

Supplementary Data

Transgenic microalgae expressing double-stranded RNA as potential feed supplements for controlling white spot syndrome in shrimp aquaculture

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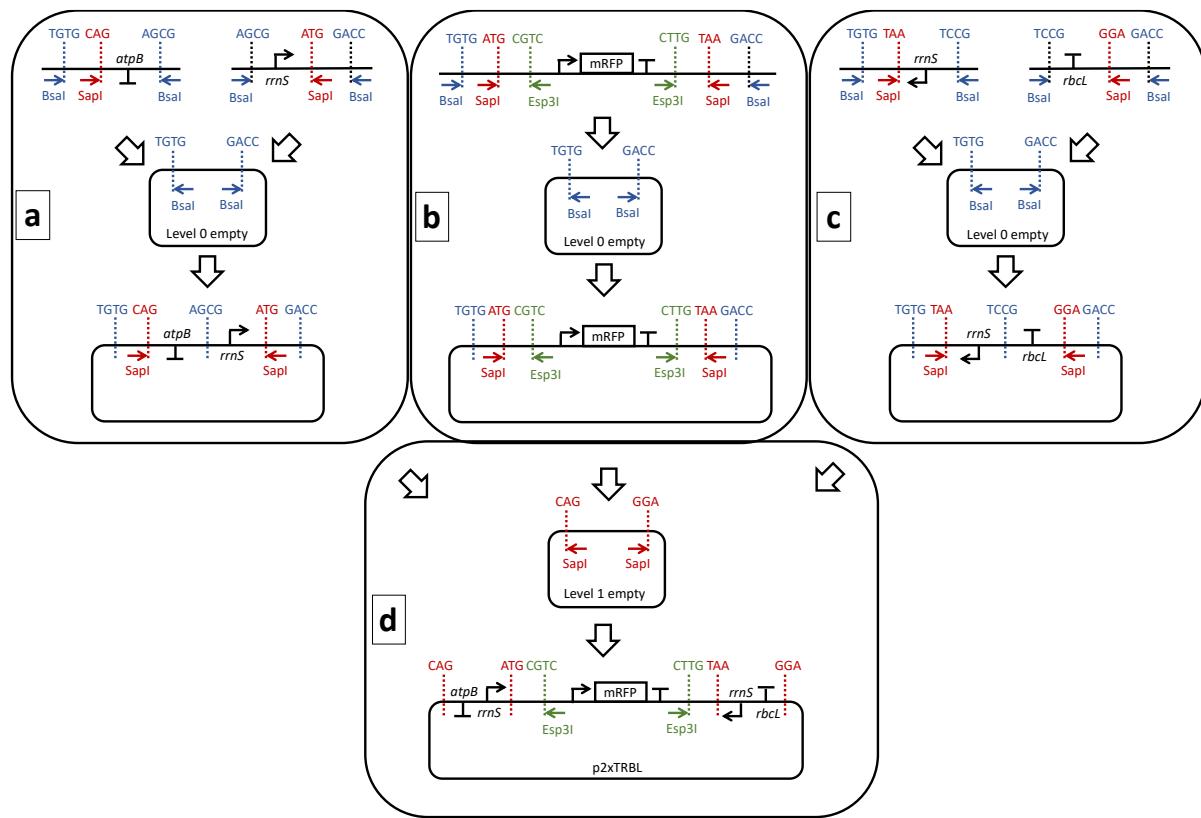


Figure S1 Golden Gate assembly of p2xTRBL. *C. reinhardtii* expression elements were amplified by PCR, with the addition of 5' extensions to allow *BsaI* cloning into the Level 0 empty vector (a, c). The mRFP cassette was synthesized *de novo*, also with the addition of Golden Gate cloning sequences (b). The three Level 0 cassettes were cloned into a Level 1 empty vector using *SapI* to generate p2xTRBL (d).

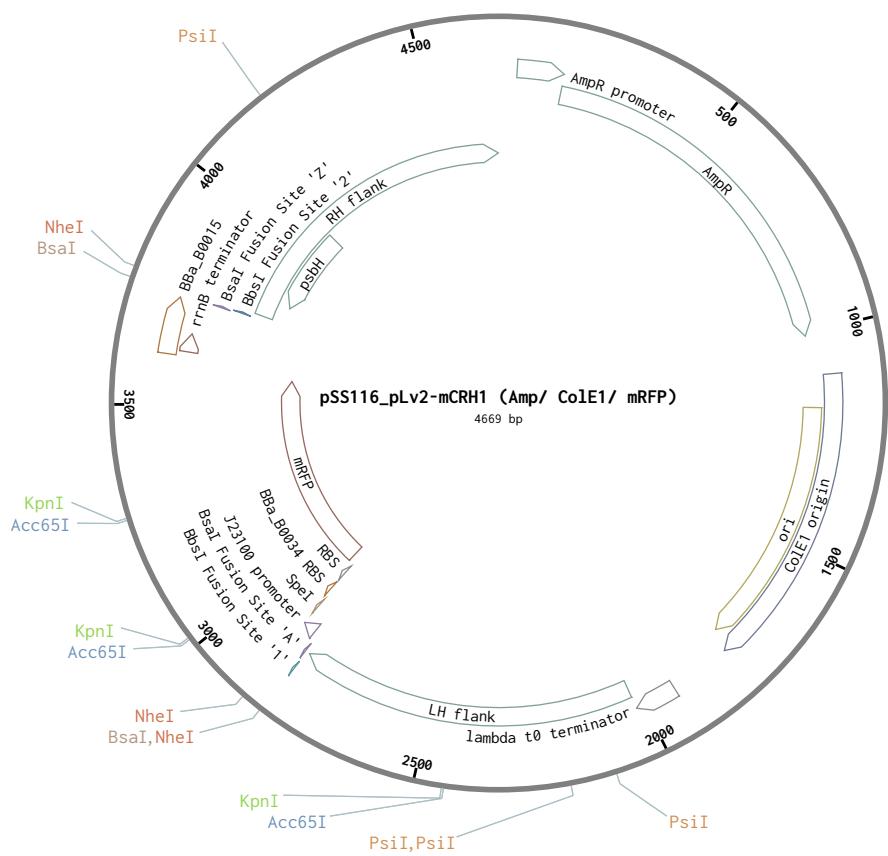


Figure S2 Map of plasmid pSS116, an integration vector for targeting transgenes into the *C. reinhardtii* chloroplast genome between *psbH* and *trnE2*.

The sequence diagram illustrates the VP28 transcription cassette. The sequence is divided into several regions:

- Inverted repeats:** Indicated by horizontal arrows pointing in opposite directions. A red arrow spans the first 15 bases, a blue arrow spans the last 15 bases, and a green arrow spans the middle 15 bases.
- Esp31 sites:** Indicated by bolded segments. A bolded **cg** is located at the start of the sequence, and a bolded **tttg** is located within the middle region.
- rrnS promoter elements:** Indicated by green boxes. A green box covers the first 15 bases, and another green box covers the middle 15 bases.
- Transcriptional start site:** Indicated by a black triangle pointing to the first base of the green promoter region.
- atpB terminator:** Indicated by a light grey box spanning the last 15 bases.
- rbcL terminator:** Indicated by a dark grey box spanning the first 15 bases.
- Transcript termination sites:** Indicated by vertical black arrows pointing downwards at the ends of the green and blue promoter regions.
- Inverted repeat termination sites:** Indicated by horizontal green arrows pointing to the ends of the green promoter region.

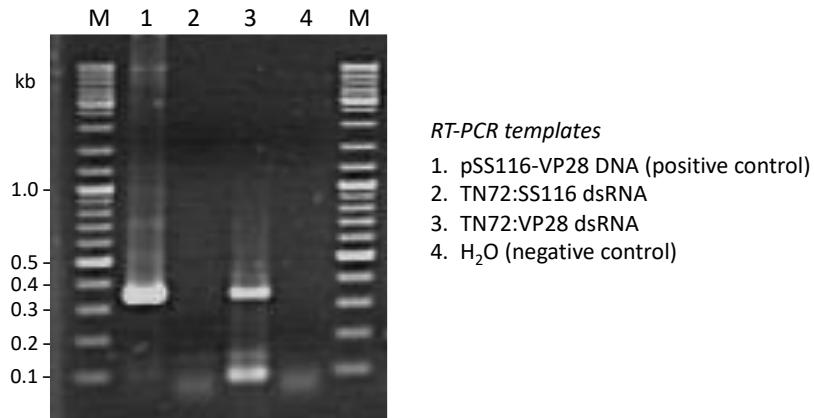
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Tataatgttatgttaattaaaataaaattggctctttaagaagaagaaaacaacttaatggtgtcc
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ttttttttgtcaaattttattttttttaataaaaattcaataacaagctgctccgtt
ttttttttcatgatgttatgtgaatagcataaacatcggtttattttatggtgtttagg
→ ← ↓
ttaaatacctaaacatcatt

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Figure S3 Sequence of the VP28 transcription cassette. The VP28 sequence is highlighted in yellow. *Esp31* fusion sites are in bold. The *rrnS* promoter elements are highlighted in green with the transcriptional start site indicated by a black triangle. The *atpB* terminator is highlighted in light grey and the *rbcL* terminator in dark grey. Inverted repeat regions are indicated by horizontal coloured arrows and the transcript termination sites by vertical black arrows.

A



B



Figure S4 RT-PCR analysis of dsRNA from transformant TN72:VP28. **A.** Lane 1: PCR amplification of VP28 DNA from pSS116-VP28 plasmid. Lane 2: RT-PCR using dsRNA fraction from strain TN72:SS116. Lane 3: RT-PCR using dsRNA fraction from strain TN72:VP28. 4 RT-PCR using ddH₂O as template. **B.** Illustration of primers used for RT-PCR.

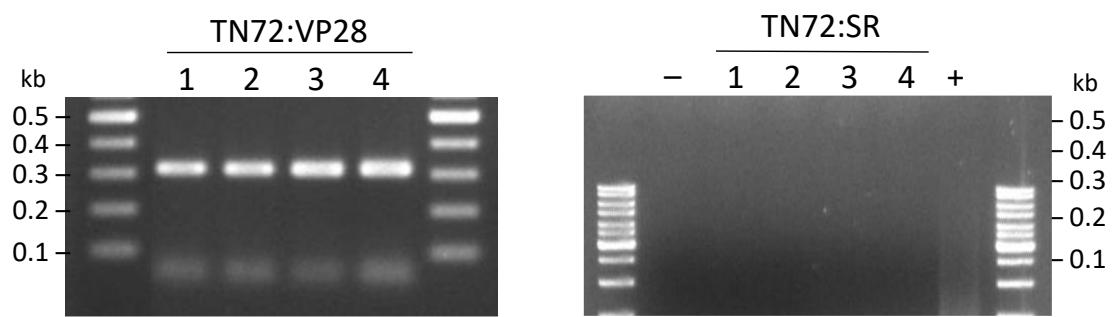


Figure S5 Detection of dsRNA from freeze-dried *C. reinhardtii*. Four replicate samples each representing 0.1 g of biomass from either TN72:VP28 (left panel) or TN72:SR (right panel) were used for dsRNA extraction and RT-PCR using specific primers VP28_Esp_F and VP28_Esp_R (See Figure S4). Controls are H₂O (–) or purified dsRNA (+) as template.

Table S1 Details of primers used

Primer	Sequence (5'-3')	Function	Reference
VP28_Esp_F	ATAC <u>CGCGTCTCTCGTCGAT</u> TTTCTTC <u>ACTCTTCG</u>	Generation of VP28 fragment for p2XTRBL cloning (<i>Esp31</i> sites underlined). RT-PCR analysis of dsRNA from <i>E. coli</i> and <i>C. reinhardtii</i> (PCR product of 338 bp)	This work
VP28_Esp_R	ATAC <u>CGCGTCTCTCAAGCCACAGGAGTGATGACAA</u>		
rbcL_F	GTCACCACCA <u>GACATACGAAG</u>	Internal control for chloroplast DNA extraction. (PCR product of 264 bp)	[18]
rbcL_R	GGTC <u>CACTACTTAAACGCTAC</u>		
TN72_F	GTCATTGC <u>AAAATACTGGTGC</u>	TN72 plastome-specific primers for assessing homoplasmic status. (PCR product of 0.88 kb)	[19]
TN72_R	CGGATGT <u>AACTCAATCGGTAG</u>		
WSSV447_F	ATGAGAAT <u>GAACTCCAAC</u> TTAA	WSSV load assay.	[26]
WSSV447_R	CAGAGCCTAGT <u>CTATCAATCAT</u>		