

Title:

Differential expression pattern of *Vago* in bumblebee (*Bombus terrestris*), induced by virulent and avirulent virus infections

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Supplementary data

Figure S1: The eight conserved cysteine residues in VWC domain of *BtVago* compared with *CqVago*

Culex	MKTFGVIIILLVCCAIGGS-----LAAEAVLQNAEHPDYPGKCYDEGTQTVVAPLESA
Bombus	MKLVFVLLLVAAIVFAAEKKEEERPKTFRRLIPADVLRDFPGMCFASRCATIEPTKSW
	** . *::*... .. : . :: *:* * : . : : * :*
Culex	KLPKSC TKVFCSTN-----LSLTYTT CGSVLVNDPHCEKIE-QDLTKDFPECHKYKCE
Bombus	DLTPFCGRSTCV PADDNSGRLFELVEDCGPLPKANPKCKLSDKTNKTATFPDCCPIFECE
	. * * : * * ** : : *:* : : * **:* : : **
Culex	LEGVVTYH-----
Bombus	EGAKLEYPEIPTLPPPTTEIVETEKTPEATPAKA
	. : *

Figure S2: Predicated signal peptide cleavage site of *BtVago*

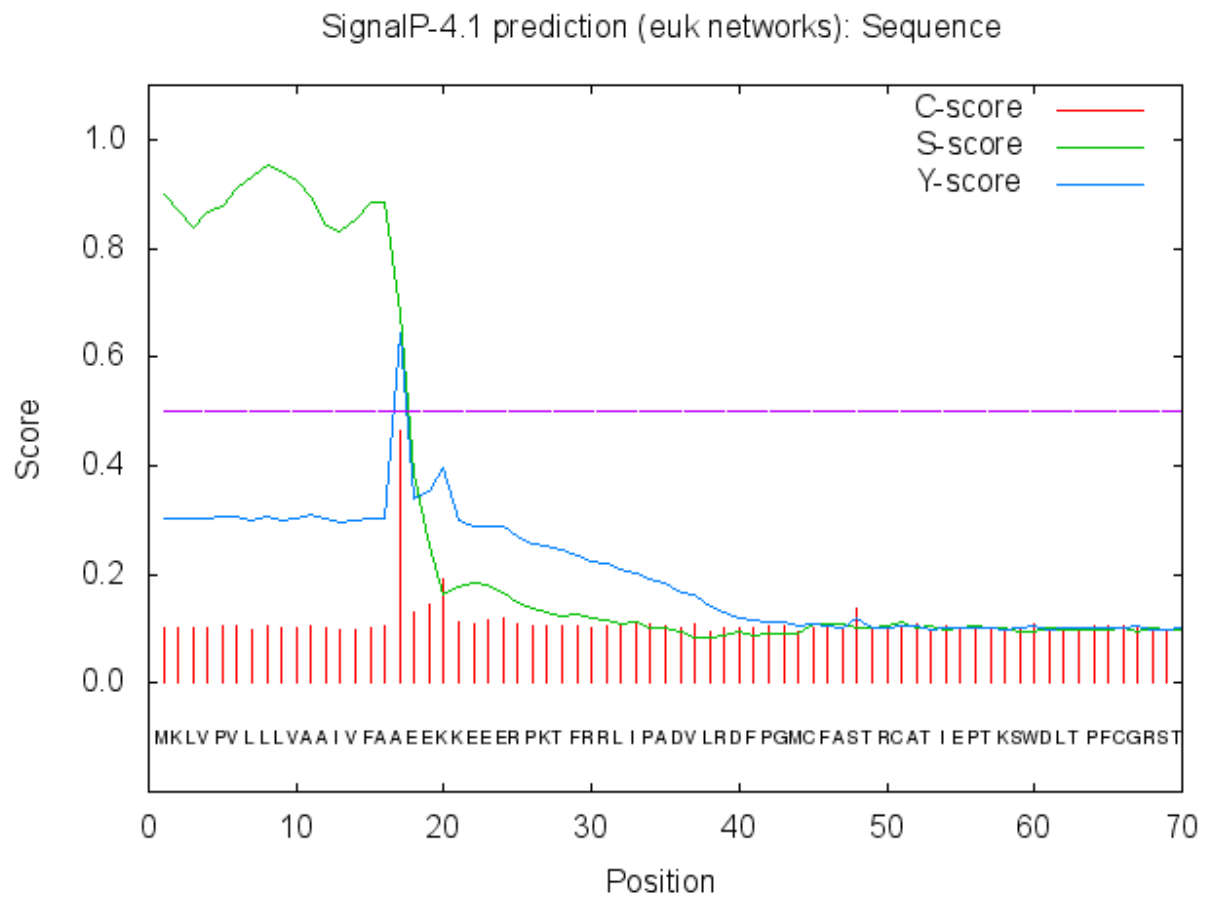


Figure S3: The expression of *BtDicer-2*, *BtVago* and *BtHop* upon the silencing of *BtDicer-2* (A) or *BtVago* (B)

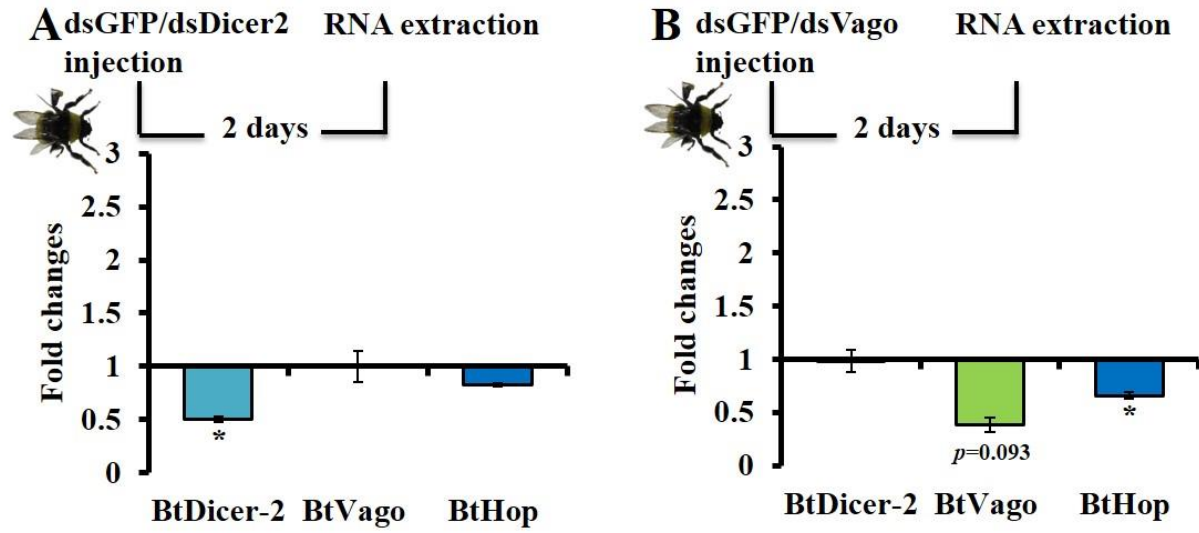


Figure S4: Silencing of *BtDicer-2* (A), *BtVago* (B), and *BtHop* (C) through injections of dsRNA

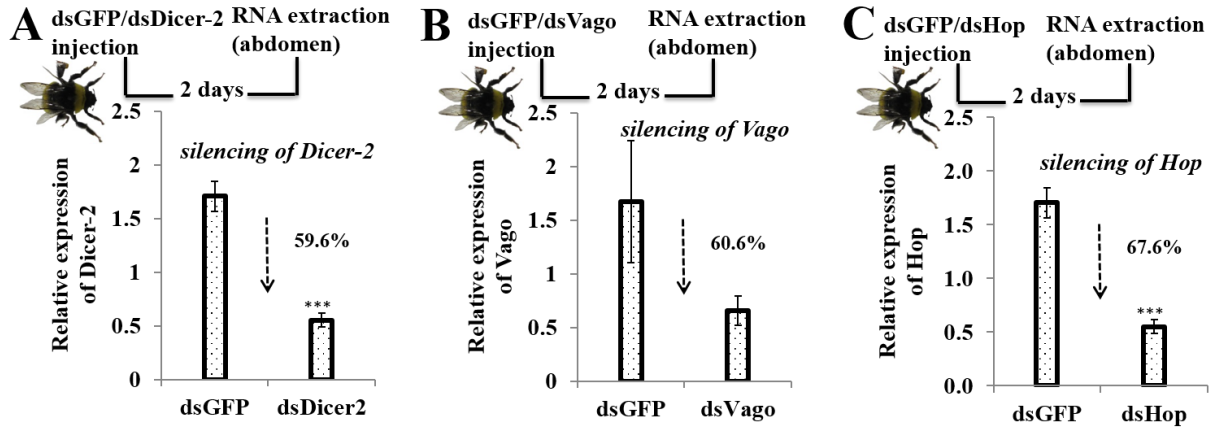


Table S1: Predicted NF- κ B binding site in *BtVago* promoter region

Factor name	Start position	End position	Dissimilarity	String	RE equally	RE query
RelA [T00595] GATA-1	438	448	12.115729	TCTAACGAAGC	0.02146	0.01273
[T00305] GATA-1	735	742	4.138263	CACGATAA	0.54932	0.69044
[T00305] GATA-1	925	932	1.520654	TTATCATT	0.30518	0.65047
[T00305] GATA-1	1609	1616	4.052395	TCGGATAA	0.54932	0.69044
[T00305] GATA-1	1896	1903	1.682543	TTATCACA	0.30518	0.65047
[T00267] GATA-1	735	743	8.557309	CACGATAAT	0.06866	0.14082
[T00267] GATA-1	924	932	4.979757	TTTATCATT	0.18311	0.36798
[T00267] GATA-1	1609	1617	3.166737	TCGGATAAG	0.11444	0.16757
[T00267] GATA-1	1895	1903	3.40581	CTTATCACA	0.10681	0.17821

Table S2: Primers used in this study

Name	Primer sequence	Amplicon length	Purpose
qPCRVagoF2	TGTTACCCTTCAACGCAATTC	194	QPCR for Vago
qPCRVagoR2	ACAGATTCCGAAACGCTGAT		
Vago-T7dsF2	TAATACGACTCACTATAGGGAGACCTAGTCCCGGAAGTC GAGA	418	dsRNA synthesis for Vago
Vago-T7dsR2	TAATACGACTCACTATAGGGAGAGTACGTACGAATTACA AGATCAACT		
qPCRHopF3	TGGCACAATGTGTCTCATCTT	186	QPCR for Hop
qPCRHopR3	GAGGTACACAACGAGGTCCAG		
Ds2ndHOP-F4	TAATACGACTCACTATAGGGAGATGTCCTTTGTTTCTGCT CTGGA	374	dsRNA synthesis for Hop
Ds2ndHOP-R4	TAATACGACTCACTATAGGGAGATGACTGTCCTTCAGAAT CTTGGA		
q-Ri-dicer2-F2	TGGTCAAACATCAAGAACAACCA	166	QPCR for Dicer-2
q-Ri-dicer2-R2	GATCGGGGCCATACGAACAT		
C-dsDicer2-2 nd -F4	TAATACGACTCACTATAGGGAGAGCGAAGGTGTCACCAA ATGT	437	dsRNA synthesis for Dicer-2
C-dsDicer2-2 nd - R4	TAATACGACTCACTATAGGGAGAGGGTGTGTAAGGCCT GCAA		

S1: Conserved proteins sequences used to construct phylogenetic tree of Vago

>Bombus_terrestris

MKLVVLLLLVAAIVFAAEKKEEERPKTFRRLLIPADVLRDFPGMCFASTRCATIEPTKSWDLTPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTATFPDCCPIFECEEGAKLEYPEIPTLPPPEIVETEKTEATPA

>PREDICTED:_uncharacterized_protein_LOC411622_Apis_mellifera.

MKFAPILLVIAIVFAAEE-KEEERPKTFRRLLIPADVLRDFPGMCFASTRCATIEPTKSWELTPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTAAPNCCPIFECEEGAKLEYPEIPTLPPPEIIEETKTSEEVPT

>PREDICTED:_uncharacterized_protein_LOC105185794_Harpegnathos_saltator.

MEHAFVLLFTTIVFIAIADEKEEERPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKTWELSPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFDCPIFECEEGAKLEYPEIPTLPPPEIEASEITAAPKV-

>PREDICTED:_uncharacterized_protein_LOC100878806_Megachile_rotundata.

MKLATILLVVAVVFAAEE-KEEERPKTFRRLLIPADVLRDFPGRCFASTKCATIEPTKSWDLTPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSEKTNKSAPFDCCPIFECEEGAKLEYPEIPTLPPPEIVETEKTEAAPA

>PREDICTED:_uncharacterized_protein_LOC105623255_Atta_cephalotes.

MKLVFALLCVAVAFVAAEDAQVQERPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKSWELSPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFPECCPIFECEEGAKLEYPEIPTLPPPSDEDAAKAQPETPKP

>PREDICTED:_uncharacterized_protein_LOC105282831_isoform_X2_Cerapachys_biroi.

MAFLPHPCASSV-----KIERNFR-----ENFPGMCFASTKCATIEPTKSWQLAPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFDCPIFECEEGAKLEYPEIPTLPTPTED-AAKGEPAAAAP

>PREDICTED:_uncharacterized_protein_LOC105148313_Acromyrmex_echinator.

MKLVFALLCVAAVFVATQDAQVQERPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKSWELAPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFPECCPIFECEEGAKLEYPEIPTLPPPSD-AAAAQPEAPKP

>PREDICTED:_uncharacterized_protein_LOC105455873_Wasmannia_auropunctata.

MKTVFALLCVAAVFVATQGAQEQRPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKSWELAPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTSSFDCCPIFECEEGAKLEYPEIPTLPPPSGEDAAAAQPQAPKP

>PREDICTED:_uncharacterized_protein_LOC105258902_Camponotus_floridanus.

MKLIFILLCAAVAFVAAEGTTEQRPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKTWELSPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSEKTNKTASFDCPIFECEEGVKEYPEIPTLPPPEAEAAANVPPEATPK

>PREDICTED:_uncharacterized_protein_LOC105561162_Vollenhovia_emoryi.

MKTIFVLLCVAVAFVAAQDVQEQRPKTFRRLLIPADVLRDFPGMCFASTKCATIEPTKSWELAPFCGRSTCVADDNSGR
LFELVEDCGPLPKANSKCKLSDKTNKTASFPDCCPIFECEGDGAKLEYDPIPTLPPPSDAI-AKAQPETPKP

>PREDICTED: *_uncharacterized_protein_LOC103577816_Microplitis_demolitor*.

NKYFAIVLLVAAVVVAEEEE----ERP KTFRRLLIPADVLRDFPGMCFASTKCATVEPTKSWELSPFCGRSTCVSDDDSGR
LFELVEDCGPLPKANPKCKLSEKTNKTASFPDCCPIFECEEGAKLEYPEIPTVPPPPS--EAEAKPEVAKV

>PREDICTED: *_uncharacterized_protein_LOC105197444_Solenopsis_invicta*.

MKI-VFALCVAVAFVAANA-EEQERPKTFRRLLIPADVLRDFPGMCFASTKCATIEPLKSWELSPFCGRSTCVLADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFPDCCPLFECEEGAKLEYPEIPTLPPPSGVDATAKAQPEAPQS

>PREDICTED: *_uncharacterized_protein_LOC100113619_Nasonia_vitripennis*.

FSLALVAIVAVVAVHAAEEKKDEDRPKTYRRLIPADVLRDFPGMCFASTKCATIEPKQSWDLAPFCGRSTCVADDNSGR
LFELVEDCGPLPKANPKCKLSEKTNKTAPFPDCCPVFECEGDGAKLEYPEIPTLPPPTAEEIAKAAAAGKPA

>PREDICTED: *_LOW_QUALITY_PROTEIN:_uncharacterized_protein_LOC105428984_partial_Pogonomyrmex_barbat
us*.

WKLTYLFTISEVLFDSFLSGFYPNKRKCHLVLFHYIVDFPGTFASTKCATIEPGKSWELSPFCGRSTCVSADDNSGR
LFELVEDCGPLPKANPKCKLSDKTNKTASFPECCPIFECEGDGATLEYDPIPTLPPPTED-AAKAQPEAPKP

>PREDICTED: *_uncharacterized_protein_LOC105268204_Fopius_arisanus*.

MKYVVAILFIAALVAED--KDEERPKTYRRLIPADILRDFPGMCFASTKCATVEPGKTWDLSPFCGRSTCVPAEDNSGR
LFELVEDCGPLPKENPKCKLSEKTKTSPFPDCCPIFECEEGAKLEYPEIPTIPPELVEPTETTTAKA--

>conserved_hypothetical_protein_Pediculus_humanus_corporis.

MKFLIVFLIGFGAFCFGQETETTERPKVFRRLIPADVLRDFPGMCFASTKCTTVEPGKSWDLTPFCGRSTCVVAEEQAGR
LLELVEDCGPLPKANPKCKLSEKTNKTAPFPDCCPIFECEKGVKLEYPELPTVPPPTDKKKA-----

>CG31997_isoform_A_Drosophila_melanogaster.

MSFHTLILTAFTVSLCAEQKKS DAGERIFKRLIPADVLRDFPGMCFASTRCATVEPGKSWDLTPFCGRSTCVQNEENDAK
LFELVEDCGPLPLANDKCKLSEKTNKTASFPYCCPIFTCDPGVKLEYPEIGKDNDKKNSE-----

>PREDICTED: *_uncharacterized_protein_LOC105220319_Bactrocera_cucurbitae*.

MKAFLVVACALFVCVAAQDDTSQTNSKIYKRLIPADVLRDFPGMCFASTRCATVEPTKTWELTPFCGRSTCVQNDENPAK
LLELVEDCGPLPLANEKCKLSEKTNKTAPFPYCCPIFTCEPGVKLEYPEIPKEEAKKE-----

>uncharacterized_protein_LOC100167365_precursor_Acyrtosiphon_pisum.

MDYRAVVLGVAIVLSADEKTPAPEARIYRRLIPADVLRDFPGLCFASTKCATVEPGHTWELSPFCGRSTCVQGE-GTDR
LLELVEDCGPYPKSNPKCKLSEKTNKTASFPPECCPVFDCEPGVKLEYPEVTVVENPESSDDSTTTTSTEKP

>PREDICTED: _uncharacterized_protein_LOC655864_Tribolium_castaneum.

MKV-FLVLALCLAVAAAEDKKEEERPKTFKRLIPADVLRDFPGMCFASRCATIEPGKTWELHPFCGRSTCVVSEDKPPR
LLELVEDCGPLPLANPKCKLEEKTNKTAAPDCCPVFKCEEGAKLEYPEIPTVAPVPE--ASSTTPKA---

>hypothetical_protein_KGM_07285_Danaus_plexippus.

MKY-LIVLAIIFSYAADD-KDDERPKTFRRLLIPADVLRDFPGLCFASRCATVEPGNTWDLAPFCGKSTCVVSEDTPPR
LLELVEDCGPLPIANPKCKLTDKTNKTAPFPDCCPIFTCEDGVKLEYPELPTPPEEEKKEKS-----

>PREDICTED: _uncharacterized_protein_LOC105665112_Ceratitis_capitata.

MKASLVIFVALFLYGAAQDAKNSELKIYKRLIPADVLRDFPGMCFASRCATVEPTKTWELTPFCGRSTCVQNEEDNPSK
LLELVEDCGPLPLANEKCKLTGKTNKTAPFPYCCPIFICEPGIKLEYPEVPREELNKE-----

>unknown_Manduca sexta.

MKYLVVVAVFALAFAAEEKE---DIPKTYKRLIPADVLRDFPGTCFASRCATVEPGNTWELSPFCGRSTCVLSEDHPPR
LLELVEDCGPLPLANPKCKLTDKTNKTAPFGCCPIFTCEEGAKLEYPELPTPPPEGE--KAEEKPKKA--

>similar_to_CG31997_Papilio_xuthus.

MKYLFLAVFALAFAAEEE-KDGERPKTFRRLLIPADVLRDFPGLCFASRCATVEPGNSWDLAPFCGRSMCVVSEDTPPR
LLELVEDCGPLPLANPKCKLTDKTNKTAPFGCCPIFTCEDGVKLEYPELPTAAPEEEK-KAEEKPKA---

>conserved_hypothetical_protein_Culex_quinquefasciatus.

GVFVVAALCVANVFAADEPTKDEGVKIYKRLIPADVLRDFPGMCFASRCATIEPGKSWDLAPFCGRSTCVVSESNAQ
LLELVEDCGPLPLANDKCKLTDKTNKTAPFPYCCPKFTCEPGVKLEYPEIKPSDASEEK-----

>AAEL000445-PA_Aedes_aegypti.

GVLIAATLCVANVFAADEPTDEDTVKIYKRLIPADVLRDFPGMCFASRCATIEPGKSWDLAPFCGRSTCVVSETNPSQ
LLELVEDCGPLPLANDKCKLTDKTNKTAPFPYCCPKFTCEPGVKLEYPDVKGPEAAPEDD--KKN-----

>PREDICTED: _uncharacterized_protein_LOC105381502_Plutella_xylostella.

MKY-LVAFVAVFALAFAAEEKAEERPKTFRRLLIPADVLRDFPGMCFASRCATVEPGNAWDLSPFCGRSTCVVSEDEPPR
LLELVEDCGPLPLANDKCKLTDKTNKTAPFGCCPVFTCTDGAKLEYPELPTPPPEGE----EKKPEEEKP

>PREDICTED: _uncharacterized_protein_LOC101888253_Musca_domestica.

MKFFAVLLVACVYGAATDKTKEEAPKIYKRLIPADVLRDFPGMCFASRCATVEVGKSWELTPFCGRSTCVQNEEDPSK
LLELVEDCGPLPLANDKCKLTEKTNKTAAPFYCCPVFTCEPGVKLEYPEAVKETKKE-----

>AGAP002085-PA_Anopheles_gambiae_str._PEST.

MRSFTVVAVLALYVQAADEATKDAAPKTYKRLIPADVLRDFPGMCFASRCATFEPGQYWDLTPFCGRSTCVLSDDAQPR

LLELVEDCGPLPLANDKCKLTEKTNKTAPFPACPTFTCEPGAKLEYPEIKTAPESTSEQN-----

>hypothetical_protein_YQE_10125_partial_Dendroctonus_ponderosae.

-----MCFASTKCATVEPGKTWELYPFCGRSTCVVSEDQPPR

LLELVEDCGPLPLANEKCKLEEKTNKTAPFPACPEFKCEAGAKLEYPEIPTVAPVPEDASTTTAKSA---

>PREDICTED:_uncharacterized_protein_LOC101741978_Bombyx_mori.

MKYLIVLAVFALAAFAEEKE---ERPKTFRRILPADVLRDFPGMCFASTRCITVEPGNTWELSPFCGRSTCVVSEDQPPR

LLELVEDCGPLPLTNPCKQLTEKTNKTAPFPGCCPIFTCEEGAKLEYPELTPPPEDK--KAEEKPKA---

>unknown_Culicoides_sp._LJH-2002.

MRPFVFFVIVSLSFASET-KNDDGIKVYKRLIPADVLRDFPGVCFASTKCAMFEPGKQWDLKPFGRSTCVTPEDGSSR

LLELVEDCGPLPIANDKCKLTEKTNKTADFPYCCPQFACQDGAKLEYPEVKTSSA-----

>hypothetical_protein_DAPPUDRAFT_214640_Daphnia_pulex.

MKFILCFSLFVLVASVE-----ITPKLTREIKADVLRDFPGVCYASTQCRTFKENEEWDLKPFCKGKSIKGA--DGI

LKERVSDCGPPAKANPECKV--NANATLPYPNCCPVYDCAPGVQLEFPDIPVA-----

>single_VWC_domain_protein_1_Litopenaeus_vannamei.

MKF-LLIACLGLVFAQQGPADLQGGPFVDRDLKADVLRDFPELCFSSTNFRLFLENQSWSLFPFCGKAECVKSG---AD

YIERVHDCGPQPKNAEACTILQRNDTILEYPSCCPKYVCPDGVTLLEYPEIQKQNOAALQAAAAAREAAAGPQ

>Chain_A_Structure_Of_A_Chordin-Like_Cysteine-Rich_Repeat_(Vwc_Module)_From_Collagen_lia.

-----YVEFQEA-----GSCVQDGQ--RYNDKDVWKPEP-CRICVCDTGTLCDDI

ICEDVKDC-----LSPEIPFGECCPI--CPA-----DLAAAA-----

S2: Sequence of dsGFP used as non-specific dsRNA

>dsGFP fragment 455 bp

TGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACG
GGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCG
GATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTGCGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACT
CCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTACCAGGGTGTC
GCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAG
CCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCG
TA