

Supplementary materials, *Marine drugs*

Transgenic *Schizochytrium* as a Promising Oral Vaccine Carrier: Potential Application in the Aquaculture Industry

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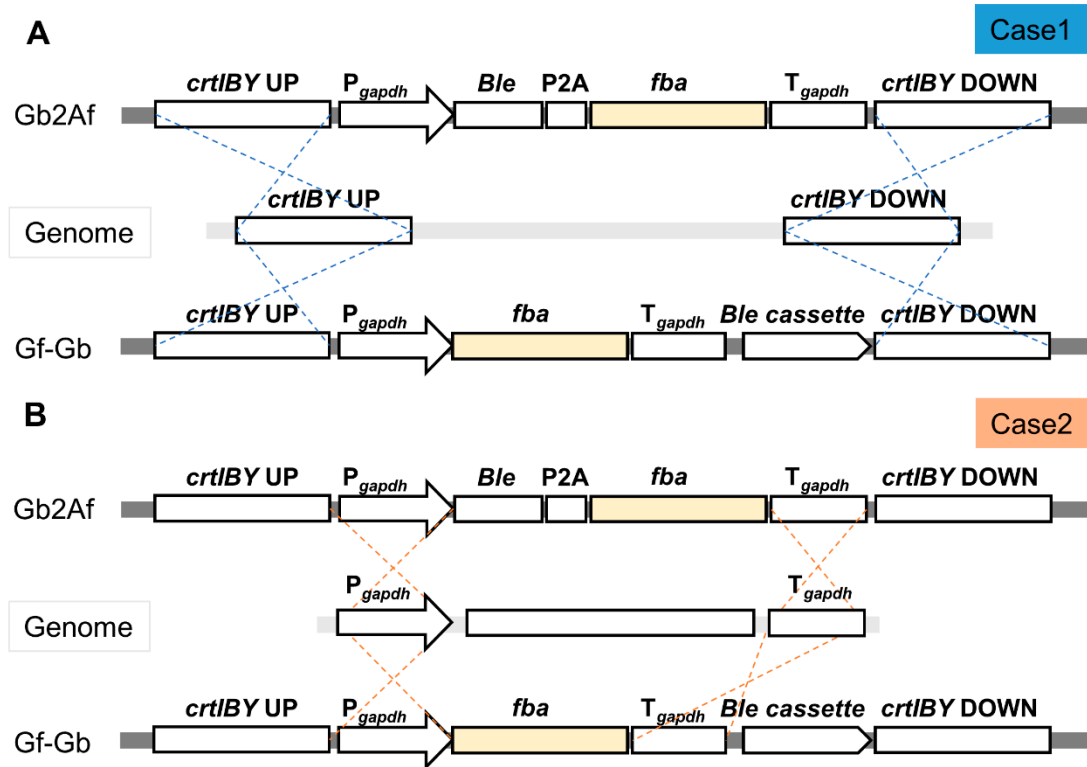


Figure S1. The scheme of integration via homologous recombination. Case 1_integration at the *crtIBY* gene locus (A), Case 2_integration at the *gapdh* gene locus (B). G2Af, the GAPDH promoter drive the co-expression of the marker gene *Ble* and the antigen gene *fba* (linked by a 2A sequence). Gf-Gb, the GAPDH promoter solely to drive the expression of the antigen gene *fba*.

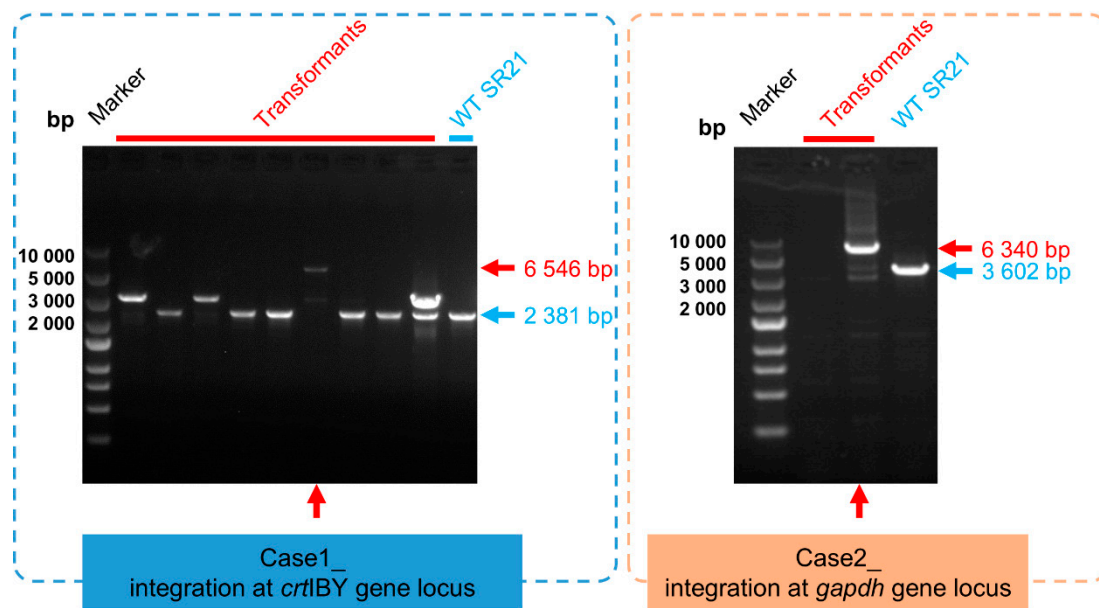


Figure S2. Identification of Gf-Gb transformants via colony PCR. The PCR products were amplified using primers flanking the modified regions of the SR21 genome. Red arrows indicate the positive transformants, blue arrows indicate the WT SR21. The DNA sequencing of the PCR products was following performed to confirm the integration.

Table S1

The primers used for construction of recombinants.

Primer	Sequence (5'-3')	Application
150841-U-F	AAAACGACGGCCAGTGAATTCGAGCTCG	Amplification of crtIBY upstream homologous
	CCACCCAGGATGTCTGTC	
150841-U-R	TGGAGCCCTCACAAGATCAAGGTACCGC	sequence
	TTAACCTCCACTAGGG	
150841-D-F	CTCTTCTAGTGCTTCAAGGGATCCGATTT	Amplification of crtIBY downstream homologous
	TTCCGCCGAGCTG	
150841-D-R	GACCATGATTACGCCAAGCTTGCTCCAG	sequence
	AGTTCTCGAGTGC	
GAPDHp-F	TAGTGGAGGGTTAAGCGGTACCTTGATCT	Amplification of endogenous
	TGTGAGGGCTCCA	
fba-GAPDHp-R	GTCAAAGATTTTAGACATTTTGCTTGGTG	promoter sequence
	TTTATGT	
fba-GAPDHt-F	CACCACCACCACCACCACTAACCATGGA	Amplification of endogenous
	TGTACCCAATACCACACCGGTAGC	
GAPDHt-R	CAGCTCGGCGGAAAAATCGGATCCCTTG	terminator sequence
	AAGCACTAGAAGAG	
Gp-fba-F	GTCAAAGATTTTAGACATTTTGCTTGGTG	Amplification of antigen gene <i>fba</i> sequence
	TTTATGT	
Gt-fba-R	CCATGGTTAGTGGTGGTGGTGGTGGTGC	(Separate cassette or linked

	AGCACGTCGATGCAGTT	by P2A sequence)
P2A-fba-F	AGCAAGCAGGAGACGTGGAAGAAAACC	
	CCGGTCCTGTCGACATGTCTAAAATCTTT	
	GAC	
P2A-Ble-R	TCCACGTCTCCTGCTTGCTTGAGCAGAG	
	AGAAGTTCGTGGCGTCCTGCTCCTCGGC	Amplification of marker gene
	CAC	<i>Ble</i> sequence
Ble-F	ACATAAACACCAAGCAAAATGGCCAAGT	
	TGACCAGT	
crt-V-F	ATGGACGAAGCTAAAGAAAA	Colony PCR identification of
crt-V-R	ACCGTACTATAAACGAGGTA	SR21transformants at <i>crtI</i> <i>BY</i>
		gene locus
gapdh-V-F	CCGGACTGCCATTTGAACGA	Colony PCR identification of
gapdh-V-R	GGCTCGGATTCGAATGAACG	SR21transformants at <i>gapdh</i>
		gene locus

Table S2

The primers used for RT-qPCR.

Primer	Sequence (5'-3')	Gene accession number
TLR2-F	TCTCCGTCTTGGTTTCAC	EU643837.1
TLR2-R	GGTCCCACAGTTGAGTATG	
TLR4-F	GGAATAATGGGCAGCCGTAAG	NM_212813.2
TLR4-R	AGCGACACCAGGAAGTATCAATG	
TLR5-F	GAAACATTCACCCTGGCACA	NM_001130595.2
TLR5-R	CTACAACCAGCACCACCAGAATG	
MyD88-F	AACAACCTTCGCTGGATAA	NM_212814.2
MyD88-R	GTTACTGGAATCGCCTCA	
IL6-F	TCAACTTCTCCAGCGTGATG	NM_001261449.1
IL6-R	TCTTTCCTCTTTTCCTCCTG	
IL1 β -F	TGGACTTCGCAGCACAAAATG	NM_212844.2
IL1 β -R	GTTCACTTCACGCTCTTGGATG	
IL8-F	GTCGCTGCATTGAAACAGAA	XM_009306855.3
IL8-R	CTTAACCCATGGAGCAGAGG	
TGF- β -F	TGGGCTGGCGGTGGAT	NM_194386.2
TGF- β -R	CCTCTGGGTTTCAGCGTGTT	
MHC1-F	GGAGTTCACCTTGCTTATGC	NM_194403.1
MHC1-R	CCCTCTGACCCATTCTGT	
CD8-F	AAGAGCATAGCACCGTAG	NM_001040049.1

CD8-R	GACTTCCGTCTGCTTTGCG	
MHC2-F	TGACTCAACTGTCCGTGATA	NM_001005943.2
MHC2-R	CCATTAGCCATCTCCATAGTG	
CD4-F	GTGGTCTTCATCTTGCTTGT	NM_001135096.1
CD4-R	AATCCCTTTGGCTGTTTGTT	
IgZ1-F	CACCCAGCATTCTACAGCAAAC	AY643752.1
IgZ1-R	GTCGGTACAAGAACCAAACTCAG	
IgZ2-F	CAGAATGGAGCAAGCCTGAC	EU732710.1
IgZ2-R	TAACTGTGCCCTCTTGGTGTATT	
β-actin-F	ATGGATGAGGAAATCGCTGCC	NM_131031.2
β-actin-R	CTCCCTGATGTCTGGGTCGTC	
