

Supplementary Material

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Expression of *Histophilus somni* IbpA DR2 protective antigen in the diatom *Thalassiosira pseudonana*

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Supplementary Figure S1 Construct maps. Design of constructs used to generate transgenic diatom lines. Uninformative regions of construct maps have been omitted for clarity.

Supplementary Figure S2 IbpA DR2 sequences. a) Codon optimized and mutagenized IbpA DR2 nucleotide sequences in FASTA format. Altered nucleotides underlined and bold. b) Amino acid alignment showing the H:A mutation in bold

Supplementary Table S1 Primers used for amplification and construct synthesis

Figure S1

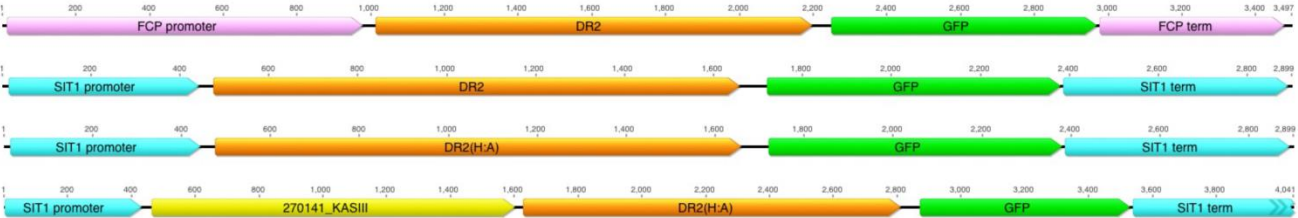


Figure S2

a) Nucleotide Sequences:

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>DR2_Domain_Codon_optimized_for_Tp_Start_Met_added
ATGTCTGTGAACGAGGTGGCTCCACTCTCTTCTGTGCCAGCTGAGCTCAAGGATGCTGCTGGAGGAAACAA
GAAGGCTGCTGAGAAGTCTGAGGGAGCTACCGGAGTGGAGAAGGAGAAGACCACCCTCTTCCAACGTGTGA
AGCAATTCTTACCAGGATCTAAGTCTGGAGCTAAGCCAGTGGCTGGAGATGAGACCGCTAACAAGGTGAAC
TACCAAGATCTCGAGGATAACCTCAACCTCAAGGGACTCATCTCTCTCGAGGATGATCGTAACGCTAACCTT
CGAGTCTAACGTGCTCAAGAACGAGAAGTTCCTCGATGAGGCTCGTGAGATCTCTAAGAAGTCTATCCCAG
AGGCTACCGTGAAGCAAATGTCTCACCTCCCAGAGTTCGATGATATCCTCACCGAGGGAGCTAAGAAGGTG
GAGTCTCGTATCAACAAGGCTATCACCTTCCGTCCATCTGTGGAGGAGTTCCTCTGAGATCCAAGATCTCGT
GAAGACCCTCCCAAAGACCAAGGTGATCGAGGATCTCTCTACCAAGACCAACGAGATCACCGAGGCTCTCG
CTGCTACCTCTAAGACCATCCAACGTACCCAGAGCTCAAGGAGCAACTCAAGACCGCTATCGAGGATTTTC
CTCCAAAACCTCTCAAGGAAAGCCACTCACCGTGCAAATGATCGAGAACCCTCAACCACGGACTCCGTCCAGA
TGAGGGAGAGGGACGTCTCCTCTACAAGAAGGAGAACCCTACCAAGGAGAACGCTGTGTTCTTTCTCCAG
AGGCTGCTAAGATCCAACCTCGCTGAGACCGTGGATTTTCATCAACCCTGCTAAGAACGAGGGAATCGAGCCA
TCTGTGGTGGGAGCTCTCGTGTACCAACGTCTCATCGCTTACCACCCATTCGCTGAGGGAAACGGACGTAT
GGCTCGTGTGATCGTGAACAAGATCCTCCTCGATGCTGGATACCCAGCTTTTACCAAGTTCCTCTGATGAGT
TCGAGCCACAAATCATCCACAAACCAAGGCTTCTACCAAGTCTGCTACCTCTTCTGAGGTGGTGGTGGAG
TTCCTCAAGGAGCTCGCTAAGAAGGGATCTAAGGAGGATAACGAGCAAAACCTCGAGAAGACCGATCGTAC
CTCTACCGATCTCACCGAGTCTGCTGTGGAGAACTCTGCTGCTCTCTCT
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>DR2_Domain_Codon_optimized_for_Tp_Start_Met_added_H:A_mutant
ATGTCTGTGAACGAGGTGGCTCCACTCTCTTCTGTGCCAGCTGAGCTCAAGGATGCTGCTGGAGGAAACAA
GAAGGCTGCTGAGAAGTCTGAGGGAGCTACCGGAGTGGAGAAGGAGAAGACCACCCTCTTCCAACGTGTGA
AGCAATTCTTACCAGGATCTAAGTCTGGAGCTAAGCCAGTGGCTGGAGATGAGACCGCTAACAAGGTGAAC
TACCAAGATCTCGAGGATAACCTCAACCTCAAGGGACTCATCTCTCTCGAGGATGATCGTAACGCTAACCTT
CGAGTCTAACGTGCTCAAGAACGAGAAGTTCCTCGATGAGGCTCGTGAGATCTCTAAGAAGTCTATCCCAG
AGGCTACCGTGAAGCAAATGTCTCACCTCCCAGAGTTCGATGATATCCTCACCGAGGGAGCTAAGAAGGTG
GAGTCTCGTATCAACAAGGCTATCACCTTCCGTCCATCTGTGGAGGAGTTCCTCTGAGATCCAAGATCTCGT
GAAGACCCTCCCAAAGACCAAGGTGATCGAGGATCTCTCTACCAAGACCAACGAGATCACCGAGGCTCTCG
CTGCTACCTCTAAGACCATCCAACGTACCCAGAGCTCAAGGAGCAACTCAAGACCGCTATCGAGGATTTTC
CTCCAAAACCTCTCAAGGAAAGCCACTCACCGTGCAAATGATCGAGAACCCTCAACCACGGACTCCGTCCAGA
TGAGGGAGAGGGACGTCTCCTCTACAAGAAGGAGAACCCTACCAAGGAGAACGCTGTGTTCTTTCTCCAG
AGGCTGCTAAGATCCAACCTCGCTGAGACCGTGGATTTTCATCAACCCTGCTAAGAACGAGGGAATCGAGCCA
TCTGTGGTGGGAGCTCTCGTGTACCAACGTCTCATCGCTTACgctCCATTTCGCTGAGGGAAACGGACGTAT
GGCTCGTGTGATCGTGAACAAGATCCTCCTCGATGCTGGATACCCAGCTTTTACCAAGTTCCTCTGATGAGT
TCGAGCCACAAATCATCCACAAACCAAGGCTTCTACCAAGTCTGCTACCTCTTCTGAGGTGGTGGTGGAG
TTCCTCAAGGAGCTCGCTAAGAAGGGATCTAAGGAGGATAACGAGCAAAACCTCGAGAAGACCGATCGTAC
CTCTACCGATCTCACCGAGTCTGCTGTGGAGAACTCTGCTGCTCTCTCT
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b) Amino acid alignment of mutant vs. native IbpA DR2 domain:

	1	10	20	30	40	50	60	70
HSDR2_CO_H:A mutant translation	MSVNEVAPLSSVPAELKDAAGNKKAAEKS	EGATGVEKEK	TLFQRVKQFF	TGSKSGAK	PVAGDETANKVNY	QDLEDN		
DR2_native	MSVNEVAPLSSVPAELKDAAGNKKAAEKS	EGATGVEKEK	TLFQRVKQFF	TGSKSGAK	PVAGDETANKVNY	QDLEDN		
HSDR2_CO_H:A mutant translation	LN	LKGLISLEDDRNANFESNVLKNEKFLDEARE	EISKKS	IPEATVVKQMSHLPEFDDILTEGAKKVESRINKAITFRPSV				
DR2_native	LN	LKGLISLEDDRNANFESNVLKNEKFLDEARE	EISKKS	IPEATVVKQMSHLPEFDDILTEGAKKVESRINKAITFRPSV				
HSDR2_CO_H:A mutant translation	EEFSEIQDLVKTLPKTKVIEDLS	TKTNEITEALAA	TSKTIQRTPELKEQLKTAIEDFLQNSQGKPLTVQMIENLNHGL					
DR2_native	EEFSEIQDLVKTLPKTKVIEDLS	TKTNEITEALAA	TSKTIQRTPELKEQLKTAIEDFLQNSQGKPLTVQMIENLNHGL					
HSDR2_CO_H:A mutant translation	R	PDEGEGRLLYKKNLTKENAVFSSPEAAKIQLAETVDFINRAKNEGIEPSVVGALVYQRLIA	Y	PPFAE	NGR	MAR	VI	
DR2_native	R	PDEGEGRLLYKKNLTKENAVFSSPEAAKIQLAETVDFINRAKNEGIEPSVVGALVYQRLIA	Y	PPFAE	NGR	MAR	VI	
HSDR2_CO_H:A mutant translation	V	NKILLDAGYPAFTKPSDEFEPQII	PQTKAS	TKSATSS	SEVVVEFLKELAKKGS	KEDNEQNLEK	TDR	TS
DR2_native	V	NKILLDAGYPAFTKPSDEFEPQII	PQTKAS	TKSATSS	SEVVVEFLKELAKKGS	KEDNEQNLEK	TDR	TS
HSDR2_CO_H:A mutant translation	S	AALS						
DR2_native	S	AALS						

Supplementary Table S1. Primers used for amplification and construct synthesis

Gene	Construct	Forward Primer	Reverse Primer
lbpA DR2	Codon optimized gene	5'- ATGTCTGTGAACGAGGTGGTC-3'	5'- AGAGAGAGCAGCAGAGTTCTCC -3'
Thaps3 270141	Chloroplast targeting	5'- ATGAGACTCTCCATCGCAACTATA -3'	5'- ACCCCACTTCAAGATTGCTGCAC -3'