Supplementary information.

Transgene-free genome editing in marine algae by bacterial conjugation – comparison with biolistic CRISPR/Cas9 transformation.

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Supplementary Figure 1. Schematic illustration of the pPtPuc3m diaCas9_sgRNA plasmid. Important elements of the episomal plasmid pPtpuc3m_diaCas9_sgRNA: diaCas9 (brown) under regulation of LHCF2 promoter and LHCF1 terminator (grey), sgRNA (red) regulated by U6 promoter (yellow), target region (green), zeocin resistance cassette (black), origin of transfer (oriT; pink), CEN/ARS (dark grey).

Supplementary Figure 2. **Overview of CRISPR-induced targeted mutations in the Myb1R_SHAQKYF5 gene identified in primary colonies.** Different types of mutations at the targets sites Myb PAM1 and Myb PAM2 present in primary colonies by using either pKS_diaCas9_sgRNA for biolistic bombardment or pPtpuc3m_diaCas9_sgRNA for bacterial conjugation. C is used as an abbreviation for colony number. The target sequence is shown in green with PAM sequence in brown font. Indels are shown in red font.

Supplementary Figure 3: Experimental design for generating non-transgenic mutant lines. Mutant lines originating from single cells were grown in the absence of antibiotics for 16 days during which they were 100-fold diluted after 7 days. After 16 days, cells were 1000-fold diluted, and 200 µl from the cultures were plated to obtain single colonies on non-selection plate. Colonies could be picked after three weeks. Cells from 25 randomly chosen colonies were patched onto a new non-selection plate in a grid pattern. After five days randomly selected colonies were re-patched on non-selection plate and selection plate. Verification of non-transgenic cells was done by PCR.

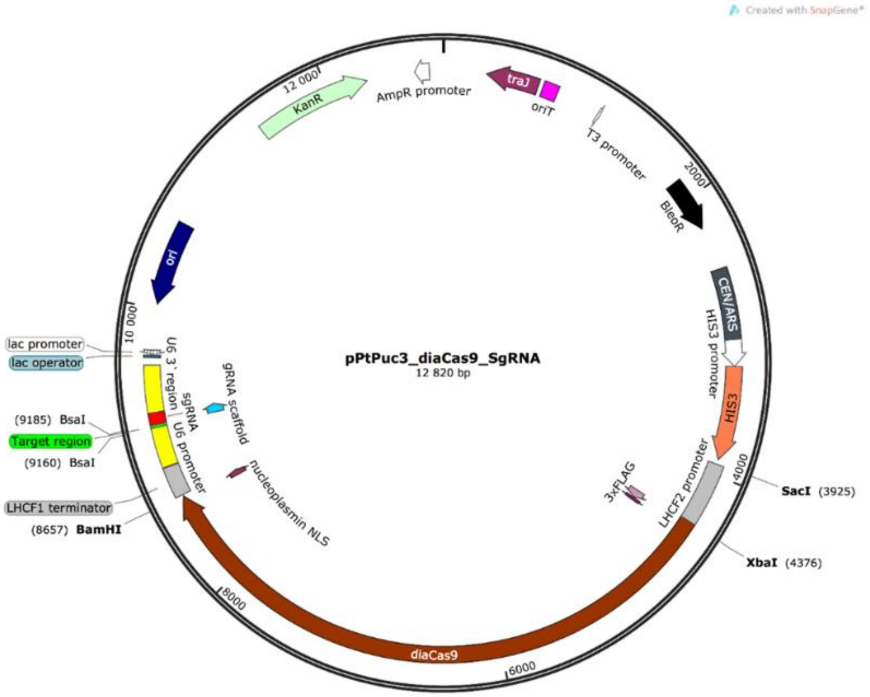
Supplementary Figure 4. Sequence for pPtPuc3m_diaCas9_sgRNA from 5' end. oriT. Colour coding used: BleoR, CEN/ARS, LHCF2 Promoter, diaCas9, LHCF1 terminator, **U6promoter,** sgRNA, U6 3' prime region, kanR

Supplementary figure 5: Plasmid rescue of pPtPuc3m_diaCas9_sgRNA episome from transformed *P. tricornutum* lines. Plasmid rescue was performed using genomic DNA from pure mutant lines containing pPtPuc3m_diaCas9_sgRNA episome and used to transform *E. coli* Dhα. 150-200ng of isolated plasmid DNA was linearized using XbaI restriction enzyme and separated by agarose gel electrophoresis. Lanes marked with L indicate 1-kb plus ladder (Fermentas) and with PL indicate pPtPuc3m_diaCas9_sgRNA plasmid as control. CON with number indicate colonies generated using bacterial conjugation and A-E represent 5 plasmids isolated from 5 random *E. coli*.

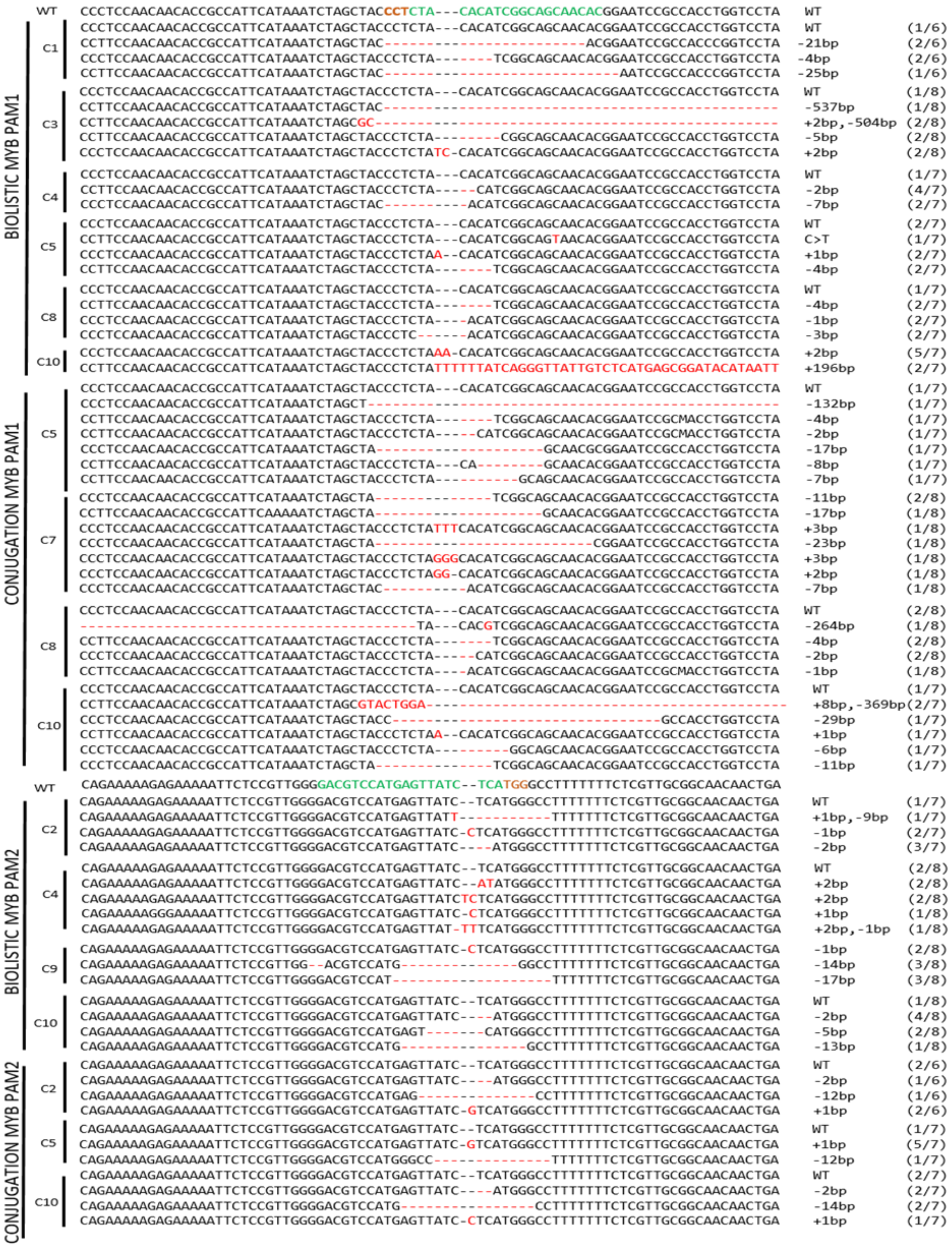
Supplementary Table 1: List of polymorphisms used to differentiate alleles for the MYB1R_SHAQKYF5 gene. Pt_Myb (XM_002181623) sequence is used as a reference gene, and the numbers represent the position of the nucleotide in the reference gene.

Supplementary Table 2: List of primers used in the experiments.

Supplementary Figure 1



Supplementary Figure 2



	WT	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	
BIOLISTIC MYB PAM1	C1	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/6)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTAC------------------------ACGGAATCCGCCACCCGGTCCTA	-21bp	(2/6)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-4bp	(2/6)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTAC----------------------------AATCCGCCACCCGGTCCTA	-25bp	(1/6)
	C3	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/8)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTAC---	-537bp	(1/8)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCGC--	+2bp,-504bp	(2/8)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA--------CGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-5bp	(2/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTATC-CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+2bp	(2/8)
	C4	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-----CATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-2bp	(4/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTAC----------ACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-7bp	(2/7)
	C5	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(2/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGTAACACGGAATCCGCCACCTGGTCCTA	C>T	(1/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTAA--CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+1bp	(2/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-4bp	(2/7)
	C8	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-4bp	(2/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA----ACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-1bp	(2/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTC------ACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-3bp	(2/7)
	C10	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTAAA-CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+2bp	(5/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTATTTTTTATCAGGGTTATTGTCTCATGAGCGGATACATAATT	+196bp	(2/7)
CONJUGATION MYB PAM1	C5	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/7)
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		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CA--------GCAACACGGAATCCGCCACCTGGTCCTA	-8bp	(1/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA----------GCAGCAACACGGAATCCGCCACCTGGTCCTA	-7bp	(1/7)
	C7	CCCTCCAACAACACCGCCATTCATAAATCTAGCTA--------------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-11bp	(2/8)
		CCTTCCAACAACACCGCCATTCAAAAATCTAGCTA--------------------GCAACACGGAATCCGCCACCTGGTCCTA	-17bp	(1/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTATTTCACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+3bp	(1/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTA--------------------------CGGAATCCGCCACCTGGTCCTA	-23bp	(1/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTAGGGCACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+3bp	(1/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTAGG-CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	+2bp	(1/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTAC----------ACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-7bp	(1/8)
	C8	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(2/8)
		--TA---CACGTCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-264bp	(1/8)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-4bp	(2/8)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA-----CATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-2bp	(2/8)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA----ACATCGGCAGCAACACGGAATCCGCMACCTGGTCCTA	-1bp	(1/8)
	C10	CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---CACATCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	WT	(1/7)
		CCTTCCAACAACACCGCCATTCATAAATCTAGCGTACTGGA---	+8bp,-369bp	(2/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACC--------------------------------GCCACCTGGTCCTA	-29bp	(1/7)
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		CCCTCCAACAACACCGCCATTCATAAATCTAGCTACCCTCTA---------GGCAGCAACACGGAATCCGCCACCTGGTCCTA	-6bp	(1/7)
		CCCTCCAACAACACCGCCATTCATAAATCTAGCTA--------------TCGGCAGCAACACGGAATCCGCCACCTGGTCCTA	-11bp	(1/7)
	WT	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA		
BIOLISTIC MYB PAM2	C2	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(1/7)
		CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATT-----------TTTTTTTCTCGTTGCGGCAACAACTGA	+1bp,-9bp	(1/7)
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		CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC----ATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	-2bp	(3/7)
	C4	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(2/8)
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	C10	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(1/8)
		CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC----ATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	-2bp	(4/8)
		CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGT-------CATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	-5bp	(2/8)
		CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATG---------------GCCTTTTTTTCTCGTTGCGGCAACAACTGA	-13bp	(1/8)
CONJUGATION MYB PAM2	C2	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(2/6)
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	C5	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(1/7)
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	C10	CAGAAAAAGAGAAAAATTCTCCGTTGGGGACGTCCATGAGTTATC--TCATGGGCCTTTTTTTCTCGTTGCGGCAACAACTGA	WT	(2/7)
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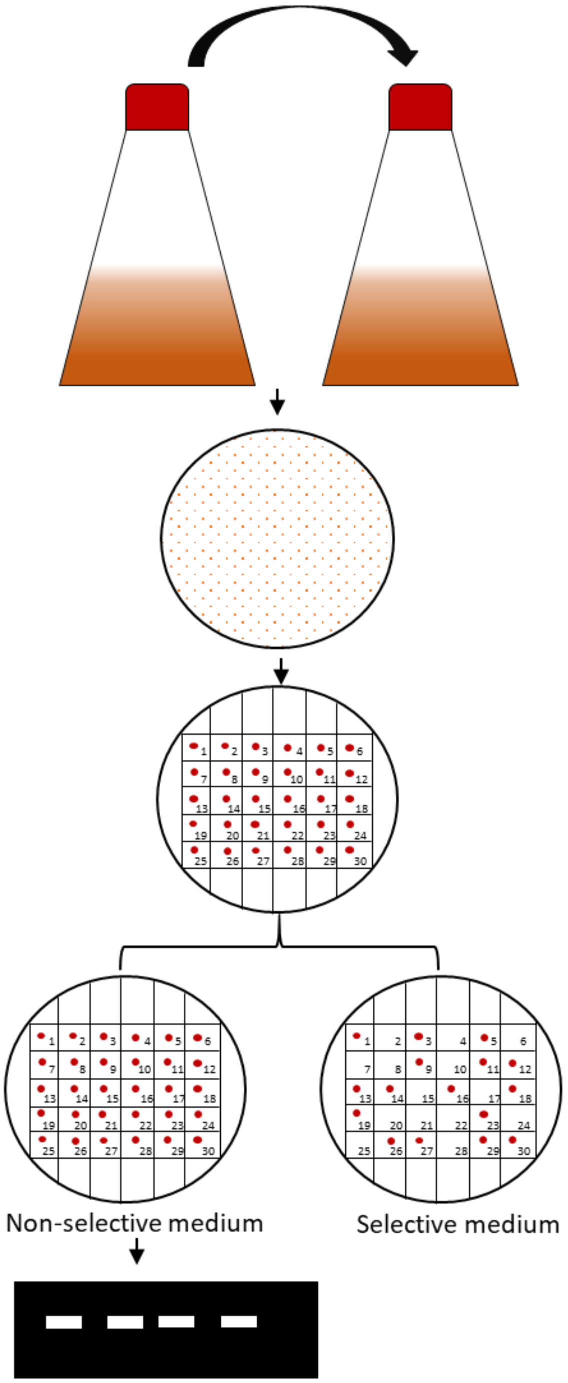
Supplementary Figure 3



Grow cells without selection for 16 days. 100 fold dilution after 7 days.

Isolate single colonies on non-selective medium

Patch single colonies on non-selective medium

Patch single colonies on non-selective medium and replica patch on selective medium

PCR verification

Supplementary Figure 4

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCAGATCGTCTTGCCTTGCTCGTCGGTGATGTACTTACAGCTCGAAGTGCCTCTTCTTGATGGAGCGCATGGGGACGTGCTTGGCAATCACGCGCACCCCCCGGCCGTTTTAGCGGCTAAAAAAGTCATGGCTCTGCCCTCGGGCGGACCACGCCCATCATGACCTTGCCAAGCTCGTCCTGCTTCTCTTCGATCTTCGCCAGCAGGGCGAGGATCGTGGCATCACCGAACCGCGCCGTGCGCGGGTCGTCGGTGAGCCAGAGTTTCAGCAGGCCGCCCAGGCGGCCCAGGTCGCCATTGATGCGGGCCAGCTCGCGGACGTGCTCATAGTCCACGACGCCCGTGATTTTGTAGCCCTGGCCGACGGCCAGCAGGTAGGCCGACAGGCTCATGCCGGCCGCCGCCGCCTTTTCCTCAATCGCTCTTCGTTCGTCTGGAAGGCAGTACACCTTGATAGGTGGGCTGCCCTTCCTGGTTGGCTTGGTTTCATCAGCCATCCGCTTGCCCTCATCTGTTACGCCGGCGGTAG**CCGGCCAGCCTCGCAGAGCAGGATTCCCGTTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAAGGAACACCCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCC**GGCTGACGCCGTTGGATACACCAAGGAAAGTCTACACGAACCCTTTGGCAAAATCCTGTATATCGTGCGAAAAAGGATGGATATACCGAAAAAATCGCTATAATGACCCCGAAGCAGGGTTATGCAGCGGAAGATGGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGTACCTAACAGGATTAGTGCAATTCGAGTTGAATCACTGGGAAAAACATTGTCTTCTTTTTTATATTATCATTTGCATTAGTGCTGCAGTCGTAGATACTTGTTGGTTGAAAGACATCAGCTGGGAGGGACTGGACTAGCGTTTGGTAAGGAGACATACCTGTTAACGTTGGTTGCAAAATTCCATTTCGCGATTTATGTTATCTGTAAATCCTGATTTGTCTGGAATTCTTGATACTTCCGTTTTTTTAGAGGCCAATGATTAGCATCGGCGATTCTCAAAATAGCATTTTCGACATGCGGTGCTGATTTCATAAACATAGACAACGCTTTTACATGTAAAAGTAACTTGCGGACTTGGAACAGTGCTCTGTTTTTGGTGTGAACGTAACTCAGCAATATTTCTGTGCTAGCAAGGTTTTTTATGATCGACCGAAGATCTCAAAACTCCGGGTCTTTCAACTGTCTGACTAGACCATGTTCGTAACGTCGGACAGCAGCTTTCGTTGTACTGGTAGAATTTCTACGTGCGAAGCACGTGTAGGCAGGTTGAACGACGATCCCTGCCGATGGATGGATTGGCACGCGGCGGAACGCTTTCGTGATCTACACCACCTGGATCTTCACATATCTTCGAAATCGAAAAATTAACCAAGTCGACGGTATCGATAATATTCTAGCTGAGGGTACCCATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGGTCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCGGGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCGAGATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACTTCGTGGCCGAGGAGCAGGACTGACCGACGCCGACCAACACCGCCGGTCCGACGCGGCCCGACGGGTCCGAGGCCTCGGAGATCTGGGCCCATGCGGCCGCAACAACTACCTCGACTTTGGCTGGGACACTTTCAGTGAGGACAAGAAGCTTCAGAAGCGTGCTATCGAACTCAACCAGGGACGTGCGGCACAAATGGGCATCCTTGCTCTCATGGTGCACGAACAGTTGGGAGTCTCTATCCTTCCTTAAAAATTTAATTTTCATTAGTTGCAGTCACTCCGCTTTGGTTTCACAGTCAGGAATAACACTAGCTCGTCTTCAGCGAGCATCACGTGCTATAAAAATAATTATAATTTAAATTTTTTAATATAAATATATAAATTAAAAATAGAAAGTAAAAAAAGAAATTAAAGAAAAAATAGTTTTTGTTTTCCGAAGATGTAAAAGACTCTAGGGGGATCGCCAACAAATACTACCTTTTACCTTGCTCTTCCTGCTCTCAGGTATTAATGCCGAATTGTTTCATCTTGTCTGTGTAGAAGACCACACACGAAAATCCTGTGATTTTACATTTTACTTATCGTTAATCGAATGTATATCTATTTAATCTGCTTTTCTTGTCTAATAAATATATATGTAAAGTACGCTTTTTGTTGAAATTTTTTAAACCTTTGTTTATTTTTTTTTCTTCATTCCGTAACTCTTCTACCTTCTTTATTTACTTTCTAAAATCCAAATACAAAACATAAAAATAAATAAACACAGAGTAAATTCCCAAATTATTCCATCATTAAAAGATACGAGGCGCGTGTAAGTTACAGGCAAGCGATCCTAGTACACTCTATATTTTTTTATGCCTCGGTAATGATTTTCATTTTTTTTTTTCCACCTAGCGGATGACTCTTTTTTTTTCTTAGCGATTGGCATTATCACATAATGAATTATACATTATATAAAGTAATGTGATTTCTTCGAAGAATATACTAAAAAATGAGCAGGCAAGATAAACGAAGGCAAAGATGACAGAGCAGAAAGCCCTAGTAAAGCGTATTACAAATGAAACCAAGATTCAGATTGCG

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113 ATGATCATCACCGTAGTGAGAGTGCGTTCAAGGCTCTTGCGGTTGCCATAAGAGAAGCCACCTCGCCCAA
114 TGGTACCAACGATGTTCCCTCCACCAAAGGTGTTCTTATGTAGTTTTACACAGGAGTCTGGACTTGACCT
115 GAGCT**CAATCTCGCCTATTCATGGTGTATAAAAGTTCAACATCCAAAGCTAGAACTTTTGGAAAGAGAAA**
116 **GAATATCCGAATAGGGCACGGCGTGCCGTATTGTTGGAGTGGACTAGCAGAAAGTGAGGAAGGCACAGGA**
117 **TGAGTTTTCTCGAGACACATAGCTTCAGCGTCGTGTAGGCTAGGCAGAGGTGAGTTTTCTCGAGACATAC**
118 **CTTCAGCGTCGTCTTCACTGTCACAGTCAACTGACAGTAATCGTTGATCCGGAGAGATTCAAAATTCAAT**
119 **CTGTTTGGACCTGGATAAGACACAAGAGCGACATCCTGACATGAACGCCGTAAACAGCAAATCCTGGTTG**
120 **AACACGTATCCTTTTGGGGGCCTCCGCTACGACGCTCGCTCCAGCTGGGGCTTCCTTACTATACACAGCG**
121 **CGCATATTTCACGGTTGCCAGAAGTCAAG**ATGGCCTCTAGAATGGACTATAAGGACCACGACGGAGATTA
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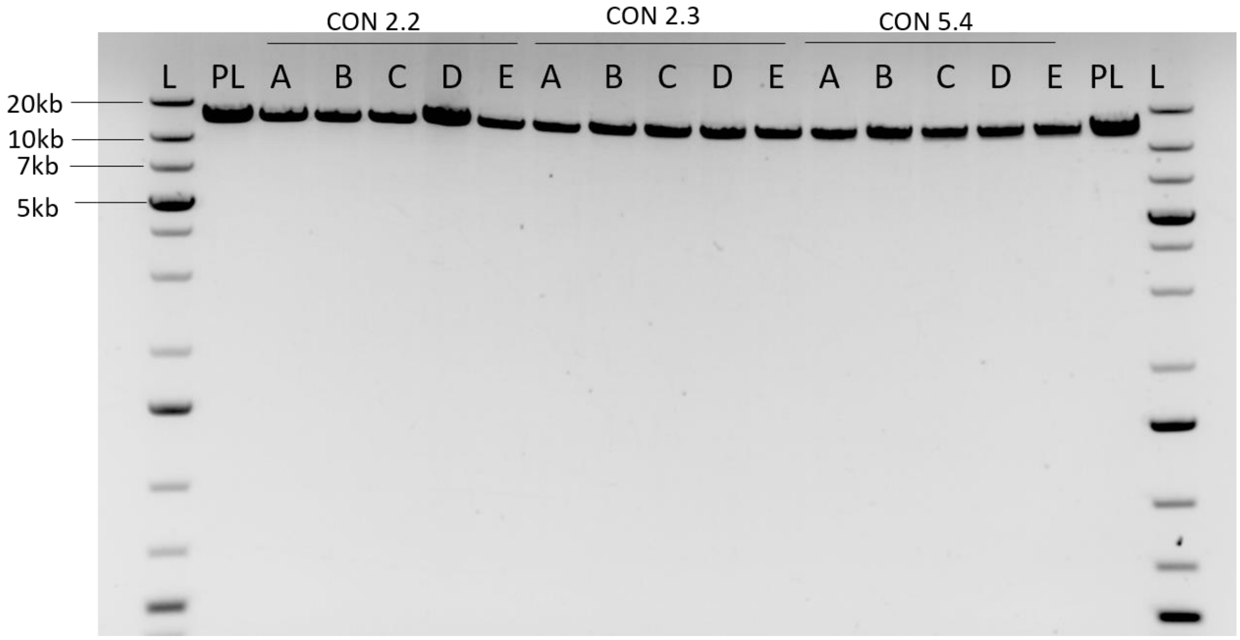
Supplementary Figure 5



Supplementary Table 1

Nucleotide Position	Allele 1	Allele 2
112	G	A
191-196	-	GCGGCAACA (Insert)
311-312	TT	CC
330	T	C
459	T	G
477	T	C
593	G	C
600	C	G
758	G	C
1095	A	G

Supplementary Table 2: List of primers used in the experiments.

Primer Name	**5' Sequence 3'**	**PCR product size(bp)**
Primary PCR amplification		
Myb PAM 1 seq Fw	CCCGATGCTGCCAATCTCAGC	790
Myb PAM 1 seq Rw	GGACGGTGGCGTGCATCAAG	
Myb PAM 2 seq Fw	GCCTACGATGCGACTGTCCG	482
Myb PAM 2 seq Rw	CCGACAGCGGTGGCCGTGCTC	
High resolution melting primers		
HRM_Myb PAM 1_Fw	CCAACAACACCGCCATTCATAAATCTAGC	93
HRM_Myb PAM 1_Rw	GCAAATCAGTAGTCTCGTAGGACCAGG	
HRM_Myb PAM 2_Fw	GCATTGATGCTTCCGCAACTTACAG	106
HRM_Myb PAM 2_Rw	CCATCAGTTGTTGCCGCAACG	
Verification of Construct(plasmid)		
Myb PAM 1 Fw	CTACACATCGGCAGCAACAC	544
M13 Rev	CAGGAAACAGCTATGAC	
Myb PAM 2 Fw	GACGTCCATGAGTTATCTCA	544
M13 Rev	CAGGAAACAGCTATGAC	
Topo sequencing		
M13 Rev	CAGGAAACAGCTATGAC	
Verification of marker free mutants		
dCas9 F1	GAAGTACTCGATTGGATTGGAC	817
dCas9R1	GAGGTCGTCGTCATATGTGTCT	
OriFw	GAATCAGGGATAACGCAGG	855
OriRW	GCATTGGTAACTGTCAGACC	
Phatr2_52110F(ENTPPL)	CGTTTGTGTGCTCCGTTCTA	188
Phatr2_52110R(ENTPPL)	CCTTAAGGCATTGGAGTCACTC	
qPCR		
qPCRphat_28684F	TGCGCTCGACACCATTTCAA	137
qPCRphat_28684R	GCTCCTTTGAAGATTGGACG	
Phatr2_24186F	TCTCCGATACGGGCTTGGA	160
Phatr2_24186R	CAGCCGATCCGTCATAACTGC	
dCas9R4	ATATTCACTTGCGGCATCGACA	97
dCas9F5	TTATCGAGACAAACGGCGAAAC	