Supplemental information

A single point mutation causes one-way alteration of pheromone receptor function in two *Heliothis* species

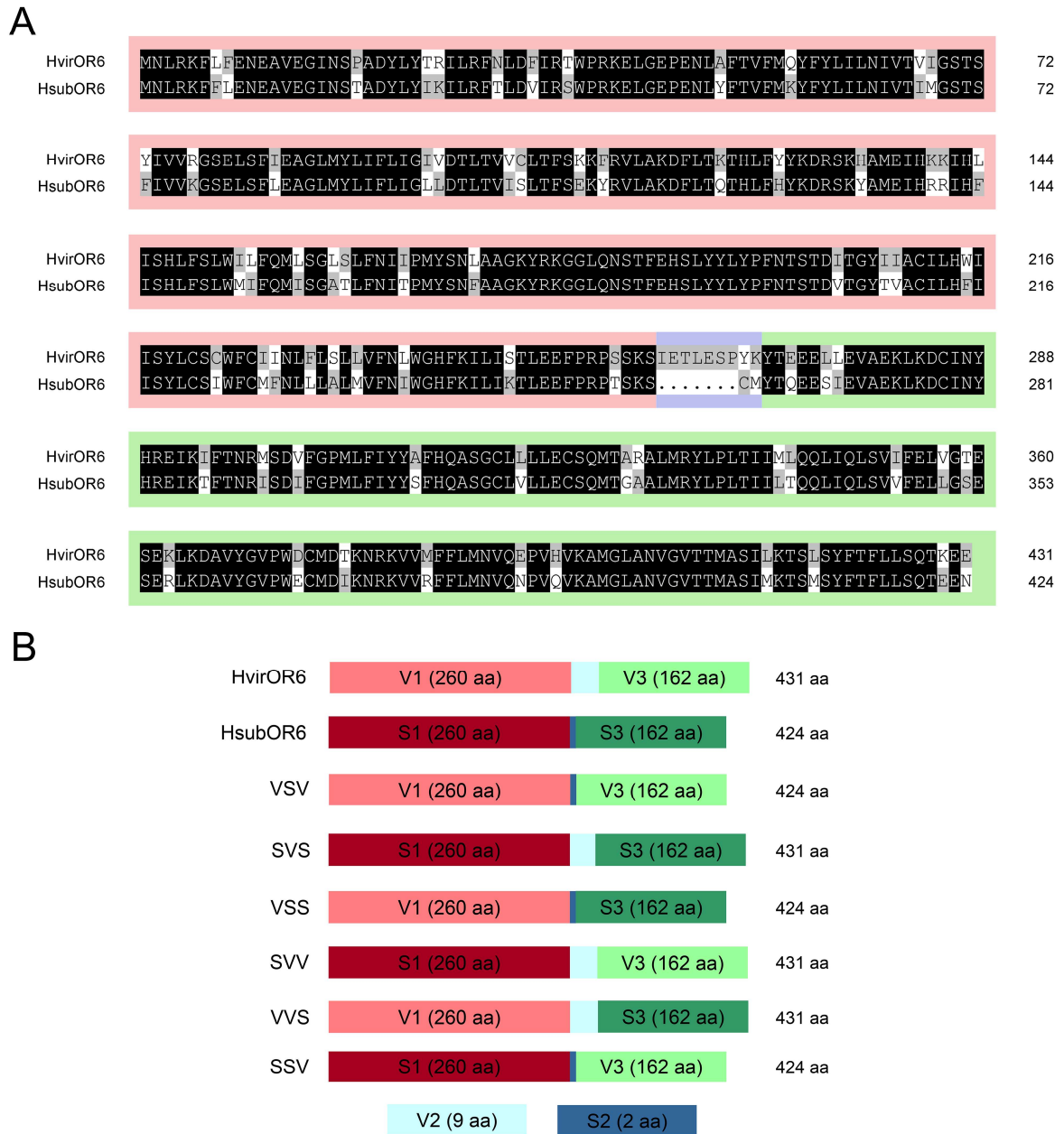Song Cao, Yang Liu, Bing Wang, and Guirong Wang

Supplementary Information

Supplementary Figures

Figure S1. Amino acid sequence alignments of the *H. virescens* and *H. subflexa* PRs. Related to Figure 1.

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HsubOR6    .MNLRKFFLENEAVEGINSTADYLYIKILRFTLDVIRSWERKE......LGEPENLYFTVFMKYFYLILNIVTIMGSTSFIVVKGSELSFLEAGLMYLIFLIGLLDTLTVISLTF    108
HvirOR6    .MNLRKFLFENEAVEGINSPADYLYTRILRFNLDFIRTWERKE......LGEPENLAFTVFMQYFYLILNIVTVIGSTSYIVVRGSELSFIPAGLMYLIFLIGIVDTLTVVCLTF    108
HsubOR11   ......MHLAGNAVTGVTGPMDYNYMKVLRFVLRIISGWEGKA......LGEKTLRIEGMGHAYYNTILSLVYLALGIAYLKTNFHRFDFLELGQLYIVLLMNMLSTSRAFTLCL    103
HvirOR11   ......MHLAGNAVTGITGPMDYKYMKVLRFVLRIISGWEGKA......LGEKTLRIEGMGHAYYNTILSLVYLALGIAYLKKNFHRFDFLELGQLYIVLLMNMLSTSRAFTLCL    103
HsubOR13   ....MKILSDGSDLECVEKVEDIFYINLARKSMWILDSWPKAFN.............ESSTFRYVVLALNVTTLIGGAIYLRNNTGVLSSFELGHTYITVFMNCITCSRGLMILS    98
HvirOR13   ....MKILSDGSDLECVEKVEDIFYINLARKSMWILDSWPKAFN............ASSKYRYFVLALNVATLIGGAIYLRNNTGVLSSFELGHTYITVFMNCITCSRCLMILS    98
HsubOR14   MSGLRDFFFNYEAKECITNPTEYPYMIMSRHLLTVITCWEKKPKKGLNARAKLKARIWVTVQKAFHLNVCFITTLGMAMYIALHKKSMSFEELGHLYISLLMTVVIFTRITTLCL    115
HvirOR14   MTGIRDFFFNYEAKDCVTNPTEYPYMIMSRHLLTVITCWEKPKPKEGLNARAKLRAKIWVIVQKIFHMSLCLLTTLGMAMYIGLHKKSMSFELGHLYISLLMTVVIFSRITTLCL    115
HsubOR15   MTGFRDFIFNYQPKDCITNPVDYPYMIIARYLMTFISMWEKKSVVYHSKRAELRARIWLWVQKFYHLLLCATAFFGGVLYITLHKKSMTFYELGHIYISLLMMACTFSRITTLCF    115
HvirOR15   MTGFRDFVFNYQPKDCITNPVDYPYLIIARYLLTFISMWEKKSVVYHSTRAELKARIWLWVRKFYHLLLCAVAFFGGVLYITLHKKSMTFYELGHIYISLLMMACTFSRITTLCF    115
HsubOR16   .MGLRQFLFEKEAVEGINTASDYLYIKILRFMLVIVNSWERKE......IGEPESPRFSVFAKYFYLFVTVLAAIGSISYVVLHNRELTFLETGHMYIVVLMSLVDASRVATLTM    108
HvirOR16   .MGLRQFLFENEAVEGINTASDYLYIKILRFTLVIVNSWERKE......IGEPESPRFSVFAKYFYLFLTVLAAIGSIAYVAVHNRELTFLETGHMYIVVLMSLVDVSRVATLTM    108


HsubOR6    SEKYRVLAKDELTQTHLFHYKDRSKYAMEIFRRIFFISHLFSLWMIFQMISGATLFNITPMYSNFAACKYRKGG.LQNSTFEHSLYYLYPFNTSTDVTGYTVACILHFIISYLCS    222
HvirOR6    SKKFRVLAKDELTKTHLFYYKDRSKHAMEIFKKIFLISHLFSLWILFQMLSGLSLFNIIPMYSNLAAGKYRKGG.LQNSTFEHSLYYLYPFNTSTDITGYIIACILHWIISYLCS    222
HsubOR11   SQKYREVAKIFIQKVHLFYFKEKSDYAMKIFIIVEKVSFISAVYLSVLLFIAAVMFNLIPMYNNYSAGRYSSFDNLENTTYEQAISCLYPWNFETNFNGYLVATLSGWYGTMLCG    218
HvirOR11   SQKYREVAKIFIQKIHLFYFKEKSDYAMKIFIVHKISFISAVYLSVLLFIAAVMFNLIPMYNNYSAGRYSSFDNLENTTYEQAISCLYPWNFETNFNGYLVATLSGWYGTMLCG    218
HsubOR13   .KDYNHVMSLEVHKIHLFHHRHKSDYAYLTHIFIFIKISHFYTVYLLGLALNGLLLFNLIPFYNCYSRGMFKDVI.PANATYDHAVFYSVPFDYTTKFKGYLAMTSFNCFISYTCT    211
HvirOR13   .KDYNHVMSLFVQKIHLFHHKHKSDYAYLTHIFIFIKISHFYTVYLLGLALNGLFLFNMIPFYNCYSRGMFRDVI.PANATYDHAVFYSVPFDYTTKFKGYLAMTSFNVFISYTCT    211
HsubOR14   HPDYRAVATEELTKIHLFYFKDDSEFSMQTHKQIHTISHLFTLYLTGQMIAGLSLFNLTPMYNNFSAGKYKKGG.LKNSTFEHTLYFAYPFNASSDVWGYIVSNILHWIISYLCS    229
HvirOR14   NPKYRAVSTEELTKIHLFYYKDDSEFSMQIHKQVEKISHLFTLYLTGQMIAGLSLFNLTPMYNNFSAGKYKKGG.LKNSTFEHSLYYSYPFNASSDVGGYIVSNICDWIISYLCS    229
HsubOR15   NDEYRVVAKDEVTKIHLFYKNRSDYSMQTHKKVFMISHVFTLYLSGQMMLGLFLFNVTPMYNNYSACKYKSGG.LKNSTYEHSLYFSWPFNASTDMRGYIISNILHWMLSYTCS    229
HvirOR15   NDEYRVVAKDEVTKIHLFYKNRSDYSMKVEMISHVFTLYLSGQMMLGLFLFNVTPMYNNYSACKYKSGG.LKNSTYEHSLYFSWPFNASTDMRGYIVSNILNWMLSYTCS    229
HsubOR16   STAYRKVASDELTKIHLFYYKDRSKHAMETHCAVEKISHLFTLWLVSQMLSGLSLFNLIPMYSNYAACRYSGEV.LKNSTFEHSLYYPYPFDTSTDISGYSIACAIHWVLSYLCS    222
HvirOR16   STTYREVARDELTKIHLFYYKERSKHAMETHRAVFKISHLFTLWLVSQMLSGLSLFNLIPMYSNYAACRYSGDV.SKNSTFEHSLYYSYPFDTSTDIRGYSIACVIHWVLSYLCS    222


HsubOR6    IWFCMFNLLLALMVFNIWGHFKILIKTLEEFPRPTS...............KSCMYTQEFSIEVAEKLKDCINYFREIKTFTNRISDIFGPMLFIYYSFHQASGCLVLLECSQMT    322
HvirOR6    CWFCIINLFLSLLVFNIWGHFKILISTFEEFPRPSSK........SIETLESPYKYTEEFLLEVAEKFKDCINYFREIKIFTNRMSDVFGPMLFIYYAFHOASGCLLLLECSQMT    329
HsubOR11   SSVSMFDLFLCLMIFNLWGHFKILIHNLEHFPRPASEVVDTEGEERSGRIVGSEMYSQTFBLEQVAVILKDCIQYFMLIFDETNNMSEAFGMALFIYYSFHQITGCLILLECSQMT    333
HvirOR11   SSVSMFDLFLCLMIFNLWGHFKILIHNLEHFPRPASEIVDAEGAERSGRIIGSEMYSQAELEQVAVILREGIQYFMLIFDETNNMSDAFGMALFIYYSFHQITGCLILLECSQMT    333
HsubOR13   SYFCVVDLTISLVIFHLWGHFMRLLTYHLANFKKPASVLESNDN....KDEIKDHSYTDEFLKEVFSKLREYIQHFNLILEFSSEMSNAFGPALLAYMVFHQVSGCIILLECSQLD    322
HvirOR13   SYFCVVDLTISLVIFHLWGHFMRLLTYHLANFKKPASVLESNDNN....KDEIKDHSYTEEELKEVFSKLREYTQHFNLILEFSSEMSNAFGPALLAYMVFHQVSGCIILLECSQLD    323
HsubOR14   TWFCTLDLFLSIMVFHVWGHFKILLHEINLFPRPSNRTTFR..LDSSNITLTSEMYSSRLGQVSERLKKCIEYFRKIVSFTDKMSKVFGPMLFVYYGFHQTSGCLLLLECSQMT    342
HvirOR14   TWFCTLDLFLSIMVFHVWGHFKILLHDIDHFPRPANLTTFK..LDNSNITLTSEKFSSIELGQVSEKLKKCIEYFRKIVSFTDEMSEVFGPMLFVYYGFHQTSGCLLLLECSQMT    342
HsubOR15   SWFCVIDFFLSLMVFHVWGHFKILLHDIDHFPRPLNKISAV..IEDSSITITNEMYSKNEMEQVFDTLNKCIDYFREIVSFTDKMSEVFGPMLFAYYGFHQASGCLLLLECSQMT    342
HvirOR15   SWFCVIDFFLSLMVFHVWGHFKILLHDIDHFPRPLNKINSV..IEDS.ITITNEMYSKTELDQVFDRLGKCIDYFREILSFTDKMSEVFGPMLFAYYGFHQASGCLLLLECSQMT    341
HsubOR16   TWFCMFDLFLSLMVFHLWGHFKILISTLNDFPRPSSK...........VEGAKYSDEELVDVAARLKDCIVYFREIILFTDRMSNVFGPMLFVYYSFHQASGCLLLLECSQMT    324
HvirOR16   TWFCMFDLFLSLMVFHLWGHFKILINTLNDFPRPSSK...........VEGAQFSDEELVDVAARLKDCIVYFREITLFTDRMSNVFGPMLFVYYSFHQASGCLLLLECSQMT    324


HsubOR6    GAALMRYLPLTIILTQQLIQLSVVFELIGSESERLKDAVYGVPWECMDIKNRKVVRFFLMNVQNPVQVKAMGLANVGVTTMASIMKTSMSYFTFLLSQTEEN.    424
HvirOR6    ARALMRYLPLTIIMLQQLIQLSVIFELVGTESEKLKDAVYGVPWDCMDTKNRKVVMFFLMNVQEPVHVKAMGLANVGVTTMASILKTSLSYFTFLLSQTKEE.    431
HsubOR11   AAALTRYLPLTIIMFGELVLLSIIFETIGTMSEKLKDAVYKVPWEYMDTKNRRTVLIFLIKVQEPIHVKAGGLVDVGVTTMASILKTSFSYBAFLRTF.....    431
HvirOR11   AAALTRYLPLTIIMFGELVLLSIIFETIGTMSEKLKDAVYKVPWEYMTKNRRTLIFLIKVQEPIHVKAGGLVDVGVTTMASILKTSFSYFAFLRTF.....    431
HsubOR13   MKTLVRYGPLTIVIFQQLIQISVIFELLGSSNDKLIDGVYLVPWEYMDTSNRKLVFSMLRQSHRSINLKMMSMLTVGVQTMTAILKTSFSYFVMLKTVAEEEQ    425
HvirOR13   TKTLVRYGPLTIVIFQQLIQISVIFELLGSSNDKLIDGVYLVPWEYMDTKNRKLVFTMLRQSHRSINLTMMSMVTVGVQTMTAILKTSFSYFVMLKTVAEEE.    425
HsubOR14   VAALVRYLPLTIILFQQLIQLSIIFELVGSVSDKLKDAVYGLPWEAMDTKNRKTVAVFLLNVQKPVHVKALGLAEVGVATMAAILKTSMSYFAFLRSK.....    440
HvirOR14   VAALVRYLPLTIMLFQQLIQLSIIFELVGSVSDKLKDAVYSLPWEAMDIKNKKTVAIFLMNVQEPVHVKALGLAEVGVTSMTAILKTSMSYFTFLRSM.....    440
HsubOR15   VAALVRYLPLTIILFQQLIQMSIIFELVGSAVDKLKDAVYGLPWEAMDTKNRKTVAFFLMNVQEPVHVKALGLAEVGVTSMTAILKTSMSYFAFLRSM.....    440
HvirOR15   VAALVRYLPLTIILFQQLIQMSIIFELVGSVTDKPRDAVYGLPWEAMDTKNRKTVAFFLMNVQEPVHVKALGLAEVGVTSMTAILKTSMPYFAFLRSM.....    439
HsubOR16   AQALMRYVPLTIILTQQLIQLSVIFELVGSESDKLHAVYGLPWECLDVKNRRVVVIFLANTQEPVHVKAMGVANVGVTSMAAILKTSMSYFTFLRSM.....    422
HvirOR16   AQALMRYVPLTIILTQQLIQLSVIFELVGSESDKLKHAVYGLPWECMDVKNRRVVLIFLANTQEPVHVKAMGVANVGVTSMAAILKTSMSYFTFLRSM.....    422
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Figure S2. Sequence alignment of HvirOR6 and HsubOR6 (A) and sequence structure sketch of the six chimeric genes (B). Fragment 1 was marked in red box. Fragment 2 was marked in purple box. Fragment 3 was marked in green box. Related to Figure 4 and Figure 5.

A

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HvirOR6   MNLRKFLFENEAVEGINSPADYLYTRILRFNLDFIRTWPRKELGEPENLAFTVFMQYFYLILNIVTVIGSTS   72
HsubOR6   MNLRKFLFENEAVEGINSTADYLYIKILRFTLDVIRSWPRKELGEPENLYFTVFMKYFYLILNIVTIMGSTS   72

HvirOR6   YIVVRGSELSFIEAGLMYLIFLIGIVDTLTVVCLTFSKKFRVLAKDFLTKTHLFYYKDRSKLAMEIIHKKIHL   144
HsubOR6   FIVVKGSELSFLEAGLMYLIFLIGLLDTLTVISLTFSEKYRVLAKDFLTQTHLFHYKDRSKYAMEIIHRRIHF   144

HvirOR6   ISHLFSLWILFQMLSGLSLFNIIPMYSNLAAGKYRKGGLQNSTFEHSLYYLYPFNTSTDITGYIIACILHWI   216
HsubOR6   ISHLFSLWMIFQMISGATLFNITPMYSNFAAGKYRKGGLQNSTFEHSLYYLYPFNTSTDVTGYTVACILHFI   216

HvirOR6   ISYLCSCWFCIINLFLSLLVFNLWGHFKILISTLEEFPRPSSKSIETLESPYKYTEEELLEVAEKLKDCINY   288
HsubOR6   ISYLCSIWFCMFNLLLALMVFNIWGHFKILIKTLEEFPRPTSKS.......CMYTQEESIEVAEKLKDCINY   281

HvirOR6   HREIKLFTNRMSDVFGPMLFIYYAFHQASGCLLLLECSQMTARALMRYLPLTIIMLQQLIQLSVIFELVGTE   360
HsubOR6   HREIKTFTNRISDIFGPMLFIYYSFHQASGCLVLLECSQMTGAALMRYLPLTIILTQQLIQLSVVFELLGSE   353

HvirOR6   SEKLKDAVYGVPWDCMDTKNRKVVMFFLMNVQEPVHVKAMGLANVGVTTMASILKTSLSYFTFLLSQTKEE   431
HsubOR6   SERLKDAVYGVPWECMDIKNRKVVRFFLMNVQNPVQVKAMGLANVGVTTMASIMKTSMSYFTFLLSQTEEN   424
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B

HvirOR6	V1 (260 aa)	V3 (162 aa)	431 aa
HsubOR6	S1 (260 aa)	S3 (162 aa)	424 aa
VSV	V1 (260 aa)	V3 (162 aa)	424 aa
SVS	S1 (260 aa)	S3 (162 aa)	431 aa
VSS	V1 (260 aa)	S3 (162 aa)	424 aa
SVV	S1 (260 aa)	V3 (162 aa)	431 aa
VVS	V1 (260 aa)	S3 (162 aa)	431 aa
SSV	S1 (260 aa)	V3 (162 aa)	424 aa

V2 (9 aa)   S2 (2 aa)

Supplementary Table

Table S1. Primers used in this study. The candidate mutated sites are marked in red. Related to STAR Methods.

Primer	Sequence (5' to 3')
Primers for gene clone	
HsubOrco F	caccATGATGACCAAAGTGAAGGCCCAG
HsubOrco R	TTACTTGAGTTGTACCAACACCATG
HsubOR6 F	caccATGAACTTACGAAAATTTTTTTTGG
HsubOR6 R	TCAGTTTTCCTCTGTCTGCGAGAG
HsubOR11 F	caccATGCACCTTGCAGGCAATGCTGTG
HsubOR11 R	CTAAAACGTGCGTAGAAAAGCGAAG
HsubOR13 F	caccATGAAAATTCTTTCGGACGGTTCTG
HsubOR13 R	TCACTGTTCTTCTTCTGCAACTGT
HsubOR14 F	caccATGTCAGGTTTACGTGACTTTTTC
HsubOR14 R	TCACTTACTGCGTAGAAAGGCGA
HsubOR15 F	caccATGACTGGTTTTCGTGATTTCATC
HsubOR15 R	TCACATGCTGCGTAGAAAAGCGAAG
HsubOR16 F	caccATGGGTCTCCGTCAATTTCTTTTCG
HsubOR16 R	CTACATACTCCTTAGAAACGTGAAG
Primers for chimeric genes construction	
HsubOR6 F	caccATGAACTTACGAAAATTTTTTTTGG
HsubOR6 R	TCAGTTTTCCTCTGTCTGCGAGAG
HvirOR6 F	caccATGAACTTACGAAAATTCTTATTCG
HvirOR6 R	TTATTCTTCCTTTGTCTGCGAAAG
VSV F	CAGTTCGAAATCTTGTATGTATACTGAAGAGGAGTTGC
VSV R	CCTCTTCAGTATACATACAAGATTTCGAACTGGGTCTC
SVS F	ATTGAGACACTAGAAAGTCCTTATAAATATACTCAAGAAGAGTCCATCG
SVS R	TTTATAAGGACTTTCTAGTGTCTCAATAGATTTCGAAGTAGGTCTTGG
SVV F	TCCGAGACCCAGTTCGAAATCTATTGAGACACTAGAAAGTCCTT
SVV R	AAGGACTTTCTAGTGTCTCAATAGATTTCGAACTGGGTCTCGGA
VSS F	TCCAAGACCTACTTCGAAATCTTGTATGTATACTCAAGAAGAGT
VSS R	ACTCTTCTTGAGTATACATACAAGATTTCGAAGTAGGTCTTGGA
VVS F	CACTAGAAAGTCCTTATAAATATACTCAAGAAGAGTCCATC
VVS R	GATGGACTCTTCTTGAGTATATTTATAAGGACTTTCTAGTG
SSV F	CCTACTTCGAAATCTTGTATGTATACTGAAGAGGAGTTGCT
SSV R	AGCAACTCCTCTTCAGTATACATACAAGATTTCGAAGTAGG
Primers for preparation of mutants (HvirOR6 to HsubOR6)	
VM1 F 153(I-M), 154(L-I)	CTCTCTATGGATGATATTTCAAATG
VM1 R	AACAGATGCGATATCAAGTGGATTT
VM2 F 161(L-A),162(S-T)	GTTATCAGGTGCCACTCTATTCAACAT

VM2 R		ATTTGAAATAGTATCCATAGAGAGAACAG
VM3 F	158(L-I),167(I-T)	CTATTCAACATA**ACA**CCTATGTATAG
VM3 R		ACTGAGACCTGA**TAT**CATTTGAAATAG
VM4 F	302(V-I)	GAATGTCAGAC**ATA**TTTGGGCCGATG
VM4 R		TATTTGTGAAAATTTTTATTTCTCTGTGG
VM5 F	312(A-S)	CAAGTGGCTGTCTCTTACTTCTCGAATG
VM5 R		CTTGGTGAAA**CGA**GTAATATATGAAC
VM6 F	321(L-V)	CAAGTGGCTGTCTC**GTA**CTTCTCGAATG
VM6 R		CTTGGTGAAACGCGTAATATATGAAC
VM7 F	353(I-V)	ATTCAGTTGTCAGTT**GTT**TTTGAATTGG
VM7 R		CAATTGCTGTAACATTATTATTGTTAG
VM8 F	344(L-T)	ATTCAGTTGTCAGTTATTTTTGAATTGG
VM8 R		CAATTGCTG**TGT**CATTATTATTGTTAG
VM9 F	343(M-L)	ATTCAGTTGTCAGTTATTTTTGAATTGG
VM9 R		CAATTGCTGTAA**CAA**TATTATTGTTAG
VM10 F	357(V-L)	GTTATTTTTGAATTG**CTT**GGGACTGAG
VM10 R		TGACAACTGAATCAATTGCTGTAAC
VM11 F	330(A-G),331(R-A)	GCAGATGACG**GGTGCA**GCTCTAATGC
VM11 R		GAACATTCGAGAAGTAAGAGACAGCC
VM12 F	173(L-F)	GTATAGTAAC**TTT**GCCGCAGGAA
VM12 R		ATAGGTATTATGTTGAATAGACTGAG
VM13 F	50(A-Y)	AGAGAATTTG**TAC**TTCACAGTTT
VM13 R		GGCTCGCCTAACTCCTTCCGAGG
VM14 F	204(I-V),209(I-V)	TATATC**GTG**GCTTGTATTTTACATT
VM14 R		CCCAGT**AAC**ATCAGTAGAAGTATTA
VM15 F	56(Q-K)	CAGTTTTTATG**AAA**TACTTTTACTTG
VM15 R		TGAAAGCCAAATTCTCTGGCTC
VM16 F	67(V-I),73(Y-F)	CAACCTCG**TTC**ATCGTGGTTCGTGG
VM16 R		AGCCTAT**AAT**GGTAACTATGTTCAAG
VM17 F	77(R-K),84(I-L)	TATCTTTC**TTA**GAAGCTGGGCTTATG
VM17 R		ATTCTGATCC**CTT**AACCACGATGTAC
VM18 F	97(I-L),98(V-L)	TTAATTGGT**CTTCTC**GATACGCTAAC
VM18 R		AAATATCAAATACATAAGCCCAGCTTC
VM19 F	104(V-I),105(C-S)	CTAACAGTG**ATATC**TTTGACATTCTC
VM19 R		CGTATCAACAATACCAATTAAAAATATC
VM20 F	215(W-F)	TTATGTTCATGTTGGTTCTGTATTATC
VM20 R		GTATGATATGAT**GAA**ATGTAAAATAC
VM21 F	223(C-I)	TTATGTTCA**ATT**TGGTTCTGTATTATC
VM21 R		GTATGATATGATCCAATGTAAAATAC
VM22 F	231(F-L)	TCAATCTC**TTG**TTATCCCTACTGGTT
VM22 R		TAATACAGAACCAACATGAACATAAG

VM23 F	233(S-A)	TCAATCTCTTCTTAGCTCTACTGGTT
VM23 R		TAATACAGAACCAACATGAACATAAG
VM24 F	227(I-M),228(I-F),235(L-M)	TTATCCCTAATGGTTTTCAATCTTTGG
VM24 R		GAAGAGATTGAACATACAGAACCAAC
VM25 F	414(L-M),418(L-M)	TCGATTATGAAAACATCGATGTCGTAT
VM25 R		AGCCATAGTAGTAACTCCAACGTTTGC
VM26 F	144(L-F)	AAAATCCACTTCATATCGCATCTG
VM26 R		TTTGTGGATCTCCATAGCATGC
VM27 F	208(I-T)	TGGGTATACAATTGCTTGTA
VM27 R		GTAATATCAGTAGAAGTATTAAAAGG
VM28 F	299(M-I)	GCCGATGTTGTTCATATATTACG
VM28 R		CCAAACACGTCTGATATTCTATTTG
VM29 F	68(I-M)	ACATAGTTACCGTTATGGGCTCAAC
VM29 R		ATCCAATGTAAAATACAAGCAATG
VM30 F	239(L-I)	CCTACTGGTTTTCAATATTTGG
VM30 R		TCATCAGGAAGAACATCACCAC
VM31 F	429(K-E),431(E-N)	AGAGGAGTTGCTTGAGGTTG
VM31 R		TTAGTTTTCCTCTGTCTGCG
Primers for preparation of mutants (HsubOR6 to HvirOR6)		
SM321 F	321(V-L)	GGCTGTCTCTTACTTCTCGAATG
SM321 R		ACTTGCTTGGTGAAACGAG