Improving galactooligosaccharide synthesis efficiency of β-galactosidase Bgal1-3 by reshaping the active site with an intelligent hydrophobic amino acid scanning

Zongmin Qin, Shuifeng Li, Xin Huang, Wei Kong, Xiangpeng Yang, Sufang Zhang, Lichuang Cao*, Yuhuan Liu*

School of Life Sciences, Institute of Aquatic Economic Animals and Guangdong Provincial Key Laboratory for Aquatic Economic Animals, National Engineering Center for Marine Biotechnology of South China Sea, Sun Yat-Sen University, Guangzhou, 510275, P. R. China.

*Correspondence should be addressed to Lichuang Cao (E-mail: caolch5@mail.sysu.edu.cn) or Yuhuan Liu (E-mail: lsslyh@mail.sysu.edu.cn).

Tel: 86-20-84113712, Fax: 86-20-84036215.

Supporting Information

Figure captions

Supplementary Figure S1. SDS-PAGE analysis of crude enzyme and purified enzyme.

Supplementary Figure S2. TLC analysis of GOS synthesized by C510V and H512I with 40% (w/v) lactose as a substrate.

Supplementary Figure S3. Kinetics of the GOS formation in milk catalyzed by Bgal1-3 (A), C510V (B) and H512I (C).

Supplementary Figure S4. Multiple sequence alignment of GH2 β-galactosidases with the ability to synthesize GOS.

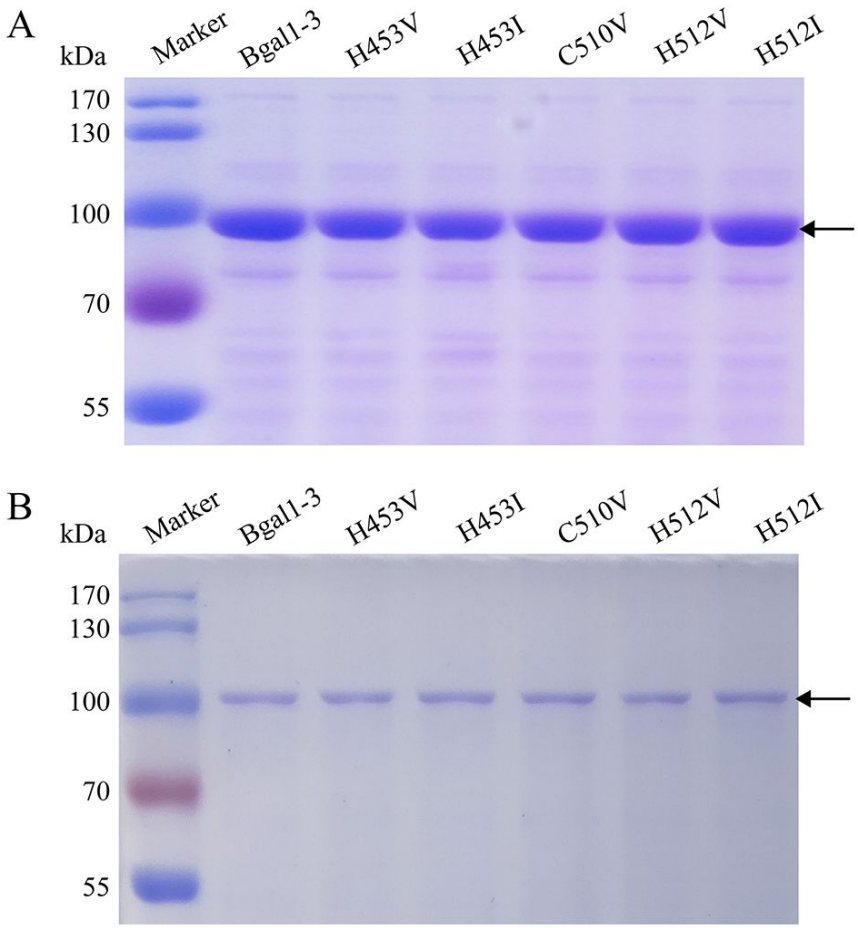
A
kDa
Marker
Bgal1-3
H453V
H453I
C510V
H512V
H512I
170
130
100
70
55
B
kDa
Marker
Bgal1-3
H453V
H453I
C510V
H512V
H512I
170
130
100
70
55

Figure S1.

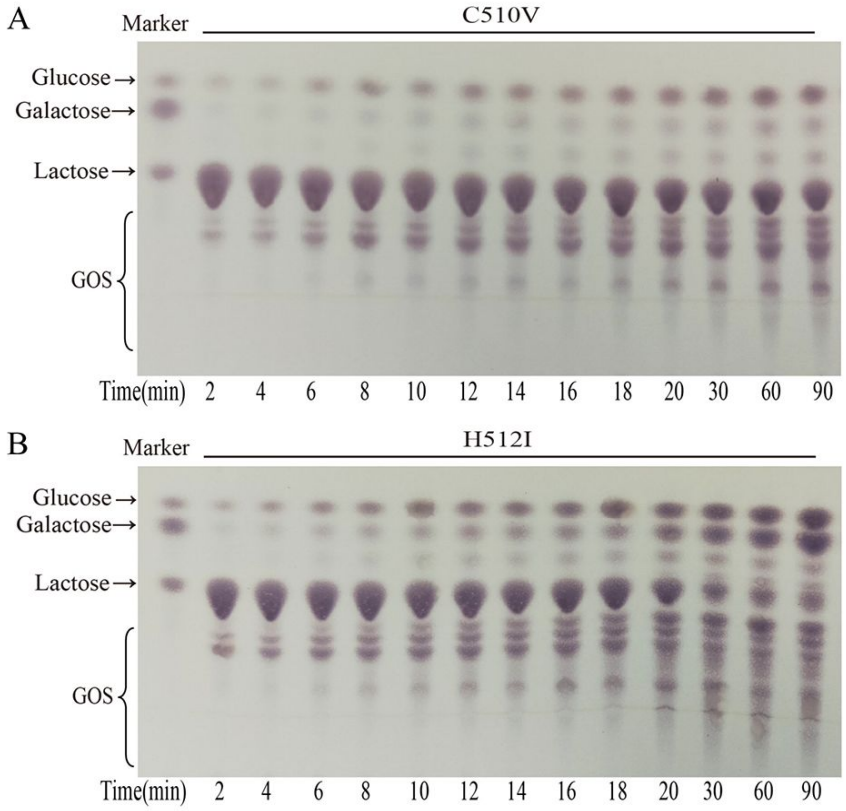
A
Marker
C510V
Glucose→
Galactose→
Lactose→
GOS
Time(min) 2 4 6 8 10 12 14 16 18 20 30 60 90
B
Marker
H512I
Glucose→
Galactose→
Lactose→
GOS
Time(min) 2 4 6 8 10 12 14 16 18 20 30 60 90

Figure S2.

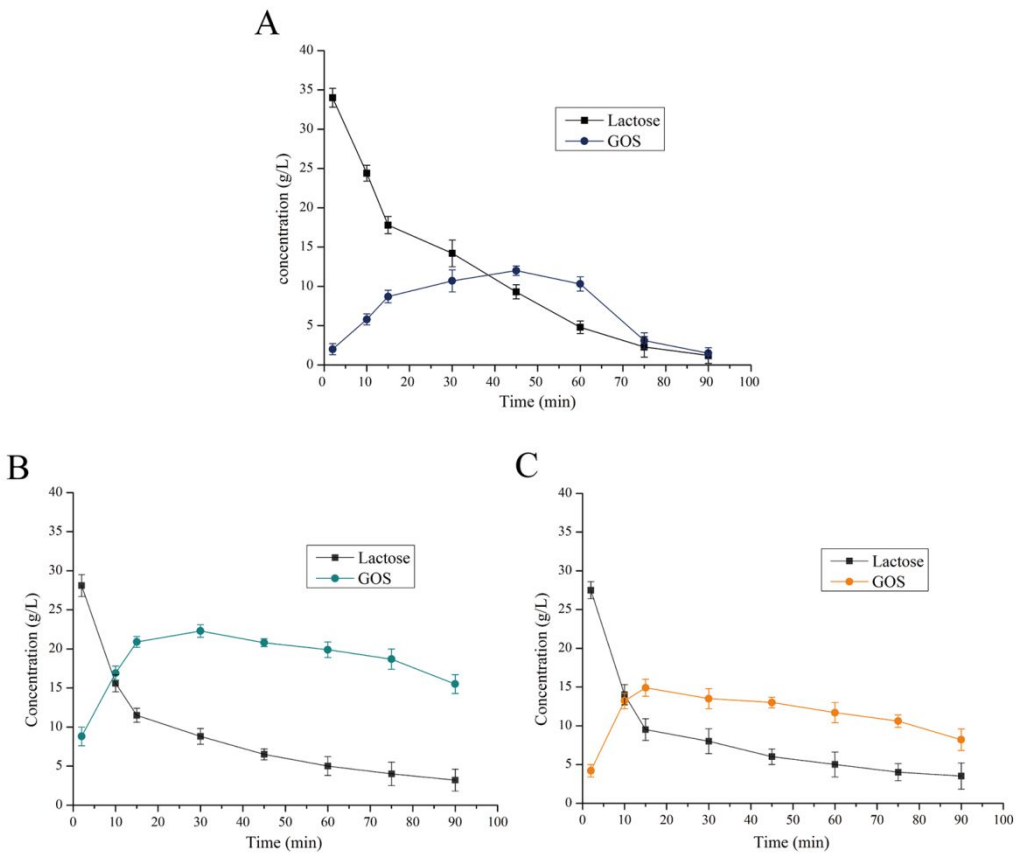
A
concentration (g/L)
Time (min)
Lactose
GOS
B
Concentration (g/L)
Time (min)
Lactose
GOS
C
Concentration (g/L)
Time (min)
Lactose
GOS

Figure S3.

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AGK30046.1    ........................................................................
AAD36268.1    ................MKNMPYEW.ENPQLVSEGTEKSHASFIPY.LDPFSGEWEY....
ABB73039.1    ......MPNTLSLTLSAILARRDW.ENPGVTQWNRLEAHAPLHSWRLEQPALDDAA....
ACE06986.1    ......MSNKLVKEKRVDQADLAWLTDPEVYEVNTIPPHSDHESFQSQEE.LEEGK....
AAL02052.1    ..........MTDVTHVDRASQAWLTDPTVFEVNRTPAHSSHKWY.ARDP.QSGQW....
ABE00939.1    MNTTDDQRKNGDPIVSPSIPTTAWLADPRVYAVHRLDAHSDHACW.SRSP.VDGES....
AHY00656.1    .MSVETPSALADSSPHTAPGSAGRSLELGAADIQDLESFEAGRGA.LPAR.AYLQS....
CAD29775.1    ..........................MTTADVSYLTDQGPGSGRRVPAR.SWLHS....
ABK59934.1    ..................MQANIKWLDDPEIFRVNQLPAHSDHPFFKNYRE.WQNNH....
BAJ61032.1    ............MKKAISCVFLISALILSSFQVPVQGQAMSKTTSAAGNSVSYDG.....
CAC14566.2    MAVRRLGGRIVAFAATVALSIPLGLLTNSAWAVEDATRSDSTTQMSSTPEVVYSSAVDSK
consensus>50  ......................w..dp...qvn....h....................

                                                                  1        10
AGK30046.1    .............................................................MRPLIDFNSGW
AAD36268.1    PEEFISLNGNWRFLFAKNPFEVPED................FFSEKFDDSNWDEIEVPSNW
ABB73039.1    SASRRSLNGVWRFNYFPAPEQIPEA...............WVTEDCADAV..PMPVPSNW
ACE06986.1    SSLVQSLDGDWLIDYAENGQGPVN.................FYAEDFDDSNFKSVKVPGNL
AAL02052.1    SDLKQSLDGEWRVEVVQAADINLEEKPS.............TAESFDDSSFERIQVPGHL
ABE00939.1    TNLRQSLDGEWRVRVETAPTGRFPDGTSDGPDWISDVSPLFAAPGFDDSSFSRVQVPSHL
AHY00656.1    DAPRLSLNGEWQFRLSPGSRVAPDDGWQL.....GEALNG.....ESLPVPSSW
CAD29775.1    DAPALSLNGDWRFRLLPAAPGTAGAGSVLPS...GETVEGVAAESYDDAAWDTLPVPSHW
ABK59934.1    SSFKQSLNGMWQFKFSKDPQSRPVD................FYKKDFNTSSFTTIPVPSEI
BAJ61032.1    .ERRVNFNENWRFQRETNGSIAGAQ.................NPGFDDSSWRKLNLPHDW
CAC14566.2    QNRTSDFDANWKFMLSDS...VQAQ..................DPAFDDSAWQQVDLPHDY
consensus>50  .....slngnwrf...........d....................fdds....idvpsnw

                  20         30                                   40        50
AGK30046.1    LFEGSETVRLP.HNAVELPFSYFDE.....................RSYQRPFTYEKRFAAD
AAD36268.1    EMKG...YGKPIYTNVVYPF....EPNPPFV............PKDDNPTGVYRRWIEIP
ABB73039.1    QMQG...FDTPIYTNVTYPI....PVNPPFV............PQE.NPTGCYSLTFDVD
ACE06986.1    ELQG...FGQPQYVNVQYPWDGSEEIFPPQI............PSK.NPLASYVRYFDLD
AAL02052.1    QTAG...LMNHKYVNVQYPWDGHENPLEPNI............PEN.NHVALYRRKFTVS
ABE00939.1    ETAG...LLAPQYVNVQYPWDGHEDPKAPAI............PEH.GHVAVYRREFDAD
AHY00656.1    PMHG...HGAPAYTNVQFPF....AVEPPHV............PEA.NPIGDHLVVFEAG
CAD29775.1    VMGQDGKYGRPIYTNVQYPF....PIDPPHV............PDA.NPTGDFRRRFDVP
ABK59934.1    ELNN...FAQNQYINTLYPWEGKIFRRPAYALNKSDAEEGSFSEGKDNTVGSYIKHFDLN
BAJ61032.1    SIELD..FNKNSLATHEGGY........................LDGGIGWYRKTFTIP
CAC14566.2    SITQK..YSQSNEA..ESAY........................LPGGTGWYRKSFTID
consensus>50  em.g...f..p.y.nvqypf.......pp.v............p...n..g.yr..fdid

                        60         70         80         90        100
AGK30046.1    PSWEGK.....EVSIVFDGAMADCKVSLNGNETQSHRDGYTPFEVRLTGKLQPGENVLTV
AAD36268.1    EDWFKK.....EIFLHFEGVRSFFYLWVNGKKIGFSKDSCTPAEFRLTDVLRP..GKNLI
ABB73039.1    DAWLQS....GQTRIIFDGVNSAFHLWCNGRWMGYSQDSRLPAEFNLSTVLRP..GENRL
ACE06986.1    EAFWD.....KEVSLKFDGAATAIYVWLNGHFVGYGEDSFTPSEFMVTKFLKK..ENNRL
AAL02052.1    TPVANAKQAGGSVSIVFHGMATAIYVWVNGAFVGYGEDGFTPNEFDITGLLHD..GENVV
ABE00939.1    GEVAQAVREGRPVTLTFQGAATAIYVWLNGSFVGYAEDSFTPSEFDVTDAIKV..DGNVL
AHY00656.1    PEFFPHAL.....LRFDGIESAGTVWLNGVELGTTRGSRLAHEFDVSGILEQ..GENTL
CAD29775.1    AQWFESTT..AALTLRFDGVESRYKVWVNGQEIGVGSGSRLAQEFDVSDALRA..GSNLL
ABK59934.1    PELRDH.....DIHIVFEGAERAMYVWLNGHFIGYAEDSFTPSEFDLTKYIKE..KDNIL
BAJ61032.1    ESMKGK.....RISLDFDGVYMNSTTYLNGEVLGTYPFGYNAFSYDISDKLYKDGRANVL
CAC14566.2    RDLAGK.....RIAINFDGVYMNATVWFNGVKLGTHPYGYSPFSFDLTGNA.KFGGENTI
consensus>50  .e..q.......v.l.FdGv..a..vwlNG..igy..dsftp.efdvt..l....gen.l

                110        120        130        140        150        160
AGK30046.1    AIDGSENPEIPPFGGQIDYLTYAGI.YRDVWLRVTSPIFVENAKIETPNVLSDHKSVRAR
AAD36268.1    TVEVLKWSDGS.YLEDQDMWWFAGI.YRDVYLYALPKFHIRDVFVRT.DLDENYRNGKIF
ABB73039.1    AVMVLRWCDGS.YLEDQDMWRMSGI.FRDVTLLHKPETQIADYRVVT.DLNAELDRAVLK
ACE06986.1    AVALYKYSSAS.WLEDQDFWRMSGL.FRSVTLQAKPRLHLEDLKLTA.SLTDNYQKGKLE
AAL02052.1    AVACYEYSSAS.WLEDQDFWRLHGL.FRSVELAARPHVHIENTQIEA.DWDPEAGTASLD
ABE00939.1    AVVCYEYSSAS.WLEDQDFWRLHGL.FRSVELNARPAAHIADLHADA.DWDLATSRGSLS
AHY00656.1    AVRVAQFSAAS.YVEDQDMWWLPGI.FRDVTLQARPAAGIDDVFVHA.GYDHITGEGI..
CAD29775.1    VVRVHQWSAAS.YLEDQDQWWLPGI.FRDVTLQARPAGGITDAWLRT.GWSARSGAGTGT
ABK59934.1    AVEVFKHSTAS.WLEDQDMFRFSGL.FRSVELLAFPETHLVDLDLKP.TVCDNYQDGIFN
BAJ61032.1    VVKVNNTQPSSRWYSGSGIYRNVYLTVTDPIHVARYGTFVTTPNLEK.SIKEDRADVNIK
CAC14566.2    VVKVENRLPSSRWYSGSGIYRDVTLTVTDGVHVGNNGVAIKTPSLAT.QNGGD.VTMNLT
consensus>50  a!.v.e.s.as.wledqd.wrl.gl.frdv.l.a.p...i.d......d...d...g...

                170        180                    190        200        210
AGK30046.1    IDVANPQNLPLSGTLLAIL.........TDSQGNTIASTDASVDGASIEIAFDDLTGISL
AAD36268.1    LDVEMRNLGEEEEKDLEVT......LITPDGDEKTLVKETVKPEDRVLSFAFD.VKDPKK
ABB73039.1    ADVALAGAGFADCEVVFTLWRKGEKCASVSRRPGSAVVDERGSWDERLTVAIP.IDRPAL
ACE06986.1    VEANIAYRLPNASFKLEVR..........DSEGDLVAEKLGPIRSEQLEFTLA.DLPVAA
AAL02052.1    AALTVLNATDAATVRATLK..........DADGNTVWQTTGDAEAQTAISSGP.LPGIEP
ABE00939.1    LDVLIDGAANAATVDFALW..........DKNGTIVWHTATKADG.TLHAEAE.IDDAAP
AHY00656.1    LKVEASRGGQAIDAVVRV.............PELALELAAGTEVRV..PA......VEP
CAD29775.1    IDPEITADATAFPVTLSV.............PELGVNVTWKSAEEV..APLA.LENVEP
ABK59934.1    AELKFTGSLNGHVHLSVE...........DVNGSAILEQDVPLDSEVEFTSST.LENIHL
BAJ61032.1    TKISNDAAEAKQVKIKSTIYDGAGNT...VQTVETEEKTAAAGTVTPFEQNTV.IKQPKL
CAC14566.2    TKVANDTEAAANITLKQTVFPKGGKTDAAIGTVTTASKSIAAGASADVTSTIT.AASPKL
consensus>50  .dv.......a...................d.d.e.........d.............i..
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                 220       230       240       250       260       270
AGK30046.1   WDLDNPALYTLAITIDTTHGQ..DESAFRFGFRSVEFTHEG.FRLNGKPLKLRGLNRHQS
AAD36268.1   WSAETPHLYVLKLKLGEDEKKV......NFGFRKIEI.KDGTLLFNGKPLYIKGVNRHEF
ABB73039.1   WSAETPELYRLTMALLGPQGEVLEVEACDVGFRRVDI.SNGLLKLNGKPLLIRGVNRLEH
ACE06986.1   WSAEKPNLYQVRLYLY.QAGSLLEVSRQEVGFRNFEL.KDGIMYLNGQRIVFKGANRHEF
AAL02052.1   WSAESPTLYELDVDVIDQAGDVIECTSQKVGFRRFRI.EDGILTINGKRIVFKGADRHEF
ABE00939.1   WSAERPDLYELSVTLLDADGKVLETARTRIGFRHVAI.EDGILKLNGKRLVFRGVNRHEF
AHY00656.1   WSAEVPKLYEAAVSAAGESVAL......QIGFRSIAI.EDAQFKVNGRRILLRGVNRHEH
CAD29775.1   WSAEVPRLYEASVSSAAESISV......RLGFRTVRI.VGDQFLVNGRRVVFHGVNRHET
ABK59934.1   WDNHHPYLYQLLIEVHDENGHLVELIPYQFGFRRIEINQDKVILLNGKCLIINGVNRHEW
BAJ61032.1   WSIDKPYRYNLVTEVI.VGGQTVDTYETKFGVRYFKFDENEGFSLNGEYMKLHGVSMHHD
CAC14566.2   WSIKNPNLYTVRIEVL.NGGKVLDTYDTEYGFRWIGFDATSGFSLNGEKVKLKGVSMHHD
consensus>50 Wsae.P.lYel.v....e.g.v.e....e.GfR.v.i..dg...lNGk.l...Gvnrhe.

                 280       290       300       310       320
AGK30046.1   FPYSGYAMGRAAQERDAEILKHQLHLNIVRTSHYPQSHWFLDRCDELGLLVFEEI.....
AAD36268.1   DPDRGHAVTVERMIQDIKLMK.QHNINTVRTSHYPNQTKWYDLCDYFGLYVIDEA.....
ABB73039.1   HPENGQVMDEATMRRDIEIMK.QHNFNAVRCSHYPNHPLWYRLCDRYGLYVVDEA.....
ACE06986.1   DSKLGRAITEEDMIWDIKTMK.RSNINAVRCSHYPNQSLFYRLCDKYGLYVIDEA.....
AAL02052.1   DAERGRAITEQDMIDDVVFCK.RHNINSIRTSHYPNQERWYELCDEYGIYLIDEA.....
ABE00939.1   DCRRGRAITEEDMLWDIRFMK.RHNINAVRTSHYPNQSRWYELCDEYGIYLIDET.....
AHY00656.1   HPRLGRVVPRDVVEAELRLMK.QHNINAIRTSHYPPHPQFLALADQLGFYVVLEC.....
CAD29775.1   HPDRGRVFDEAGAREDLALMK.RFNVNAIRTSHYPPHPRLLDLADEMGFWVILEC.....
ABK59934.1   NAKTGRSITLNDMEKDIDTFK.ENNINAVRTCHYPNQIPWYYLCDQNGIYMMAEN.....
BAJ61032.1   LGALGAATNARGVERQMQIMK.DMGVNAIRVTHNPASPELLEAANKLGLFIIEEAFDSWA
CAC14566.2   QGSLGAVANRRAIERQVEILQ.KMGVNSIRTTHNPAAKALIDVCNEKGVLVVEEVFDMWN
consensus>50 d...Grai..e.m..#i..mk.q.niNa!RtsHyPn....ydlc#e.G.yvidE......

                 330                 340       350       360
AGK30046.1   .....[...]PGWQHIGG.........[...]EAWKDESVENVRRMITRDWNHPSIVIWGVR
AAD36268.1   .....NIESHGIDWDP.......EVTLANRWEWEKAHFDRIKRMVERDKNHPSIIFWSLG
ABB73039.1   .....NIETHGMV..P.......MSRLADDPRWLPAMSERVTRMVQRDRNHPSIIIWSLG
ACE06986.1   .....NLESHGTWEKVGGHEDPSFNVPGDDQHWLGASLSRVKNMMARDKNHASILIWSLG
AAL02052.1   .....NLEAHGSWSLPGDVLTEDTIVPGSKREWEGACVDRVNSMMRRDYNHPSVLIWSLG
ABE00939.1   .....NLETHGSWNSPGDIPV.GTSVPGDDEAWLGACIDRLDSMILRDRNHPSVLVWSLG
AHY00656.1   .....DLETHGFESAGW......AQNPSDDPQWEDALVDRMRRTVERDKNHASVVMWSLG
CAD29775.1   .....DLETHGFEAGGW......VENPSDVPAWRDALVDRMERTVERDKNHPSIVMWSLG
ABK59934.1   .....NLESHGTWQKMGQVE.PSDNVPGSVPEWREAVIDRARNNYETFKNHTSILFWSLG
BAJ61032.1   QSKK..PYDYGRFFN.........................AWAE...HDIKEMVDRGKNEPAIIMWSIG
CAC14566.2   RSKNGNIEDYGKWFGQAIAGDNAVLGGDKDETWAK...FDLTSTINRDRNAPSVIMWSLG
consensus>50 .....nle.hG..............p.d...W..a.vdrv..mverdkNhps!i.Wslg

                 370                 380       390                 400
AGK30046.1   INESQDDHD........FYVRTNKLARELDTTRPTGGVR......YITESELLEDVY...
AAD36268.1   .NEAGDGVN.........FEKAALWIKKRDNTRLIHYEG.......TTRRGESYYV....
ABB73039.1   .NESGHGAN.........HDALYRWLKTTDPTRPVQYEG.......GGANTAATDIVCPM
ACE06986.1   .NESYAGTV.........FAQMADYVRKADPTRVQHYEG.......VTHNRKFDDAT...
AAL02052.1   .NESYVGDV.........FRAMYKHVHDIDPNRPVHYEG.......VTHNRDYDDVT...
ABE00939.1   .NESYAGEV.........LKAMSAHAHRLDPGRPVHYEG.......VNWNHAYDGIS...
AHY00656.1   .NEAGTGRN.........LAAMSRWTKDRDPSRPIHYEG.......DWSSEHVDVYSRM
CAD29775.1   .NESGTGSN.........LAAMAAWAHARDSSRPVHYEG.......DYTGAYTDVYSRM
ABK59934.1   .NESYAGSN.........IVAMNEFYKEHDSSRLVHYEG.......VVHRPELKDQIS..
BAJ61032.1   .NEIYDTTNAAGV...ETARNLVGWVKEIDTTRPTTIGEDKTRGDKVNVTPINSYIKEIF
CAC14566.2   .NEMMEGISGSVSGFPATSAKLVAWTKAADSTRPMTYGDNKI...KANWNESNTMGDNLT
consensus>50 .NEs..g.n...........am..w.k..D.tRpvhyeg.......v.......dv....

                 410       420       430       440       450       460
AGK30046.1   ......[.]TMNDFILGNEELGGNRPRTPLRPQQEVTGLDRVVPYMITEYGGHMYPTKVHDE
AAD36268.1   ......DVFSLMYPKMDILLEYA.....SKKREKPFIMCEYAHAMGNSVGNLKDYWDV.I
ABB73039.1   YARVDWDQPFPAVPKWSIKKWIG.....MPDETRPLILCEYAHAMGNSFGGFAKYWQA.F
ACE06986.1   ......QIESRMYVPAKVIEEYL.....TNKPAKPFISVEYAHAMGNSVGDLAAY.TA.L
AAL02052.1   ......DIETCMYLHADEIEKYL.....MDDPKKPYLSCEYMHAMGNSVGNMDEY.TA.L
ABE00939.1   ......DFESRMYAKPAEIQDWLEHGDERGEASKPFVSCEYMHAMGNSCGGLSEF.ID.L
AHY00656.1   YASQA.ETALIGQGIEPALN...DAALDARRRAMPFVLCEYVHAMGNGPGGMSEYQAL.F
CAD29775.1   YSSIP.ETDSIGRNDSHALLLGCDSAESARQRTKPFILCEYVHAMGNGPGAMDQYEAL.V
ABK59934.1   ......DIESHMYLPPKEVAEYL.....RNNPQKPFMECEYMHDMGNSDGGMGSYIKL.I
BAJ61032.1   NIVDVVGLNYSEN.NYDGYHKQNPSWKLYGSETSSATRSRGVYTHPYQYNQSTKYADLQQ
CAC14566.2   ANGGVVGTNYSDGANYDKIRTTHPSWAIYGSETASAINSRGIYNRTTGGAQSS.[.].DKQL
consensus>50 ......d.e.......................q..kpfi.cey.hamgns.g.m..y.....

                 470       480       490       500       510       520
AGK30046.1   EQRQAEHVRRHLEVMNAAYGDPQIAGCIGWCAFDYNTHKDFGSGDRICHHGVMDMFREPK
AAD36268.1   EKYPYLHGGCIWDWVDQGIRKKDENGREF.WAYGG.DFGDTPNDGNFCINGVVLPDRTPE
ABB73039.1   RSHPRLQGGFVWDWVDQALTKKDEKGNAF.WAYGG.DFGDTPNDRQFCLNGLVFPDRTPH
ACE06986.1   EKYPHYQGGFIWDWIDQGL...EKDG..H.LLYGG.DFDDRPTDYEFCGNGLVFADRTES
AAL02052.1   ERYPKYQGGFIWDFIDQAIYATQPDGTRS.LRYGG.DFGDRPSDYEFSGDGLLFADRKPS
ABE00939.1   ERYERYSGGFIWDYIDQGLVQRLPDGSER.LSVGG.EWGDRPTDYEFVGNGIVFADRTPS
AHY00656.1   EKYPRLMGGFVWEWLEHGITVSTADGVDH.YGYGG.DFGEEVHDGNFVTDGLVDADRRPR
CAD29775.1   DKYPRLHGGFVWEWRDHGIRTRTAEGMEF.FAYGG.DFGEVVHDSNFVMDGMVLSDSTPT
ABK59934.1   DEYPQYVGGFIWDFIDQALLVYDPISKQNVLRYGG.DFDDRHSDYEFSGDGLMFADRTPK
BAJ61032.1   SSYDNDYVGWGRTAEDAWKYDRDLKHIAGQFIWTGFDYIGEPTPY.....YNSYPA.K
CAC14566.2   TSYDNSAVGWGAVASSAWYDVVQRDFVAGTYVWTGFDYLGEPTPWNGTGSGAVGSWPSPK
consensus>50 e.yp...ggfiwd.idq.....d.dg......ygg.dfgd.p.d.nf..nglv..drtp.
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                 530       540       550       560       570       580
AGK30046.1   FAAYAYASQCEPDEEVILRPVTFWARGERNIGGVLPLIVLTNCDEVELRYGTNPPKRFRP
AAD36268.1   PELYEV.......KKVYQNVKIRQVSKDTY....EVENRYLFTNLEMFDGAWKIRKDGEV
ABB73039.1   PALYEA.......QRAQQFFTFTLVSTSPLM..IEVQSGYLFRPTDNEVLSWTVARDGKV
ACE06986.1   PKLANV.......KALYANLKLEVKDGQLF.....LKNDNLFTNSSSYYFLTSLLVDGKL
AAL02052.1   PKAQEV.......KQLYSNVHIDVTKDSVS.....VKNDNLFTATGDYVFVLSVLADGKP
ABE00939.1   PKAQEV.......KQLYSPVKLAPDGHGVT.....IENRNLFAGTDGYVFAARLLEDGHE
AHY00656.1   PGLLDF.......KKVIEPLRIDVARDWTG...FTLRNGQDFADTSAFSFRYEVEADGGA
CAD29775.1   PGLYEF.......KQIVSPIRLGLSLPAGGKPTLAVANLRHTADASDVVLRWRVEHDGAV
ABK59934.1   PAMQEV.......RYYYGLHK.......................................
BAJ61032.1   SSYFGAVDTAGFPKDIFYYYQSQWKKEP.MVHLLPHWNWK.....EGEKVRVLAYTNASK
CAC14566.2   NSYFGIVDTAGFPKDTYYFYQSQWNDDVHTLHILPAWNENVVAKGSGNNVPVVVYTDAAK
consensus>50 p.lyev.......kq.y....................n...f...........v..dg..

                 590       600       610       620       630       640
AGK30046.1   DRDAFPHLPHAPVVMDRKDFTADELGRWGMSWESATITGYVGGKAVKTIDFVADPVPATL
AAD36268.1   IEEK.TFKIF....AEPGEKRLLKIPLPEMD.......DSEYFLEISFSLSEDTPWAEKG
ABB73039.1   LASG.EVTLA....IAPEGVQRLEIALPELK.....AGPGEIWLNVEVRQPRATPWSPAG
ACE06986.1   TYQSRPLTFG....LEPGESGTFALPWPEVA.....DEKGEVVYRVTAHLKEDLPWADEG
AAL02052.1   VWQS.TRRFD....VPAGETRTFDVAWPVAAYR...ADAREVLVLQVSQRLAKATDWAESG
ABE00939.1   IWHA.DYRFD....VAAGDTQHHDIAFPDIDAD...GDTREVTYEVDLLLAEATAWAPAG
AHY00656.1   LDGGTVDVAP....VAPQSETVVELPGSVAALAAGLSDGRPAVLTVRAVLGADSAWADAG
CAD29775.1   AASG..EVAA....EGSDGPLRAGESATIALPAMPAAPLGETWLTVEAVLRDATGWAPAG
ABK59934.1   ......................................................
BAJ61032.1   VELVLNGES......LGEKNYDNKQTSWGAPYK.......ETKDGKTYLEWAVPFKPGK
CAC14566.2   VKLYFTPKGSTEKRLIGEKSFTKKTTAAGYTYQVYEGSDKDSTAHKNMYLTWNVPWAEGT
consensus>50 ........................................e....v...l..a.pwa..g

                 650       660       670       680       690       700
AGK30046.1   QVEADTPDLPGLGETVRVMVRALDQAGNKLPFFSDPVSIEVSGAAERLGPALVPLRAGAT
AAD36268.1   HVVAWEQFL...LKAPA....FEKKSISDGVS..LREDGKHLTVEAKDTVYVFSKLTGLL
ABB73039.1   HRCAWEQWP...LPAPL....FIAPPASTGEPPVLTQNDRILEVTHRQQRWQFDRASGYL
ACE06986.1   FTVAEAE.......EVA....QKLPEFKPEGRPDLVDSDYNLGLKGNNFQILFSKVKGWP
AAL02052.1   YELAFGQAV....VPAD....ATATPDTKPADGTITVGRWNAGVRGAGREVLLSRTQGGM
ABE00939.1   YELAFGQLTGTLNPEQD....ITETSHDDDGRATRTLSRWNAGIRRDDEEILLSRTQGGI
AHY00656.1   HEVAWGQ..............SVREPGAPV.PPAPVEPVQVQDSELTLGPVVFSRATGMP
CAD29775.1   HPLGAVQL.............DLSAPAVPTRSPRPATPLDGA.LPVSLGPATFD..AGTL
ABK59934.1   ......................................................
BAJ61032.1   LEAVAKDENGKVIARDQ......VVTAGEPASVRLTADRKVVKADGTDLSFITADIVDSK
CAC14566.2   ISAEAYDENNRLIPEGSTEGNASVTTTGKAAKLKADADRKTITADGKDLSYIEVDVTDAN
consensus>50 ...a..q.......................................d.........g..

                 710       720       730
AGK30046.1   GFWVRATGQGPVTVTVINERLGRQSITLTAN...........................
AAD36268.1   EQILHRRKKILKSPVVPNFWRVPTDNDIGNRMPQ............RL......AIWKRA
ABB73039.1   TQWWRNGVETLLSPVTDNVSRAPLDNDIGVSEAT............RIDPNAWVERWKAA
ACE06986.1   VSLKYAGREYLKRLPEFTFWRALTDNDRGAGYGY............DL......ARWENA
AAL02052.1   VSYTFAGNEFVLRRPAITSFRPLTDHDRGAGHGF............ER......VQCWAP
ABE00939.1   VSWKRDDREMVIRRPELVTFRPLTDNDRGNHSGF............DR......AAWFAA
AHY00656.1   TSI....GGVPVEKLGLTLWWAPTDNDLGREWG.............GADERPLATQWKDA
CAD29775.1   VSL....AGQPVSGPRLELWRAPTDNDRGAGFGAYGPGDPWLNSGRGVPAPSSEAVWKQA
ABK59934.1   ......................................................
BAJ61032.1   GIVVPDADHLITFNVTGQGELAGVDNGNASSVERYKDNK...RKAFSGKALAIVQSSKLS
CAC14566.2   GHIVPDAANRVTFDVKGAGKLVGVDNGSSPDHDSYQADN...RKAFSGKVLAIVQSTKEA
consensus>50 ........e...........r..tdnd.g...........................wk.a

AGK30046.1   .............................................................
AAD36268.1   SKERKLFKM.HWKKEENR.....VSVHSVFQLPGNSWVYTT..YTVFGNGDVLVDLSLIP
ABB73039.1   GMYDLTSRMLHCEAEQHAR.EVVVTTLNVLEHRGRALFLSRKIWRLDEQGVLHGDIQVDI
ACE06986.1   GKYARLKDISCEVKEDS......VLVKTAFTLPVALKGDLTVTYEVDGRGKIAVTADFPG
AAL02052.1   DVRPLRGQRARADRDRT......IKGTYTYELATAQRTKVTVSYTAHTDGRVNLHVEYPG
ABE00939.1   GRYAIVTETKIHESDDG......LVAEYQYELADPNHTPVSVTYHVNSDMRMQLTVEYPG
AHY00656.1   GLNRLHTRLLGISANPGQDGGETLTVRTRVSAADKQYGVLVDYTWSTDGETVGLRTQVRR
CAD29775.1   GLDRLTRRVEDVAALP..DG...IRVRTRYAAADSTHSVAVEENWQLDGGELCLRIDITP
ABK59934.1   .............................................................
BAJ61032.1   GKITVHASVAGLSSDSTSVFT..VTPADHDKKIVAGIDDVNLTVDVNEAPKLPSEIKVYY
CAC14566.2   GEITVTAKADGLQSSTVKIATTAVPGTSTEKTVRSFYYSRNYYVKTGNKPILPSDVEVRY
consensus>50 g.............e........v...............................ie...

AGK30046.1   ................................................................
AAD36268.1   .AEDV.....PEIPRIGFQFTVPEEFGTVEWYGRGPHETYWDRKESGLFARYRKAVGEMM
ABB73039.1   .ASDI.....PKPARIGLSVHLAETPEKVDWLGLGPHENYPDRKLAAQQGRWTLPLADMH
ACE06986.1   .AEEA.....GLLPAFGLNLALPKELTDYRYYGLGPNESYPDRLEGNYLGIYQGAVKKNF
AAL02052.1   EQGDL.....PTIPPFGIEWTLPVQYTNLRFFGTGPEETYLDRKHA.KLGVWSTNAFADH
ABE00939.1   NATDM.....ASLPAFGIEWELPGEYDRLRYYGPGPEETYRDRKQGGKLGIWDATAKASM
AHY00656.1   DGTWVNRGFEVEWARIGLEFVLGEETELVSWFGQGPHQSYPDTGQGARAGWFSLPLAKMD
CAD29775.1   SAGW.....NLVWPRIGVRWDLPTDVDGAAWFGAGPRESYPDSMHATVVARHAASLEELN
ABK59934.1   ................................................................
BAJ61032.1   SDESAA...AKNVTWDEVDPKQYSTVGEFTVEGSVEGTSLKAKAFVIVKGIVAVKPYSTA
CAC14566.2   SDGTSD...RQNVTWDAVSDDQIAKAGSFSVAGTVAGQKISVRVTMIDE.IGALLNYSAS
consensus>50 ................g.e..l..e......yg.gp.e.y.dr......g..........
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AGK30046.1     .....................................................
AAD36268.1     HRY.VRPQETGNRSDVRWFALSD....GETKLFVSGMPQID..FSVWPFSMEDLERVQHI
ABB73039.1     TPY.IFPTENGLRCDTRKLVLGA....HQLN......GAFH..FSVGRYSQQQLRETTHH
ACE06986.1     SPY.LRPQETGNRSKVRWYQLFDEKG.GLEFTA..NGADLN..LSALPYSAAQIEAADHA
AAL02052.1     APY.LMPQETGNHEDVCWAEITDDHGHGMRVSRADGAAPFA..VSLLPYSSFMLEEAQHQ
ABE00939.1     APY.LMVQETGSHEDVRWLEATDIQGHGLRVTQR.GDRHFT..ASLLPWNTYTIEAARRH
AHY00656.1     VEY.VRPQECGARSGSRSAALQLGGRT.LEICGDPFALTVRP.......YSQDVLDAAAHR
CAD29775.1     VPY.ARPQETGHRSDVRWLELDRAGAPWLRIDAEPDAAGRRPGFSLARHTAQEIAAAGHP
ABK59934.1     .....................................................
BAJ61032.1     TKVGVQPVLPEKATLLYSDGTTKGAT..VTWDEIPEDKLAKEGRFTVEGSVEGTDLKANV
CAC14566.2     TPVGTPAVLPGSRPAVLPDGTVTSANFAVHWTKPADTVYNTAGTVKVPGTATVFGKEFKV
consensus>50   .py...pqe.g.r..vr.........................s....s....e...h.
```

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AGK30046.1     .....................................................
AAD36268.1     SELPERDFVTVNVDFRQMGLGGDDSWGAMPHLEYRLLPKPYRFSFRMRISE....EIPSW
ABB73039.1     HLLREEPGGWLNLDAFHMGVGGDDSWSPSVSPEFILQTRQLRYTFSWQQNP.........
ACE06986.1     FELTN.NYTWVRALSAQMGVGGDDSWGQKVHPEFCL.DAQKARQLRLVIQP....LLLK.
AAL02052.1     DELPKPKHMFLRVLAAQMGVGGDDSWMSPVHPQYHI.PADKPISLDVDLEL....I....
ABE00939.1     EDLPKPRHNYLRLLAAQMGVGGDDSWGAPVHTAYQL.PAGRPLTLDVNLEL....I....
AHY00656.1     PDLKADGRTYLYVDHALRGVG.TAACGPGVLEQYRLKPRDADFILTLKVRS.........
CAD29775.1     HELPTPSHSYLYVDAAQHGLG.SRACGPDVWPDFALRPEARTLKLRISPA..........
ABK59934.1     .....................................................
BAJ61032.1     YVRVTNEVKSVNIMLQEQGSAYPKLE.ATFTNPADNLQHLNDGIKSYTNNP.....VNRW
CAC14566.2     TATIRVQRSQVTIGSSVSGNALRLTQNIPADKQSDTLDAIKDGSTTVDANTGGGANPSAW
consensus>50   .el........v...q.g.g........v..ey.l.............n..........
```

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AGK30046.1     .....................................................
AAD36268.1     RVLAAIPET.....LHVEMSSEDVIREGDTLRVKFSLLNDTPLSKEKQVVLFVDGNEY..
ABB73039.1     .....................................................
ACE06986.1     .....................................................
AAL02052.1     .....................................................
ABE00939.1     .....................................................
AHY00656.1     .....................................................
CAD29775.1     .....................................................
ABK59934.1     .....................................................
BAJ61032.1     TNWTRTPRDAGDS.ITVNFGKKHVINNLDLFVFTDSGTV.VPEKAEVQYW...DGTAWKD
CAC14566.2     TNWAYSKAGHNTAEITFEYATEQQLGQIVMYFFRDSNAVRFPDAGKTKIQISADGKNWTD
consensus>50   .....................................................
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AGK30046.1     .....................................................
AAD36268.1     ...........SVRRVVIPPFKKEEL..VFKVEGLKKGEHLIHTNLNTRKTIYVR.....
ABB73039.1     .....................................................
ACE06986.1     .....................................................
AAL02052.1     .....................................................
ABE00939.1     .....................................................
AHY00656.1     .....................................................
CAD29775.1     .....................................................
ABK59934.1     .....................................................
BAJ61032.1     V.........ENLTQPSPYVVEKNEL..TFDAVATEKLKFHLTPSVKGKFLALTEAEVYA
CAC14566.2     LAATETIAAQESSDRVKPYTYDFAPVGATFVKVTVTNADTTTPSGVVCAGLTEIELKTAT
consensus>50   .....................................................
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AGK30046.1     .....................................................
AAD36268.1     .....................................................
ABB73039.1     .....................................................
ACE06986.1     .....................................................
AAL02052.1     .....................................................
ABE00939.1     .....................................................
AHY00656.1     .....................................................
CAD29775.1     .....................................................
ABK59934.1     .....................................................
BAJ61032.1     DQIVMGETAKLQSITVNGKALEGFDHAKKNYELVLPYGSELPKIEAAAADNATVTILPAF
CAC14566.2     SKFVTNTSAALSSLTVNGTKVSDSVLAAGSYNTP....AIIADVKAEGEGNASVTVLPA.
consensus>50   .....................................................
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AGK30046.1     .....................................................
AAD36268.1     .....................................................
ABB73039.1     .....................................................
ACE06986.1     .....................................................
AAL02052.1     .....................................................
ABE00939.1     .....................................................
AHY00656.1     .....................................................
CAD29775.1     .....................................................
ABK59934.1     .....................................................
BAJ61032.1     SYPGTAKLFVTSEDGKVTTEYSIGVSTEEPKLVSAELSADKTNVMEDDIIDLKVIGLFES
CAC14566.2     .HDNVIRVITESEDHVTRKTFTINLGTEQ......EFPAD.SDERDYPAADMTVT...VG
consensus>50   .....................................................
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AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    KEKIDVTDSQPTYEFDQQIIKIEGNKLYALETGNVKVKVTVTYKGVSVTTPALEFTIAKN
CAC14566.2    SEQTSGTATEGPKKF.....AVDGNTSTYWHSNWTPTTVNDLWIAFELQKPTKLDALRYL
consensus>50  ............................................................

AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    PAPKYITSLEPVTVVVKKGEAPELPATVVAHYNRGIPRDVKVKWERINPSKYQQLGEFTV
CAC14566.2    PRPAGSKNGSVTEYKVQVSDDGTNWTDAGSGTWTTDYGWKLAEFNQPVTTKHVRLKAVHT
consensus>50  ............................................................

AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    SGMVEGTDIKAQAKVIVKGAVAVEDIRMAVLLKQMPQLPGKVTVYYSDGAEEQRAVKWEE
CAC14566.2    YADSGNDKFMSASEIRLRKAVDTTDISGATVTVPAKLTVDRVDADHPATFATKDVTV..T
consensus>50  ............................................................

AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    IPQEELENVGEFKVKGDVNGVKLKATATIRVTDEVGGEQNISRAKNGYEYPKAEASFTNN
CAC14566.2    LGDATLRYGVDYLLDYAGNTAVGKATVTVRGIDKYSGTVAKTFTIELKNAPAPEPTLTSV
consensus>50  ............................................................

AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    GPGSS.DRIEAINDDVISYEAN..PHNRWTNWQPVPRAGDWVSITFGDYEPTEYDVDSME
CAC14566.2    SVKTKPSKLTYVVGDAFDPAGLVLQHDRQADRPPQPLVGEQADERGLTCGTRCDRVEQLR
consensus>50  ............................................................

AGK30046.1    ............................................................
AAD36268.1    ............................................................
ABB73039.1    ............................................................
ACE06986.1    ............................................................
AAL02052.1    ............................................................
ABE00939.1    ............................................................
AHY00656.1    ............................................................
CAD29775.1    ............................................................
ABK59934.1    ............................................................
BAJ61032.1    IHWFADHGTSYPERFQIEYKSGDSWKEVTSLKSDPASPALGKANVYSFDRVKTSAIRVKM
CAC14566.2    KHENREAHRTGLDHLEFVGAADGAVGEQATFKVH........VHADQGDGRHDDADERDI
consensus>50  ............................................................
```

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AGK30046.1    ..................................................................
AAD36268.1    ..................................................................
ABB73039.1    ..................................................................
ACE06986.1    ..................................................................
AAL02052.1    ..................................................................
ABE00939.1    ..................................................................
AHY00656.1    ..................................................................
CAD29775.1    ..................................................................
ABK59934.1    ..................................................................
BAJ61032.1    TAQAGKSLAITELKVFSKWPKAGTEPEVTDIKVGGKSILEDFEQKGDHYEVTIDAGDANV
CAC14566.2    DPHVPVDHAVGELARAACHHVIGLRVDTHRLKASGFQIPAD.....DMAEIDRITGFHRF
consensus>50  ..................................................................
```

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AGK30046.1    ..............................................
AAD36268.1    ..............................................
ABB73039.1    ..............................................
ACE06986.1    ..............................................
AAL02052.1    ..............................................
ABE00939.1    ..............................................
AHY00656.1    ..............................................
CAD29775.1    ..............................................
ABK59934.1    ..............................................
BAJ61032.1    MPKINVKAKDQTSITIVPAVTSPSTAKVIAKSEDGKKVKVYSIHYK
CAC14566.2    ERHVG.........................................
consensus>50  ..............................................
```

Figure S4.

Table S1. Primers used for the construction of mutants.

Primers	Sequence (5' – 3')
D124I-F	CAGATC **ATC** TACCTGACCTATG
D124V-F	CAGATC **GTG** TACCTGACCTATG
D124K-F	CAGATC **AAA** TACCTGACCTATG
D124-R	GCCGCCGAAGGGCGGGATCTCGGGG
H306I-F	CACCTCG **ATC** TATCCGCAGTCGCACTGG
H306V-F	CACCTCG **GTG** TATCCGCAGTCGCACTGG
H306K-F	CACCTCG **AAA** TATCCGCAGTCGCACTGG
H306-R	CGGACGATGTTGAGGTGAAGCTGGT
G452I-F	GGC **ATC** CACATGTACCCCACCAAGG
G452V-F	GGC **GTG** CACATGTACCCCACCAAGG
G452K-F	GGC **AAA** CACATGTACCCCACCAAGG
G452-R	ATATTCGGTGATCATGTAGGGAACG
H453I-F	GGG **ATC** ATGTACCCCACCAAGGTGC
H453V-F	GGG **GTG** ATGTACCCCACCAAGGTGC
H453K-F	GGG **AAA** ATGTACCCCACCAAGGTGC
H453-R	GCCATATTCGGTGATCATGTAGGGA
F503I-F	GAC **ATC** GGCTCCGGCGACCGCATCT
F503V-F	GAC **GTG** GGCTCCGGCGACCGCATCT
F503K-F	GAC **AAA** GGCTCCGGCGACCGCATCT
F503-R	CTTGTGGGTGTTGTAGTCGAAGGC
C510I-F	CATC **ATC** CACCACGGCGTCATGGAC
C510V-F	CATC **GTG** CACCACGGCGTCATGGAC
C510K-F	CATC **AAA** CACCACGGCGTCATGGAC
C510-R	CGGTCGCCGGAGCCGAAGTCCTTG
H512I-F	GCCAC **ATC** GGCGTCATGGACATGTT
H512V-F	GCCAC **GTG** GGCGTCATGGACATGTT
H512K-F	GCCAC **AAA** GGCGTCATGGACATGTT
H512-R	AGATGCGGTCGCCGGAGCCGAAGTC