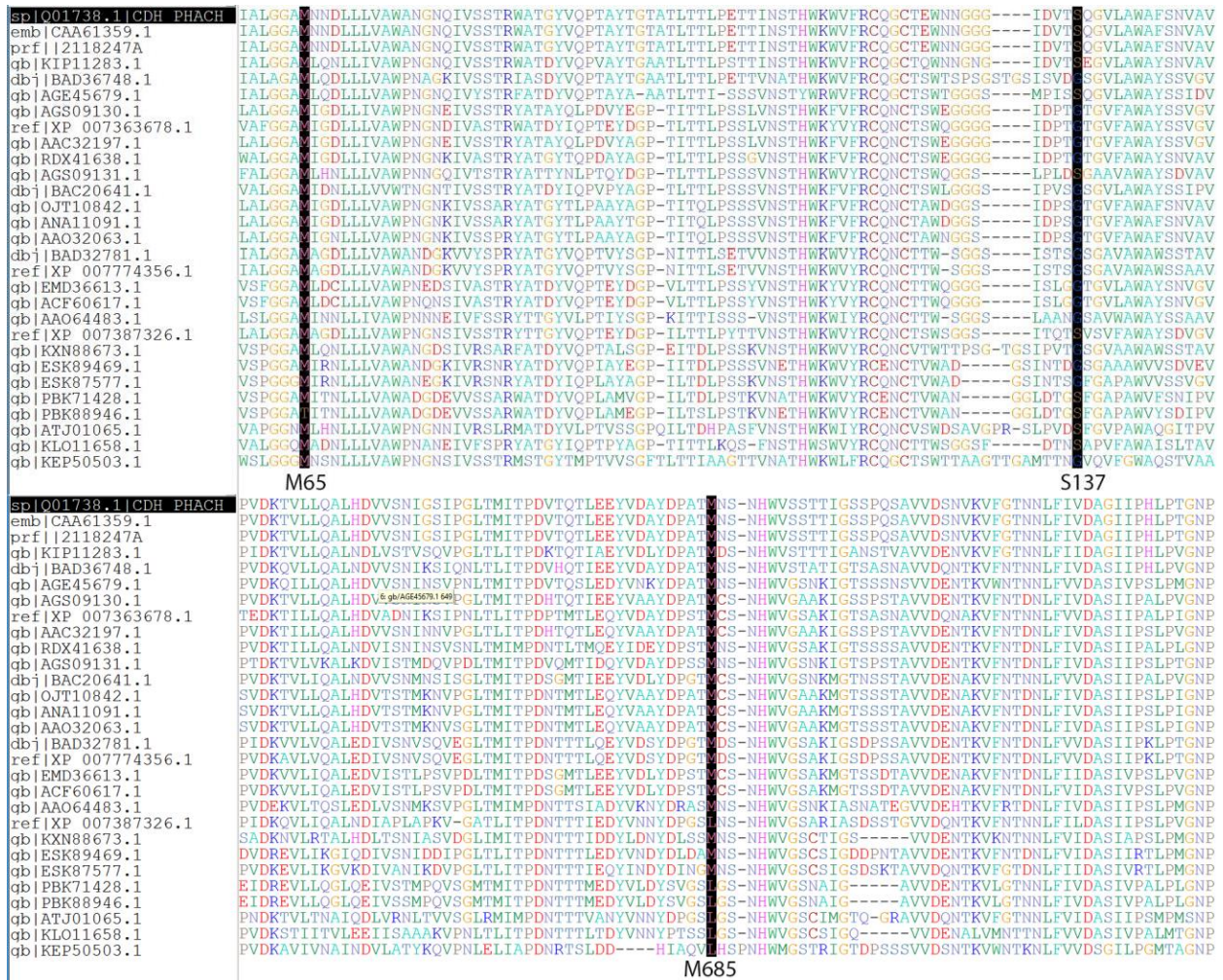


Fig. S13 Sequence alignment of 28 CDH sequences using Pubmed COBALT server (<https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?CMD=Web>). **Q01738.1** Cellobiose dehydrogenase cellobiose dehydrogenase [*Phanerochaete chrysosporium*]; CAA61359.1 cellobiose dehydrogenase [*Phanerochaete chrysosporium*]; 2118247A cellobiose dehydrogenase [*Phanerochaete chrysosporium*]; KIP11283.1 cellobiose dehydrogenase [*Phlebiopsis gigantea* 11061_1 CR5-6]; BAD36748.1 cellobiose dehydrogenase [*Irpex lacteus*]; AGE45679.1 cellobiose dehydrogenase [*Phlebia lindtneri*]; AGS09130.1 cellobiose dehydrogenase [*Trametes sanguinea*]; XP_007363678.1 cellobiose dehydrogenase [*Dichomitus squalens* LYAD-421 SS1]; AAC32197.1 cellobiose dehydrogenase [*Trametes cinnabarina*]; RDX41638.1 cellobiose dehydrogenase [*Polyporus brumalis*]; AGS09131.1 cellobiose dehydrogenase [*Cerrena unicolor*]; BAC20641.1 cellobiose dehydrogenase [*Grifola frondosa*]; JTI10842.1 Cellobiose dehydrogenase [*Trametes pubescens*]; ANA11091.1 cellobiose dehydrogenase [*Trametes velutina*]; AO32063.1 cellobiose dehydrogenase [*Trametes versicolor*]; BAD32781.1 cellobiose dehydrogenase [*Coniophora puteana*]; XP_007774356.1 cellobiose dehydrogenase [*Coniophora puteana* RWD-64-598 SS2]; EMD36613.1 cellobiose dehydrogenase [*Gelatoportia subvermispota* B]; ACF60617.1 cellobiose dehydrogenase [*Gelatoportia subvermispota*]; AO64483.1 cellobiose dehydrogenase [*Athelia rolfsii*]; XP_007387326.1 cellobiose dehydrogenase [*Punctularia strigosozonata* HHB-11173 SS5]; KXN88673.1 Cellobiose dehydrogenase [*Leucoagaricus sp. SymC.cos*]; ESK89469.1 cellobiose dehydrogenase [*Moniliophthora roreri* MCA 2997]; ESK87577.1 cellobiose dehydrogenase [*Moniliophthora roreri* MCA 2997]; PBK71428.1 cellobiose dehydrogenase [*Armillaria solidipes*]; PBK88946.1 cellobiose dehydrogenase [*Armillaria*

gallica]; ATJ01065.1 cellobiose dehydrogenase [*Volvariella volvacea*]; KLO11658.1 cellobiose dehydrogenase [*Schizopora paradoxa*]; KEP50503.1 cellobiose dehydrogenase [*Rhizoctonia solani* 123E];

Table S1. Distance of mutations from putative glycosylation sites calculated in UCSF Chimera using UniProt PDB data for heme and flavin fomain of CDH.

N Glycosylation site	Mutants (Å)			O glycosylation site	Mutants (Å)		
	S137N	M65S	M685V		S137N	M65S	M685V
N111	34.629	28.313	23.176	T7	20.742	28.506	33.770
N191	49.383	51.276	90.393	T193	43.546	47.123	87.213
N419	39.476	44.000	43.215	T194	40.172	44.915	84.670
N434	41.808	54.394	41.818	T195	37.059	41.735	82.629
N492	35.069	45.325	30.967	T197	32.186	38.935	80.532
N517	54.654	53.317	7.385	T200	27.141	37.528	78.440
N553	53.085	41.246	38.356	S201	27.832	40.020	79.213
N593	49.581	42.759	12.784	T202	26.000	39.577	76.679
N599	58.641	48.578	21.248	S203	24.891	40.161	75.793
				S204	24.125	39.809	73.530
				S205	27.556	43.518	75.973
				T207	31.574	48.964	78.584
				T208	32.685	50.689	77.766
				T211	38.748	57.598	78.663
				T215	44.330	63.760	79.864
				T338	57.855	57.114	30.228

NetNGlyc 1.0 Server - prediction results

Name: wtCDH Sequence Length: 755

```

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SEHTDFGFFGIDYSTAHSANYQNYLNGDSGNPTTTSTKPTSTSSSVTTGPTVSATPYDYIIVGAGPGGIIAADRLSEAGK      240
KVLLELRGGPSTKQTGGTYVAPWATSSGLTKFDIPGLFESLFTDSNPFWWCKDITVFAGCLVGGGTSVNGALYWPNDGD      320
FSSSVGWSPSSWTNHAPYTSKLS SRLPSTDHPS TDGQRYLEQSFNVVSQLLKGGQYNQATINDNPNYKDHVFGYSAFDLN      400
GKRAGPVATYLTALARPNFTFKTNVMSNVVRNGSQILGVQFNPTDPTLGPNGFIPVTPKGRVILSAGAFGTSRILFQSGI      480
GPTDMIQTVQSNPTAAAALPPQNQWINLQVGMNAQDNPSINLVFTHPSIDAYENWADVWSNPRPADAQYLANQSGVFAG      560
ASPKLNFWRAYSGSDGFTRYAQTGTVRPGAASVNSSLPYNASQIFTITVYLSTGIQSRGRIGIDAALRGTVLTPPWLVNPV      640
DKTVLLQALHDVVSNIGSIPGLTMITPDVTQTLEEYVDAYDPATMNSNHVVSTTIGSPQSAVVDSNVKVFGTNNLFIV      720
DAGIIPHLPTGNPQGTLMSAAEQAAAKILALAGGP
..... 80
.....N..... 160
.....N..... 240
..... 320
..... 400
.....N..... 480
.....N.....N..... 560
.....N..... 640
..... 720
..... 800
  
```

(Threshold=0.5)

SeqName	Position	Potential	Jury agreement	N-Glyc result	
Sequence	111 NSTH	0.5406	(7/9)	+	
Sequence	191 NPTT	0.5042	(5/9)	+	WARNING: PRO-X1.
Sequence	419 NTFE	0.4533	(6/9)	-	
Sequence	434 NGSQ	0.5574	(7/9)	+	
Sequence	492 NPAT	0.5879	(6/9)	+	WARNING: PRO-X1.
Sequence	517 NPSI	0.6024	(8/9)	+	WARNING: PRO-X1.
Sequence	553 NQSG	0.4408	(7/9)	-	
Sequence	593 NSSL	0.4753	(6/9)	-	
Sequence	599 NASQ	0.6196	(8/9)	+	

NetOGlyc 3.1 Server - prediction results

Name: **wtCDH** Sequence

Length: 755

QSASQFTDPTTGFQFTGITDPVHDVTYGFVFPPLATSGAQSTEFIGEVVAPIASKWIGIALGGAMNNDLLLVAWANGNOI
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 GPTDMIQTQVSNPTAAALPPQNWINLPGMNAQDNPSINLVFTHPSIDAYENWADVWSNPRPADAAQYLANQSGVFAG
 ASPKLNFWRAYSGSDGFTRYAQGTVRPGAASVNSSLPYNASQIFITVYVYLSSTGIQSRGRIGIDAALRGTVLTPPWLNVNV
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 DAGIIPHLPTGNPQGTLSAAEQAAAKILALAGGP

.....T.....

TTT.T..TSTSSS.TT..T...T.....

T.....

 ..T.....

Name	S/T	Pos	G-score	I-score	Y/N	Comment
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Sequence	S	4	0.435	0.031	.	-
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Sequence	T	10	0.440	0.397	.	-
Sequence	T	11	0.440	0.021	.	-
Sequence	T	16	0.345	0.055	.	-
Sequence	T	19	0.361	0.190	.	-
Sequence	T	26	0.348	0.059	.	-
Sequence	T	36	0.406	0.300	.	-
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Sequence	T	101	0.400	0.035	.	-
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Sequence	S	658	0.182	0.041	.	-
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Sequence	T	672	0.367	0.223	.	-
Sequence	T	684	0.354	0.170	.	-
Sequence	S	687	0.282	0.023	.	-
Sequence	S	692	0.335	0.053	.	-
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Sequence	T	695	0.402	0.108	.	-
Sequence	S	698	0.289	0.028	.	-
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Sequence	S	702	0.234	0.063	.	-
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Sequence	T	730	0.264	0.414	.	-

Sequence	T	736	0.310	0.031	.	-
Sequence	S	739	0.221	0.113	.	-

NetNGlyc 1.0 Server - prediction results

Name: S137N CDH Sequence Length: 755

QSASQFTDPTTGFQFTGITDPVHDVITYGFVFPPLATSQAQSTEFIGEUVAPIASKWIGIALGGAMNNDLLLVAWANGNQI 80
 VSSTRWATGYVQPTAYTGTATLTTLPETTIN**ST**HWKWFVRCQGCETWNNGGGIDVT**N**QGVLAWAFAFNSNAVDDPSDPQSTF 160
 SEHTDFGFFGIDYSTAHSANYQNYLNGD**SGNPT**TTSTKPTSTSSSVTTGPTVSATPYDYIIIVGAGPGGIIAADRLSEAGK 240
 KVLLLERGGPSTKQTGGTYVAPWATSSGLTKFDIPLGFESLFTDSNPFWWCKDITVFAGCLVGGGTSVNGALYWYPNDGD 320
 FSSSVGWPSWTNHAPYTSKLSRRLPSTDHPSDGDQRYLEQSFNVVSQLKGGQYNAQATINDNPNYKDHVFGYSAFDFLN 400
 GKRAGPVATYLLQTALARP**NFT**FKTNVMVSNVVR**NGS**QILGVQTNDPDLGPNGFIPVTPKGRVILSAGAFGTSRILFQSGI 480
 GPTDMIQTVQ**SNPT**AAAALPPQNQWINLPGVMNAQD**NPS**INLVFTHPSIDAYENWADVWSNPRPADAAQYLAN**QS**GVFAG 560
 ASPKLNFWRAYSGSDGFTRYAQGTVRPGAASV**NSSL**PN**AS**QIFITITVYVLTSTGIQSRGRIGIDAALRGTVLTPPWLVPV 640
 DKTVLLQALHDVVSNIQSIPLGTMITPDVVTQLEEYVDAYDPATMNSNHWVSSTIGSPQSAVVDSNVKVFGTNNLFIV 720
 DAGIIPHLPTGNPQGTLMQAAEQAAAKIILALAGGP 800
 80
**N**..... 160
**N**..... 240
 320
 400
**N**..... 480
**N**.....**N**..... 560
**N**..... 640
 720
 800

(Threshold=0.5)

SeqName	Position	Potential	Jury	N-Glyc	
					agreement result
Sequence	111 NSTH	0.5406	(7/9)	+	
Sequence	191 NPTT	0.5041	(5/9)	+	WARNING: PRO-X1.
Sequence	419 NPTT	0.4533	(6/9)	-	
Sequence	434 NGSQ	0.5577	(7/9)	+	
Sequence	492 NPAT	0.5879	(6/9)	+	WARNING: PRO-X1.
Sequence	517 NPSI	0.6024	(8/9)	+	WARNING: PRO-X1.
Sequence	553 NQSG	0.4408	(7/9)	-	
Sequence	593 NSSL	0.4753	(6/9)	-	
Sequence	599 NASQ	0.6196	(8/9)	+	

NetOGlyc 3.1 Server - prediction results

Name: **S137N CDH** Sequence

Length: 755

QSASQFTDPTTGFQFTGITDPVHDVYGFVFPPLATSGAQSTEFIGEVVAPIASKWIGIALGGAMNNDLLVAVANGNQI
 VSSTRWATGYVQPTAYTGTATLTTLPETTINSTHWKWFRCQCGTEWNNGGGIDVTNQGLAWAFNSNVAVDDPSDPQSTF
 SEHTDFGFFGIDYSTAHSANYQNYLNGDSGNPTTSTKPTSTSSSVTTGPTVSATPYDYIIVGAGPGGIIAADRLSEAGK
 KVLLLERGGPSTKQTGGTYVAPWATSSGLTKFDI PGLFESLFTDSNPFWWCKDITVFAGCLVGGGTSVNGALYWYPNDGD
 FSSSVGWPSWTHNHPYTSKLSRSLPSTDHPSTDGQRYLEQSFNVVSQLKQGQYNQATINDNPNYKDHVFGYSAFDFLN
 GKRAQPVATYLTALARPNTFFKTNVMVSNVVRNGSQILGVQVNDPTLGPNGFIPVTPKGRVILSAGAFGTSRILFQSGI
 GPTDMIQTVQSNPTAAAALPPQNQWNLVPGMNAQDNPSINLVFTHPSIDAYENWADVWSNPRPADAAQYLANQSGVFAG
 ASPKLNFWRAYSGSDGFTRYAQGTVRPGAASVNSSLPYNASQIFTTITVYLSTGIQSRGRIGIDAALRGTVLTPPWLVPV
 DKTVLLQALHDVVSNIGSIPGLTMITPDVVTQLEEYVDAYDPATMNSNHVVSSTTIGSSPQSAVVDSNVKVFGTNNLFIV
 DAGIIPHLPTGNPQGTLMSSAEQAAAKILALAGGP

.....T.....

TTT.T..TSTSSS.TT..T...T.....

T.....

 ..T.....

Name	S/T	Pos	G-score	I-score	Y/N	Comment
Sequence	S	2	0.461	0.019	.	-
Sequence	S	4	0.435	0.031	.	-
Sequence	T	7	0.507	0.064	T	-
Sequence	T	10	0.440	0.397	.	-
Sequence	T	11	0.440	0.021	.	-
Sequence	T	16	0.345	0.055	.	-
Sequence	T	19	0.361	0.190	.	-
Sequence	T	26	0.348	0.059	.	-
Sequence	T	36	0.406	0.300	.	-
Sequence	S	37	0.251	0.025	.	-
Sequence	S	41	0.241	0.042	.	-
Sequence	T	42	0.333	0.068	.	-
Sequence	S	54	0.118	0.078	.	-
Sequence	S	82	0.129	0.053	.	-
Sequence	S	83	0.145	0.040	.	-
Sequence	T	84	0.245	0.034	.	-
Sequence	T	88	0.336	0.077	.	-
Sequence	T	94	0.455	0.201	.	-
Sequence	T	97	0.467	0.124	.	-
Sequence	T	99	0.443	0.032	.	-
Sequence	T	101	0.400	0.035	.	-
Sequence	T	103	0.396	0.098	.	-
Sequence	T	104	0.372	0.090	.	-
Sequence	T	108	0.321	0.019	.	-
Sequence	T	109	0.264	0.060	.	-
Sequence	S	112	0.176	0.023	.	-
Sequence	T	113	0.228	0.022	.	-
Sequence	T	125	0.150	0.051	.	-
Sequence	T	136	0.159	0.117	.	-
Sequence	S	146	0.215	0.049	.	-
Sequence	S	154	0.214	0.108	.	-
Sequence	S	158	0.198	0.021	.	-
Sequence	T	159	0.318	0.207	.	-
Sequence	S	161	0.239	0.064	.	-

Sequence	T	164	0.356	0.022	.	-
Sequence	S	174	0.107	0.023	.	-
Sequence	T	175	0.152	0.044	.	-
Sequence	S	178	0.118	0.048	.	-
Sequence	S	189	0.381	0.030	.	-
Sequence	T	193	0.530	0.138	T	-
Sequence	T	194	0.515	0.067	T	-
Sequence	T	195	0.544	0.086	T	-
Sequence	S	196	0.474	0.279	.	-
Sequence	T	197	0.613	0.073	T	-
Sequence	T	200	0.690	0.270	T	-
Sequence	S	201	0.624	0.025	S	-
Sequence	T	202	0.716	0.347	T	-
Sequence	S	203	0.605	0.080	S	-
Sequence	S	204	0.596	0.021	S	-
Sequence	S	205	0.573	0.033	S	-
Sequence	T	207	0.686	0.303	T	-
Sequence	T	208	0.653	0.219	T	-
Sequence	T	211	0.605	0.316	T	-
Sequence	S	213	0.435	0.253	.	-
Sequence	T	215	0.545	0.024	T	-
Sequence	S	236	0.163	0.062	.	-
Sequence	S	251	0.239	0.026	.	-
Sequence	T	252	0.335	0.078	.	-
Sequence	T	255	0.327	0.081	.	-
Sequence	T	258	0.341	0.031	.	-
Sequence	T	265	0.395	0.238	.	-
Sequence	S	266	0.226	0.051	.	-
Sequence	S	267	0.200	0.028	.	-
Sequence	T	270	0.305	0.037	.	-
Sequence	S	280	0.136	0.047	.	-
Sequence	T	283	0.169	0.078	.	-
Sequence	S	285	0.101	0.032	.	-
Sequence	T	295	0.191	0.057	.	-
Sequence	T	306	0.145	0.032	.	-
Sequence	S	307	0.111	0.032	.	-
Sequence	S	322	0.208	0.071	.	-
Sequence	S	323	0.208	0.033	.	-
Sequence	S	324	0.222	0.044	.	-
Sequence	S	329	0.269	0.049	.	-
Sequence	S	330	0.259	0.019	.	-
Sequence	T	332	0.406	0.075	.	-
Sequence	T	338	0.539	0.072	T	-
Sequence	S	339	0.390	0.068	.	-
Sequence	S	342	0.356	0.067	.	-
Sequence	S	343	0.349	0.048	.	-
Sequence	S	347	0.280	0.049	.	-
Sequence	T	348	0.361	0.264	.	-
Sequence	S	352	0.251	0.165	.	-
Sequence	T	353	0.338	0.056	.	-
Sequence	S	362	0.121	0.031	.	-
Sequence	S	367	0.091	0.052	.	-
Sequence	T	379	0.148	0.030	.	-
Sequence	S	394	0.123	0.021	.	-
Sequence	T	409	0.218	0.036	.	-
Sequence	T	413	0.215	0.046	.	-
Sequence	T	421	0.237	0.078	.	-

Sequence	T	424	0.186	0.053	.	-
Sequence	S	429	0.121	0.073	.	-
Sequence	S	436	0.156	0.033	.	-
Sequence	T	443	0.355	0.076	.	-
Sequence	T	447	0.326	0.089	.	-
Sequence	T	457	0.375	0.368	.	-
Sequence	S	465	0.185	0.086	.	-
Sequence	T	471	0.284	0.101	.	-
Sequence	S	472	0.172	0.030	.	-
Sequence	S	478	0.192	0.052	.	-
Sequence	T	483	0.353	0.616	T	-
Sequence	T	488	0.358	0.304	.	-
Sequence	S	491	0.272	0.056	.	-
Sequence	T	494	0.383	0.306	.	-
Sequence	S	519	0.129	0.369	.	-
Sequence	T	525	0.232	0.057	.	-
Sequence	S	528	0.143	0.063	.	-
Sequence	S	540	0.186	0.115	.	-
Sequence	S	555	0.169	0.032	.	-
Sequence	S	562	0.129	0.089	.	-
Sequence	S	572	0.183	0.052	.	-
Sequence	S	574	0.193	0.037	.	-
Sequence	T	578	0.259	0.053	.	-
Sequence	T	584	0.330	0.399	.	-
Sequence	S	591	0.236	0.044	.	-
Sequence	S	594	0.228	0.025	.	-
Sequence	S	595	0.215	0.092	.	-
Sequence	S	601	0.213	0.031	.	-
Sequence	T	605	0.257	0.065	.	-
Sequence	T	607	0.237	0.036	.	-
Sequence	S	611	0.132	0.078	.	-
Sequence	T	612	0.197	0.026	.	-
Sequence	S	616	0.140	0.056	.	-
Sequence	T	629	0.278	0.142	.	-
Sequence	T	632	0.226	0.195	.	-
Sequence	T	643	0.258	0.040	.	-
Sequence	S	654	0.172	0.058	.	-
Sequence	S	658	0.182	0.041	.	-
Sequence	T	663	0.298	0.132	.	-
Sequence	T	666	0.294	0.079	.	-
Sequence	T	670	0.338	0.081	.	-
Sequence	T	672	0.367	0.223	.	-
Sequence	T	684	0.354	0.170	.	-
Sequence	S	687	0.282	0.023	.	-
Sequence	S	692	0.335	0.053	.	-
Sequence	S	693	0.301	0.034	.	-
Sequence	T	694	0.438	0.068	.	-
Sequence	T	695	0.402	0.108	.	-
Sequence	S	698	0.289	0.028	.	-
Sequence	S	699	0.293	0.027	.	-
Sequence	S	702	0.234	0.063	.	-
Sequence	S	707	0.229	0.023	.	-
Sequence	T	714	0.251	0.088	.	-
Sequence	T	730	0.264	0.414	.	-
Sequence	T	736	0.310	0.031	.	-
Sequence	S	739	0.221	0.113	.	-

NetNGlyc 1.0 Server - prediction results

Name: **M65S** Sequence Length: 755

```

QSASQFTDPTTGFQFTGITDPVHDVTYGFVFPPLATSGAQSTEFIGEVPVAPIASKWIGIALGGASNNDLLLVAWANGNQI      80
VSSTRWATGYVQPTAYTGTATLTLPETTINSTHWKWFVRCQGCTEWNNGGGIDVTSQGVLAWAFSNVAVDDP SDPQSTF      160
SEHTDFGFFGIDYSTAHSANYQNYLNGDSGNPTTTSTKPTSTSSSVTTGPTVSATPYDYIIVGAGPGGIIAADRLSEAGK      240
KVLLLERGGPSTKQTGGTYVAPWATSSGLTKFDI PGLFESLFTDSNPFWWCKDITVFAGCLVGGGTSVNGALYWYPNDGD      320
FSSSVGWSSWTNHAPYTSKLSRRLPSTDHPSTDGQRYLEQSFNVVSQLLKGQGYNQATINDNPNYKDHVFGYSAFDFLN      400
GKRAGPVATYLTALARPNFTFKTNVMVSNVVRNGSQILGVQTNDP TLGPNGFIPVTPKGRVILSAGAFGTSRILFQSGI      480
GPTDMIQTVQSNPTAAAAALPPQNQWINLFPVGMNAQDNPSINLVFTHPSIDAYENWADVWSNPRPADAAQYLANQSGVFAG      560
ASPKLNFWRAYSGSDGFTRYAQGTVRPGAASVNSSLPYNASQIFITIVYLSTGIQSRGRIGIDAALRGTVLT PPWL VNPV      640
DKTVLLQALHDVSNIGSIPGLTMITDPDVTQTLEEYVDAYDPATMNSNHVVSSTTIGSSPQSAVVDSNVKVFGTNNLFIV      720
DAGIIPHLPTGNPQGTLMSSAAEQAAAKILALAGGP
.....
.....N.....80
.....N.....240
.....320
.....400
.....N.....480
.....N.....560
.....N.....640
.....720
.....800

```

(Threshold=0.5)

SeqName	Position	Potential	Jury agreement	N-Glyc result	
Sequence	111 NSTH	0.5407	(7/9)	+	
Sequence	191 NPPT	0.5042	(5/9)	+	WARNING: PRO-X1.
Sequence	419 NTF	0.4533	(6/9)	-	
Sequence	434 NGSQ	0.5574	(7/9)	+	
Sequence	492 NP	0.5878	(6/9)	+	WARNING: PRO-X1.
Sequence	517 NPSI	0.6024	(8/9)	+	WARNING: PRO-X1.
Sequence	553 NQSG	0.4408	(7/9)	-	
Sequence	593 NSSL	0.4753	(6/9)	-	
Sequence	599 NASQ	0.6197	(8/9)	+	

NetOGlyc 3.1 Server - prediction results

Name: M65S CDH Sequence

Length: 755

QSASQFTDPTTGFQFTGITDPVHDVTYGTFVFPPLATSQAQSTEFIGEIVVAPIASKWIGIALGGA\$NNDDL LLVAVANGNQI
 VSSTRWATGYVQPTAYTGATLTTLPETTINSTHWKWFRCQGCTEWNNGGGIDVTSQGVLAFAFSNVAVDDP SDPQSTF
 SEHTDFGFFGIDYSTAHSANYQNYLNGDSGNPTTSTKPTSTSSSVTTGPTVSATPYDYIIVGAGPGGIIAADRLSEAGK
 KVL L LERGGPSTKQTGGTYVAPWATSSGLTKFDI PGLFESLFTDSNPFWWCKDITVFAGCLVGGGTSVNGALYWYPNDGD
 FSSSVGW PSSWTHAPYTSKLS SRLPSTDHPSTDGQRYLEQSFNVVSQLKQGQYNQATINDNPNYKDHVFGYSAFDFLN
 GK RAGPVATY LQTALARPNTFFKTNVMVSNVVRNGSQILGVQ TNDPTLGPNGFIPVTPKGRVILSAGAFGTSRILFQSGI
 GPTDMIQTVQSNPTAAALPPQNW INLPVGMNAQDNPSINLVFTHPSIDAYENWADVWSNPRPADAQYLANQSGVFAG
 ASPKLNFWRAYSGSDGTRYAQGTVRPGAASVNS SLPYNASQIFTTITVYLS TGIQSRGRIGIDAALRGTVLTPPWLVPV
 DKTVLLQALHDVVSNI GSI PGLTMITPDVTQ TLEEYVDAYDPATMNSNHVVSSTIGSPQS AVVDSNVKVFGTNNLFIV
 DAGI IPHLPTGNPQGT LMSAAEQAAAKI LALAGGP

.....T.....

TTT.T..TSTSSS.TT..T...T.....

T.....

 ..T.....

Name	S/T	Pos	G-score	I-score	Y/N	Comment
Sequence	S	2	0.461	0.019	.	-
Sequence	S	4	0.435	0.031	.	-
Sequence	T	7	0.507	0.064	T	-
Sequence	T	10	0.440	0.397	.	-
Sequence	T	11	0.440	0.021	.	-
Sequence	T	16	0.345	0.055	.	-
Sequence	T	19	0.361	0.190	.	-
Sequence	T	26	0.348	0.059	.	-
Sequence	T	36	0.406	0.300	.	-
Sequence	S	37	0.251	0.025	.	-
Sequence	S	41	0.241	0.042	.	-
Sequence	T	42	0.333	0.068	.	-
Sequence	S	54	0.137	0.078	.	-
Sequence	S	65	0.087	0.031	.	-
Sequence	S	82	0.129	0.053	.	-
Sequence	S	83	0.145	0.040	.	-
Sequence	T	84	0.245	0.034	.	-
Sequence	T	88	0.336	0.077	.	-
Sequence	T	94	0.455	0.201	.	-
Sequence	T	97	0.467	0.124	.	-
Sequence	T	99	0.443	0.032	.	-
Sequence	T	101	0.400	0.035	.	-
Sequence	T	103	0.396	0.098	.	-
Sequence	T	104	0.372	0.090	.	-
Sequence	T	108	0.321	0.019	.	-
Sequence	T	109	0.264	0.060	.	-
Sequence	S	112	0.176	0.023	.	-
Sequence	T	113	0.228	0.022	.	-
Sequence	T	125	0.167	0.051	.	-
Sequence	T	136	0.178	0.180	.	-
Sequence	S	137	0.122	0.083	.	-
Sequence	S	146	0.244	0.049	.	-
Sequence	S	154	0.213	0.108	.	-

Sequence	S	158	0.198	0.021	.	-
Sequence	T	159	0.318	0.207	.	-
Sequence	S	161	0.239	0.064	.	-
Sequence	T	164	0.356	0.022	.	-
Sequence	S	174	0.107	0.023	.	-
Sequence	T	175	0.152	0.044	.	-
Sequence	S	178	0.118	0.048	.	-
Sequence	S	189	0.381	0.030	.	-
Sequence	T	193	0.530	0.138	T	-
Sequence	T	194	0.515	0.067	T	-
Sequence	T	195	0.544	0.086	T	-
Sequence	S	196	0.474	0.279	.	-
Sequence	T	197	0.613	0.073	T	-
Sequence	T	200	0.690	0.270	T	-
Sequence	S	201	0.624	0.025	S	-
Sequence	T	202	0.716	0.347	T	-
Sequence	S	203	0.605	0.080	S	-
Sequence	S	204	0.596	0.021	S	-
Sequence	S	205	0.573	0.033	S	-
Sequence	T	207	0.686	0.303	T	-
Sequence	T	208	0.653	0.219	T	-
Sequence	T	211	0.605	0.316	T	-
Sequence	S	213	0.435	0.253	.	-
Sequence	T	215	0.545	0.024	T	-
Sequence	S	236	0.163	0.062	.	-
Sequence	S	251	0.239	0.026	.	-
Sequence	T	252	0.335	0.078	.	-
Sequence	T	255	0.327	0.081	.	-
Sequence	T	258	0.341	0.031	.	-
Sequence	T	265	0.395	0.238	.	-
Sequence	S	266	0.226	0.051	.	-
Sequence	S	267	0.200	0.028	.	-
Sequence	T	270	0.305	0.037	.	-
Sequence	S	280	0.136	0.047	.	-
Sequence	T	283	0.169	0.078	.	-
Sequence	S	285	0.101	0.032	.	-
Sequence	T	295	0.191	0.057	.	-
Sequence	T	306	0.145	0.032	.	-
Sequence	S	307	0.111	0.032	.	-
Sequence	S	322	0.208	0.071	.	-
Sequence	S	323	0.208	0.033	.	-
Sequence	S	324	0.222	0.044	.	-
Sequence	S	329	0.269	0.049	.	-
Sequence	S	330	0.259	0.019	.	-
Sequence	T	332	0.406	0.075	.	-
Sequence	T	338	0.539	0.072	T	-
Sequence	S	339	0.390	0.068	.	-
Sequence	S	342	0.356	0.067	.	-
Sequence	S	343	0.349	0.048	.	-
Sequence	S	347	0.280	0.049	.	-
Sequence	T	348	0.361	0.264	.	-
Sequence	S	352	0.251	0.165	.	-
Sequence	T	353	0.338	0.056	.	-
Sequence	S	362	0.121	0.031	.	-
Sequence	S	367	0.091	0.052	.	-
Sequence	T	379	0.148	0.030	.	-
Sequence	S	394	0.123	0.021	.	-

Sequence	T	409	0.218	0.036	.	-
Sequence	T	413	0.215	0.046	.	-
Sequence	T	421	0.237	0.078	.	-
Sequence	T	424	0.186	0.053	.	-
Sequence	S	429	0.121	0.073	.	-
Sequence	S	436	0.156	0.033	.	-
Sequence	T	443	0.355	0.076	.	-
Sequence	T	447	0.326	0.089	.	-
Sequence	T	457	0.375	0.368	.	-
Sequence	S	465	0.185	0.086	.	-
Sequence	T	471	0.284	0.101	.	-
Sequence	S	472	0.172	0.030	.	-
Sequence	S	478	0.192	0.052	.	-
Sequence	T	483	0.353	0.616	T	-
Sequence	T	488	0.358	0.304	.	-
Sequence	S	491	0.272	0.056	.	-
Sequence	T	494	0.383	0.306	.	-
Sequence	S	519	0.129	0.369	.	-
Sequence	T	525	0.232	0.057	.	-
Sequence	S	528	0.143	0.063	.	-
Sequence	S	540	0.186	0.115	.	-
Sequence	S	555	0.169	0.032	.	-
Sequence	S	562	0.129	0.089	.	-
Sequence	S	572	0.183	0.052	.	-
Sequence	S	574	0.193	0.037	.	-
Sequence	T	578	0.259	0.053	.	-
Sequence	T	584	0.330	0.399	.	-
Sequence	S	591	0.236	0.044	.	-
Sequence	S	594	0.228	0.025	.	-
Sequence	S	595	0.215	0.092	.	-
Sequence	S	601	0.213	0.031	.	-
Sequence	T	605	0.257	0.065	.	-
Sequence	T	607	0.237	0.036	.	-
Sequence	S	611	0.132	0.078	.	-
Sequence	T	612	0.197	0.026	.	-
Sequence	S	616	0.140	0.056	.	-
Sequence	T	629	0.278	0.142	.	-
Sequence	T	632	0.226	0.195	.	-
Sequence	T	643	0.258	0.040	.	-
Sequence	S	654	0.172	0.058	.	-
Sequence	S	658	0.182	0.041	.	-
Sequence	T	663	0.298	0.132	.	-
Sequence	T	666	0.294	0.079	.	-
Sequence	T	670	0.338	0.081	.	-
Sequence	T	672	0.367	0.223	.	-
Sequence	T	684	0.354	0.170	.	-
Sequence	S	687	0.282	0.023	.	-
Sequence	S	692	0.335	0.053	.	-
Sequence	S	693	0.301	0.034	.	-
Sequence	T	694	0.438	0.068	.	-
Sequence	T	695	0.402	0.108	.	-
Sequence	S	698	0.289	0.028	.	-
Sequence	S	699	0.293	0.027	.	-
Sequence	S	702	0.234	0.063	.	-
Sequence	S	707	0.229	0.023	.	-
Sequence	T	714	0.251	0.088	.	-
Sequence	T	730	0.264	0.414	.	-

Sequence	T	736	0.310	0.031	.	-
Sequence	S	739	0.221	0.113	.	-
