Supporting information

Development of a terpenoid-production platform in *Streptomyces reveromyceticus* SN-593

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1 **Table S1. RNA-seq analysis**
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Gene ID	Putative function	Median normalized *RPKM		
		Day1	Day2	Day3
rvr6185	ribosomal protein L10	31,421	1,557	25,349
rvr4994	1L-*myo*-inositol-1-phosphate synthase	14,688	501	22,384
rvr6704	ferredoxin	12,950	4,000	31,302
rvr6682	peptide ABC transporter solute-binding protein	10,381	844	3,051
rvr6236	translation initiation factor IF-1	5,491	516	14,459
rvr4938	cytochrome bd-I oxidase subunit I	4,497	260	19,873
rvr6277	co-chaperonin GroES	3,445	407	2,911
rvr6238	ribosomal protein S13	3,422	222	2,723
rvr6217	ribosomal protein S17	2,326	861	2,862
rvr3590	hypothetical protein	2,314	381	3,816
rvr2313	cytochrome c oxidase subunit III	2,216	276	17,977
rvr6204	ribosomal protein S10	2,102	472	3,909
rvr6199	elongation factor EF-Tu	1,992	762	5,314
rvr6195	ribosomal protein S12	1,839	388	4,733
rvr6197	translation elongation factor G	1,818	621	2,747
rvr6222	ribosomal protein L5	1,318	603	1,913
rvr2133	branched-chain amino acid ABC transporter substrate-binding protein	1,278	287	924
rvr6219	ribosomal protein L14	1,278	1,044	2,221
rvr2046	glyceraldehyde 3-phosphate dehydrogenase	1,245	328	5,425
rvr9025	lipoprotein	1,223	164	6,018
rvr5758	transcriptional regulator	1,162	391	4,149
rvr3991	class I heat-shock protein	871	846	714
rvr3291	citrate synthase	321	179	3,282

3 List of highly expressed gene in *Streptomyces reveromyceticus* SN-593 SR1 strain. *RPKM, reads per kilobase
4 of transcript per million mapped reads. The data were median normalized for each data set.

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1 **Table S2. Bacterial strains and plasmids used in this study**

Strain and plasmid	Relevant characteristics	Reference
Strains		
Streptomyces sp. SN-593	Wild-type reveromycin A-producing strain	[1]
SR1	*Streptomyces* sp. SN-593 that disrupted *revC* and *revD* genes (Δ*revC*Δ*rev)*	[2]
SR1-VC	SR1::pTYM19	This study
SR1-NC	SR1::pTYM19::*aphIIp-rvr4129*	This study
SR1-1	SR1::pTYM19::*aphIIp-fur22 (rvr4131)*	[3]
SR1-2	SR1::pTYM19::*rvr1215p-fur22*	This study
SR1-3	SR1::pTYM19::*rvr1226p-fur22*	This study
SR1-4	SR1::pTYM19::*rvr2028p-fur22*	This study
SR1-5	SR1::pTYM19::*rvr2030p-fur22*	This study
SR1-6	SR1::pTYM19::*rvr2133p-fur22*	This study
SR1-7	SR1::pTYM19::*rvr2353p-fur22*	This study
SR1-8	SR1::pTYM19::*rvr2436p-fur22*	This study
SR1-9	SR1::pTYM19::*rvr3032p-fur22*	This study
SR1-10	SR1::pTYM19::*rvr3087p-fur22*	This study
SR1-11	SR1::pTYM19::*rvr3168p-fur22*	This study
SR1-12	SR1::pTYM19::*rvr3291p-fur22*	This study
SR1-13	SR1::pTYM19::*rvr3493p-fur22*	This study
SR1-14	SR1::pTYM19::*rvr3650p-fur22*	This study
SR1-15	SR1::pTYM19::*rvr3991p-fur22*	This study
SR1-16	SR1::pTYM19::*rvr4050p-fur22*	This study
SR1-17	SR1::pTYM19::*rvr4941p-fur22*	This study
SR1-18	SR1::pTYM19::*rvr5758p-fur22*	This study
SR1-19	SR1::pTYM19::*rvr6185p-fur22*	This study
SR1-20	SR1::pTYM19::*rvr6204p-fur22*	This study
SR1-21	SR1::pTYM19::*rvr6279p-fur22*	This study
SR1-22	SR1::pTYM19::*rvr6682p-fur22*	This study
SR1-23	SR1::pTYM19::*rvr7845p-fur22*	This study
SR1-24	SR1::pTYM19::*rvr7933p-fur22*	This study
SR1-25	SR1::pTYM19::*rvr7936p-fur22*	This study
SR1-26	SR1::pTYM19::*rvr7941p-fur22*	This study
SR1-27	SR1::pTYM19::*rvr9107p-fur22*	This study
SR1-28	SR1-1::pKU492Acos::*fur1p-fpps-ssl1-ssl3*	This study
SR1-29	SR1-5::pKU492Acos::*fur1p-fpps-ssl1-ssl3*	This study
SR2	The *fur1* gene deletion was introduced in SR1	This study
SR2-PF	SR2::pKU492Acos::*fur1p-fpps-ssl1-ssl3*	This study
SR2-1	SR2::pTYM19::*aphIIp-fur22*	This study
SR2-2	SR2::pTYM19::*rvr2030p-fur22*	This study
SR2-3	SR2-PF::pTYM19::*aphIIp-fur22*	This study
SR2-4	SR2-PF::pTYM19::*rvr2030p-fur22*	This study
SR2-5	SR2-PF::pTYM19::*rvr2353p-fur22*	This study
SR2-6	SR2-PF::pTYM19::*rvr3650p-fur22*	This study
SR2-7	SR2-PF::pTYM19::*rvr9107p-fur22*	This study
E. coli DH5α	Cloning host; F^- Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 endA1 recA1 deoR hsdR17*(r_K^- m_K^+) *phoA*, *supE44* λ^- *thi-1 gyrA96 relA1*	
E. coli -conjugation	*E. coli* GM2929 *hsdS*::Tn10 carrying pUB307-*aph*::Tn7	[4]
E. coli-BW25113	The derivative of the F^- , λ^- , *E.coli* K-12 strain BD792 [CGSC6159]	[5]

Plasmids		
pKD13	Template plasmid for FRT-flanked kanamycin resistant gene	CGSC [5]
pKD78	Red recombinase expression plasmid	CGSC
pCP20	Ampicillin and chloramphenicol resistance plasmid that shows temperature sensitive replication and thermal induction of FLP synthase	CGSC [6]
pIM	DNA region from BamHI and KpnI site was removed from pKU250	[2]
pIM-*fur1*	pIM containing approximately 6 kb DNA containing *fur1* gene in HindIII site	This study
pIM-Δ*fur1*	*fur1* gene disruption vector	This study
pTYM19	Actinomycete-*Escherichia coli* integration vector	[7]
pTYM19::*aphIIp*	The *aphII* promoter was ligated into EcoRI and BamHI site	[2]
pTYM19::*aphIIp-fur22*	The *fur22* (*rvr4131*) transcriptional regulator gene was inserted in BamHI and HindIII site of pTYM19::*aphIIp*	[3]
pTYM19::*aphIIp-rvr4129*	# The *rvr4129* gene was inserted in BamHI and HindIII site of pTYM19::*aphIIp*	This study
pTYM19::*fur22*	The *aphII* promoter was removed from pTYM19::*aphIIp-fur22*	This study
pKU492Acos_*aac(3)IV*	Actinomycete TG1 integration vector	[8]
pKU492Acos::*fur1p*	pKU492Acos with *fur1* promoter regulating mevalonate gene cluster in EcoRI and NdeI site	This study
pKU492Acos::*fur1p-fpps-ssl1-ssl3*	The *fpps-ssl1-ssl3* gene fragment was ligated in NdeI and HindIII site of pKU492Acos::*fur1p*	This study
	Promoter construct initiated by RNA-seq analysis	
pTYM19::*rvr3291p-fur22*	*The promoter region (500 bp) obtained from *rvr3291* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr3991p-fur22*	*The promoter region (500 bp) obtained from *rvr3991* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr5758p-fur22*	*The promoter region (493 bp) obtained from *rvr5758* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr6185p-fur22*	*The promoter region (406 bp) obtained from *rvr6185* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr6204p-fur22*	*The promoter region (486 bp) obtained from *rvr6204* gene was inserted in the upstream of *fur22* gene.	This study
	Promoter construct initiated by 2-D gel electrophoresis analysis	
pTYM19::*rvr1215p-fur22*	*The promoter region (300 bp) obtained from *rvr1215* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr1226p-fur22*	*The promoter region (348 bp) obtained from *rvr1226* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr2028p-fur22*	*The promoter region (519 bp) obtained from *rvr2028* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr2030p-fur22*	*The promoter region (250 bp) obtained from *rvr2030* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr2133p-fur22*	*The promoter region (500 bp) obtained from *rvr2133* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr2353p-fur22*	*The promoter region (400 bp) obtained from *rvr2353* gene was inserted in the upstream of *fur22 gene*.	This study
pTYM19::*rvr2436p-fur22*	*The promoter region (500 bp) obtained from *rvr2436* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr3032p-fur22*	*The promoter region (405 bp) obtained from *rvr3032* gene was inserted in the upstream of *fur22 gene*.	This study
pTYM19::*rvr3087p-fur22*	*The promoter region (478 bp) obtained from *rvr3087* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr3168p-fur22*	*The promoter region (500 bp) obtained from *rvr3168* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr3493p-fur22*	*The promoter region (495 bp) obtained from *rvr3493* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr3650p-fur22*	*The promoter region (370 bp) obtained from *rvr3650* gene was inserted in the upstream of *fur22* gene.	This study

pTYM19::*rvr4050p-fur22*	*The promoter region (399 bp) obtained from *rvr4050* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr4941p-fur22*	*The promoter region (349 bp) obtained from *rvr4941* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr6279p-fur22*	*The promoter region (250 bp) obtained from *rvr6279* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr6682p-fur22*	*The promoter region (398 bp) obtained from *rvr6682* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr7845p-fur22*	*The promoter region (343 bp) obtained from *rvr7845* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr7933p-fur22*	*The promoter region (500 bp) obtained from *rvr7933* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr7936p-fur22*	*The promoter region (500 bp) obtained from *rvr7936* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr7941p-fur22*	*The promoter region (398 bp) obtained from *rvr7941* gene was inserted in the upstream of *fur22* gene.	This study
pTYM19::*rvr9107p-fur22*	*The promoter region (249 bp) obtained from *rvr9107* gene was inserted in the upstream of *fur22* gene.	This study

The putative transcriptional regulator *rvr4129* gene exists in the upstream of *fur22* gene. The gene expression by *aphII* promoter does not enhance FQ production.
*The gene was inserted into EcoRI and BamHI sites.

1 **Table S3. The primers used in this study**

Primers used for genes amplification		
Target gene or vectors	Primer	Comment
fur1	Fur1 Hind-F: 5´-CCCAAGCTTCCCGATCACGTCCA-3´ Fur1 Hind-R: 5´- CCCAAGCTTCGGTGCTGTGGAAG-3´	*fur1* gene was amplified from fosmid clone PCC1FOS-16C11
fur1p	Eco-Pfur1-F :5´- CCGGAATTCGAGGGTCTGGACGGATCGGTT-3´ Nde-Pfur1-R :5´-GGAATTCCATATGCGGGGTCCACTTCCTCAGAGT -3´	765 bp fragment of *fur1* promoter (*fur1p*) was amplified from fosmid clone PCC1FOS-16C11
Primers used for the target gene inactivation by λ-Red system		
aphII	Fur1-For-P4 : 5´-AGGCTATGCAAGCCGGCGATGCGTGCGCCCGAACACGTCATCA CGCTGGAAATTCCGGGGATCCGTCGACC-3´ Fur1-Rev-P1: 5´-AGCTGTGGCGGAACACGTTGCGGTCCACTTCGAGGAACTTCGC CAGGTCGTTGTAGGCTGGAGCTGCTTC-3´	*aphII* gene was amplified from pKD13 having flanking region of 51 bp homology with target gene on both sides.
Primers used for confirmation of gene deletion		
Wild-type *fur1* and Δ*fur1*	F1: 5´-CCCGTTGACGACGTGGTCGACCG-3´ R1: 5´-GGGCGATGTGGGAGTACAGCGCG-3 F2: 5´-TCGACCGCCGTCCGCCGGTT-3´ R2: 5´-TCCCCCAAGGGCGACCGGGT-3´	
Primers used for the evaluation of promoters		
Target gene	Primer sequence	
rvr1215	rvr1215-F: 5´-CCGGAATTCGACCTCGGGGTGCCCGACGA-3´ rvr1215-R: 5´-CGCGGATCCGGTTGCTCTCCGTTCCAGTCT-3´	
rvr1226	rvr1226-F: 5´-CCGGAATTCGGCCGCGCCGGATCGGCGGT-3´ rvr1226-R: 5´-CGCGGATCCTCGTCCGCCTCCCGACCGCC-3´	
rvr2028	rvr2028-F: 5´- CCGGAATTCAGGTAGCCGCCGACCACGGTCGCCA-3´ rvr2028-R: 5´- CGCGGATCCTGGGTCAGGGCTCCTCTCCACAT-3´	
rvr2030	rvr2030-F: 5´-CCGGAATTCAGAGCGTGCTGCCGTCCACG-3´ rvr2030-R: 5´-CGCGGATCCTGGGATCTGCATCTCCTGAAATG-3´	
rvr2133	rvr2133-F: 5´-CCGGAATTCTTGGAGCGGTCGGCGCCCC-3´ rvr2133-R: 5´-CGCGGATCCAAGCGGTCCTTTCCCCTGGCT-3´	
rvr2353	rvr2353-F: 5´-CCGGAATTCACCTGGACATCGCGGGCCCG-3´ rvr2353-R: 5´-CGCGGATCCGTCACGTCCTCCATGCATGGG-3´	
rvr2436	rvr2436-F: 5´-CCGGAATTCGGAGATGATCCAGGAAGTGC-3´ rvr2436-R: 5´-CGCGGATCCTACGCGGATTCCTTGTCTGATGT-3´	
rvr3032	rvr3032-F: 5´-CCGGAATTCAAGTGCCGTGCGAGGTGGGC-3´ rvr3032-R: 5´-CGCGGATCCTGGCGGTGCTCCTTCAAGCTC-3´	
rvr3087	rvr3087-F: 5´-CCGGAATTCGCCACCCGCACCCCCTG-3´ rvr3087-R: 5´-CGCGGATCCGCCAGTGCCGCGCGCGTTCT-3´	
rvr3168	rvr3168-F: 5´-CCGGAATTCAGCGGGCGACGGGAATCGAA-3´ rvr3168-R: 5´-CGCGGATCCGGTTGGGTCTCCTTGGGGG-3´	
rvr3291	rvr3291-F: 5´-CCGGAATTCGGGCGCCAACGGCGTCTTCA-3´ rvr3291-R: 5´-CGCGGATCCGTCATTCCCTCACCGACGGT-3´	
rvr3493	rvr3493-F: 5´-CCGGAATTCACGCAGCGGCTGACGGTGCT-3´ rvr3493-R: 5´-CGCGGATCCTGACGACCTTCTTCCGGTTCC-3´	
rvr3991	rvr3991-F: 5´-CCGGAATTCGCTACGGCTTCATCGCGGT-3´ rvr3991-R: 5´-CGCGGATCCGGTGAAGTGTCCTCCGCGGA-3´	
rvr3650	rvr3650-F: 5´-CCGGAATTCACCAGCATCCCCAAGGGCGA-3´ rvr3650-R: 5´-CGCGGATCCGTTGTCTCCTTCTGAGATGTCT-3´	
rvr4050	rvr4050-F: 5´-CCGGAATTCACCATCTGTCCGCCCAATT-3´ rvr4050-R: 5´-CGCGGATCCGCACGCCCCTTCCTCT-3´	
rvr4129	rvr4129-F: 5´-CGCGGATCCGTGGAGTTCAGAGCCCTGGGC-3´ rvr4129-R: 5´-CCCAAGCTTTCACCCGGGGTCGCCGGGCT-3´	
rvr4941	rvr4941-F: 5´-CCGGAATTCGCGCGCTCCAGCCGGCGT-3´ rvr4941-R: 5´-CGCGGATCCACCCTACGAGGGTCGCCGCA-3´	
rvr5758	rvr5758-F: 5´-CCGGAATTCGACAGCGCCGCGACGGAT-3´ rvr5758-R: 5´-CGCGGATCCGTGACAGGTACCCCTTCCGT-3´	
rvr6185	rvr6185-F: 5´-CCGGAATTCGTGCGCGGACCGGTACTCCGACCA-3´ rvr6185-R: 5´-CGCGGATCCGAGTCTCGGCCTCCTTCCGGGTGA-3´	

rvr6204	rvr6204-F: 5′-CCGGAATTCTGTCCGTTCCCAGGCCGCGGGAT-3′ rvr6204-R: 5′-CGCGGATCCGGCTACTCCGTAGTCCTTGTCTCT-3′
rvr6279	rvr6279-F: 5′-CCGGAATTCACGTCGCCGTCGGCGACGTC-3′ rvr6279-R: 5′-CGCGGATCCGCCTGCGAACTACCTCGTCTACT-3′
rvr6682	rvr6682-F: 5′-CCGGAATTCTGCCCAACATCTGGAGCACCAT-3′ rvr6682-R: 5′-CGCGGATCCTGAAATCCTGCCTGTCTGGACTG-3′
rvr7845	rvr7845-F: 5′-CCGGAATTCAGATCGGACTCCGTGCTGTG-3′ rvr7845-R: 5′-CGCGGATCCAGGCGCTGTTCCTTCCGTGA-3′
rvr7933	rvr7933-F: 5′-CCGGAATTCAAGGCTGATGTTCTGGTTGC-3′ rvr7933-R: 5′-CGCGGATCCGACAGTCTCCTTCATGCATGTT-3′
rvr7936	rvr7936-F: 5′-CCGGAATTCTGCTGCGCAAGTGAGTCCGGT-3′ rvr7936-R: 5′-CGCGGATCCTGCTCAAGTCCTCAGCCTTCTTC-3′
rvr7941	rvr7941-F: 5′-CCGGAATTCTGCAACCTGCTCAACCCGA-3′ rvr7941-R: 5′-CGCGGATCCTCTGAGGTTGCCTCCCTGACA-3′
rvr9107	rvr9107-F: 5′-CCGGAATTCGTACCCATCTGCCGCTCCCGC-3′ rvr9107-R: 5′-CGCGGATCCTGGCGGGGTCCTCCGGAGGT-3′

Primers used to check RNA-seq guided transformants

Expected amplification size of DNA	Template genomic DNA	Primer sequence (amplification between putative promoter and *fur22* gene)
rvr3291-fur22 (525 bp)	SR1-13	rvr3291-F: 5′-AATTCGGGCGCCAACGGCGTCTTCA -3′ fur22-F: 5′-GCAGCGCGCCAAGCAGCTGAAT -3′
rvr3991-fur22 (525 bp)	SR1-16	rvr3991-F: 5′-GAATTCGCTACGGCTTCATCGCGGT -3′ fur22-F: 5′-GCAGCGCGCCAAGCAGCTGAAT -3′
rvr5758-fur22 (518 bp)	SR1-19	rvr5758-F: 5′-GAATTCGACAGCGCCGCGACGGAT -3′ fur22-F: 5′-GCAGCGCGCCAAGCAGCTGAAT -3′
rvr6185-fur22 (431 bp)	SR1-20	rvr6185-F: 5′-CGTGCGCGGACCGGTACTCCGACCA-3′ fur22-F: 5′-GCAGCGCGCCAAGCAGCTGAAT -3′
rvr6204-fur22 (511 bp)	SR1-21	rvr6204-F: 5′-CTGTCCGTTCCCAGGCCGCGGGAT-3′ fur22-F: 5′-GCAGCGCGCCAAGCAGCTGAAT -3′

The underlines show homologous sequences to the target DNA region or restriction enzyme sites.

Table S4. Codon optimized *fpps-ssl1-ssl3* gene sequences for *Streptomyces reveromyceticus* SN-593.

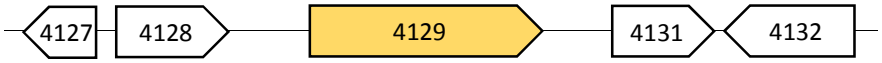
fur1p-fpps-ssl1-ssl3

GAATTCGAGGGTCTGGACGGATCGGTTCCGAAGTCCCGGGAGGAGCGCGGCCGTCGGCCGGGAGGAGCACGGGTC
CGCCCGGTCGGCGACCGCCCCGGGACGGTGCCGCCGTCCCCGCCTCCGGACGGGGCCGAGACGTTCGGCCGCCCG
GTGTGGCAGGTCGGGGACGACGTTAGGAGGCGCGCGTTCCCTGAACGTTCCCTCGCCTGGGGCCCGTTGGGCCCG
GTCGGTGCGGTGCTCGTCGAGCCGGGGTGTCCTCGGCCTCCACGGGGAGTCGAGTGTGACCCAGGTCACTCCATG
TAATGCTTGGCGCCAAGCCTGCTGCCGTGTCACTATTTCCGCACACCTTCCCTGCCCGAAAGCAGTCACCAGGCA
GCGGATCGACCGCCGTCCGCCGGTTCCCCGCACAGGCGGGACCCGCACGCAGAACTCCCGGCGATCCACAGCAGC
CGACTGCTCCCGCCGAGCAGCTCACGTCCCTTCCCGTTCAGGACAGGCGCCAATCGCCGTCGGCGGACTCCGTCC
GACCGGCCGCCCCAGCGAACATCTCCCGTGCCCCGGCCCCGCGGCCCGCCGCCGTCCCTCTCCCCCTCGCGGGGG
GCAGGACGGCGGCGGGCCGCGCCCCGTTTCCGCCCGGCCGCCGAGCCGGCGGGCCTGCCGGCCGCCGTGAAGAAC
GCGTCAACGACCCCACCGAGGACGGCGGGTGAAGGATCCGCCGTCTGCGCGACGCCCCACTGCCCTTGCTGTCGA
GACTCTGAGGAAGTGGACCCCGCATATGATGCACAAGTTCACCGGCGTGAACGCCAAGTTCCAGCAGCCGGCCCT
GCGCAACCTGTCCCCGGTGGTGGTCGAGCGCGAGCGCGAGGAGTTCGTGGGCTTCTTCCCGCAGATCGTGCGCGA
CCTGACCGAGGACGGCATCGGCCACCCCGAGGTGGGCGACGCCGTGGCCCGCCTGAAGGAGGTGCTGCAGTACAA
CGCCCCGGGCGGCAAGTGCAACCGCGGCCTGACCGTGGTGGCCGCCTACCGCGAGCTGTCCGGCCCGGGCCAGAA
GGACGCCGAGTCCCTGCGCTGCGCCCTGGCCGTGGGCTGGTGCATCGAGCTGTTCCAGGCCTTCTTCCTGGTGGC
CGACGACATCATGGACCAGTCCCTGACCCGCCGCGGCCAGCTGTGCTGGTACAAGAAGGAGGGCGTCGGCCTGGA
CGCGATCAACGACTCCTTCCTGCTGGAGTCCTCCGTGTACCGCGTGCTGAAGAAGTACTGCCGCCAGCGCCCGTA
CTACGTGCACCTCCTGGAGCTGTTCCTCCAGACCGCCTACCAGACCGAGCTGGGCCAGATGCTGGACCTGATCAC
CGCCCCGGTGTCCAAGGTGGACCTGTCCCACTTCTCCGAGGAGCGCTACAAGGCCATCGTGAAGTACAAGACCGC
CTTCTACTCCTTCTACCTGCCGGTGGCCGCCGCCATGTACATGGTGGGCATCGACTCCAAGGAGGAGCACGAGAA
CGCCAAGGCCATCCTGCTGGAGATGGGCGAGTACTTCCAGATCCAGGACGACTACCTGGACTGCTTCGGCGACCC
GGCCCTGACCGGCAAGGTGGGCACCGACATCCAGGACAACAAGTGCTCCTGGCTGGTCGTGCAGTGCCTGCAGCG
CGTGACCCCCGAGCAGCGCCAGCTGCTGGAGGACAACTACGGCCGCAAGGAGCCCGAGAAGGTCGCCAAGGTGAA
GGAGCTGTACGAGGCCGTCGGCATGCGCGCCGCCTTCCAGCAGTACGAGGAGTCCTCCTACCGCCGCCTGCAGGA
GCTGATCGAGAAGCACTCCAACCGCCTGCCGAAGGAGATCTTCCTGGGCCTGGCGCAGAAGATCTACAAGCGCCA
GAAGTGAACCACACGCGCCAACAACGGAAGCGGAGTCC**ATG**ACCATGCACCAGGACCACGGCGTGATGAAGGACC
TGGTGAAGCACCCGAACGAGTTCCCGTACCTGCTGCAGCTGGCCGCCACCACCTACGGCTCCCCGGCCGCCCCGA
TCCCGAAGGAGCCGGACCGCGCCTTCTGCTACAACACCCTGCACACCGTGTCCAAGGGCTTCCCGCGCTTCGTGA
TGCGCCTGCCGCAGGAGCTGCAGGACCCGATCTGCATCTTCTACCTGCTGCTGCGCGCCCTGGACACCGTCGAGG
ACGACATGAACCTGAAGTCCGAGACCAAGATCTCCCTGCTGCGCGTGTTCCACGAGCACTGCTCCGACCGCAACT
GGTCGATGAAGTCCGACTACGGCATCTACGCCGACCTGATGGAGCGCTTCCCGCTGGTCGTGTCCGTGCTGGAGA
AGCTGCCGCCGGCCACCCAGCAGACCTTCCGCGAGAACGTGAAGTACATGGGCAACGGCATGGCCGACTTCATCG
ACAAGCAGATCCTGACCGTGGACGAGTACGACCTGTACTGCCACTACGTGGCCGGCTCGTGCGGGATCGCCGTGA
CCAAGGTGATCGTGCAGTTCAACCTGGCCACCCCCGAGGCCGACTCCTACGACTTCTCCAACTCCCTGGGCCTGC
TGCTGCAGAAGGCCAACATCATCACCGACTACAACGAGGACATCAACGAGGAGCCGCGCCCGCGCATGTTCTGGC
CGCAGGAGATCTGGGGCAAGTACGCCGAGAAGCTGGCGGACTTCAACGAGCCCGAGAACATCGACACCGCCGTGA
AGTGCCTGAACCACATGGTGACCGACGCCATGCGCCACATCGAGCCGTCCCTGAAGGGCATGGTGTACTTCACCG
ACAAGACCGTGTTCCGCGCCCTGGCGCTGCTGCTGGTGACCGCCTTCGGCCACCTGTCCACCCTGTACAACAACC
CGAACGTGTTCAAGGAGAAGGTCCGCCAGCGCAAGGGCCGGATCGCCCGCCTGGTGATGTCCTCCCGCAACGTGC
CGGGCCTGTTCCGGACCTGCCTGAAGCTGGCCAACAACTTCGAGTCCCGCTGCAAGCAGGAGACCGCCAACGACC
CGACCGTGGCCATGACCATCAAGCGCCTGCAGTCCATCCAGGCGACCTGCCGCGACGGCCTGGCCAAGTACGACA
CCCCGTCCGGCCTGAAGTCCTTCTGCGCCGCGCCGACCCCGACCAAG**GGCGGCTCCGGCGGCGGCAGCGGCGGCG**
GCTCGGGCATGAAGCTGCGCGAGGTCCTGCAGCACCCGGGCGAGATCATCCCGCTGCTGCAGATGATGGTGATGG
CCTACCGCCGCAAGCGCAAGCCGCAGGACCCGAACCTGGCCTGGTGCTGGGAGACCCTGATCAAGGTGTCCCGCT
CCTACGTGCTGGTGATCCAGCAGCTGCCCGAGGTGCTCCAGGACCCCATCTGCGTGAACTACCTGGTCCTGCGGG
GCCTCGACACCCTGCAGGACGACATGGCCATCCCGGCCGAGAAGCGCGTGCCCCTGCTGCTGGACTACTACAACC
ACATCGGCGACATCACCTGGAAGCCGCCGTGCGGCTACGGCCAGTACGTCGAGCTGATCGAGGAGTACCCGCGCG
TGACCAAGGAGTTCCTGAAGCTGAACAAGCAGGACCAGCAGTTCATCACCGACATGTGCATGCGCCTGGGCGCCG
AGATGACCGTGTTCCTGAAGCGCGACGTGCTGACCGTGCCGGACCTGGACCTGTACGCCTTCACCAACAACGGCC
CGGTGGCCATCTGCCTGACCAAGCTGTGGGTGGACCGCAAGTTCGCCGACCCGAAGCTGCTGGACCGCGAGGACC
TGTCCGGCCACATGGCCATGTTCCTCGGCAAGATCAACGTGATCCGCGACATCAAGGAGGACGTCCTGGAGGACC
CGCCGCGCATCTGGTGGCCCAAGGAGATCTGGGGGAAGTACCTGAAGGACCTGCGCGACATCATCAAGCCCGAGT
ACCAGAAGGAGGCCCTGGCCTGCCTGAACGACATCCTCACCGACGCGCTCCGGCACATCGAGCCCTGCCTGCAGT
ACATGGAGATGGTGTGGGACGAGGGCGTGTTCAAGTTCTGCGCCGTGCCCGAGCTGATGTCCCTGGCCACCATCT
CCGTGTGCTACAACAACCCCAAGGTGTTCACCGGCGTGGTGAAGATGCGCCGCGGCGAGACCGCCAAGCTGTTCC
TGTCCGTGACCAACATGCCGGCCCTGTACAAGTCCTTCTCCGCGATCGCCGAGGAGATGGAGGCCAAGTGCGTGC
GCGAGGACCCGAACTTCGCCCTGACCGTGAAGCGGCTGCAGGACGTGCAGGCCCTGTGCAAGGCCGGCCTGGCGA
AGTCCAACGGCAAGGTGTCCGCCAAGGGCGCC**TGA**AAGCTT

The underlines show restriction enzyme sites. The sequences indicate the promoter of *fur1* gene promoter from *S. reveromyceticus* SN-593 (orange), codon optimized FPP synthase gene (*fpps*) from chicken (blue), and codon optimized SSL-1 and SSL-3 fusion synthase gene (*ssl1-ssl3*) from *Botryococcus* race B (purple). The shade sequence between *fur1* and *fur2* gene organization found in FQ gene cluster was adopted to assure transcription of *ssl-1*and *ssl-3* gene. The peptide fusions of SSL-1 and SSL-3 was connected by a triplet repeat linker of GGSG (bold letter) [9].

Table S5. Gene expression profiles and gene organization for promoter screening.

DNA sequence of *rvr4129* gene used as negative control



rvr4129

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AATCTCCGCAGGGTGCTGGAGCCGGGCCGGGCGCCCAGGACGCCCTCGACGGTGCTGCGCACCACCGACCGGGGCTACCAGTTGGACGCCCGGG
CGGTCGAGGTGGACGTCAACCGCTTCGAGCAGCACGCCACTTCGGGATGGCAGGCGTGGAACCGGGGCGAACCGCACCAGGCGCTCGGCGAGTT
CGAGGCCGGACTGGCGTGGTGGCGCGGCGAGGCGTACACGGAGGTGTCCCAGTCCGTCCATGTGGCGGCGGAGGTGACACGGCTGGAGGAGTTG
CGGCTGTCCGTGGTGGAGGGGCGCTGCGCGGCGCTGCTGGCCGTGGGGGCCCACGAGATGGCGGCCGCCGAACTGGAGGGCTTCATGCAGGCCC
ATCCGCTGCGCGAGTACGGCTGCGAGCTCCTCAGCCTGGCGCTGTACCGGTCCGGGCGGCAGGCCGACGCGCTGGCGGTGCTCCGCACCAACCA
GAGGCGCCTGGGAGAGGAACTGGGCATCGACCCCGTACCGGCGCTGCAACACCTCGAACGGGAGATCCTGAGCCACACCCCGGCGCTGGACTGG
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CCGTGGAGGAGGACGCCTTCGTCGGCCGCGAGGACGCGCTCCGGCAGTTGGACGCGGCGACGGCGGCGGCGGCGCTCGGGCGGGGACAGGTGGT
GACCGTGTCCGGTGAACCCGGCATCGGCAAGACCAGCCTGCTGCGGCGCCTGACCTCGGCGGCGGGCGCGCCGGTGCTGTGGGGTTCCTACCCG
GCCCACGTCGCCACGCCGCCGTTGTGGCCCTGGCGGCAGGTGCTGGACATGGCGGGTGCCCACGGCCCGCGGCGCCCCGTCCCGAGCGCCGTCG
CCGAAGTGCTCAACGGGGAGATCCCGCTCCCGCGGGACGGCGCGGACCCGGGCGCCGCCACCTCGCGGCGGTTGGAGGTGATCGCGGACTACCT
GATCGGCGCGGCCGAGGTCACGCCGCTGGTGATCGTCCTCGACGGGCTGCACCAGGCGGACCCGGGGTCGCTGCGGCTGCTGGCGCGCCTGACC
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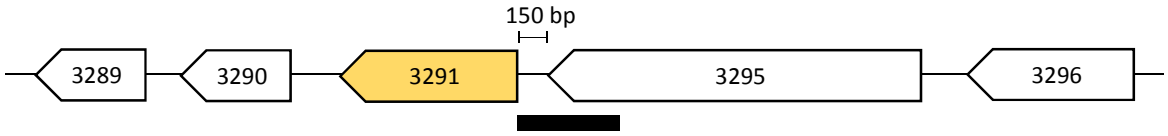
CGGAGACGACGCGCGTAGAGCTGGGCGGCCTGAGCGACGCGGAGACCCGGGCTCTGGCGAGCGCGCTGCTCCGCCGCGAGGTGAGCGCGGCCAC
CGCCCGGCAGTTACGGGGCCGCACCGGCGGCAACCCGTTCTACCTGCGCGAACTCGTCAAGGTACTCGACGGCCAGGACGACCCGGGCCGGGCC
CGCACGGCACCGGTGCCCGGGCCGGTGCGCGAGATGGTGCTCGGCCGCGTCGCACGACTCGCCCCGGCCGCGGGCGAGTTGCTGTCCGTCGCCG
CCGTCTCCGGTCCCGGCTTCACCGTCGACGTCGTCGCGGAGGTCTCCTCGACCGAGTACGGGCGGGCGCTCGGGATCGTCGGCACCCTGGTCGC
CGCGGGCCTGGTACGCGAGGACCAGCAGCGGCCGGGCTGGTACTCCTTCGTCCACGGCCTGACCGCCGACGTGCTGTTCCACGAGATGGGGCGG
CTGCGCCGGGTCCACCTGCTGCGCCGGGCCCGCACCGCGGCGGCCCGGCCGAGGAAGGCGCGGGAGCCCGGCGACCCCGGGTGA

Promoter selected from RNA-seq analysis

rvr3291

Day	Gene ID	3291	3295	3296
		Median normalized RPKM		
1		321	178	105
2		179	152	116
3		3282	175	125



The *rvr3291* gene has the gap of 150 bp after the upstream gene. Based on the RNA seq data at day3, the *rvr3291* mRNA level was dramatically increased. However, the mRNA for the upstream *rvr3295* gene was only modestly represented. This suggested that transcription of the *rvr3291* gene was not controlled by the promoter of the upstream *rvr3295* gene.

GGGCGCCAACGGCGTCTTCAACGGCACCGTGGGCGTGGTGACCGCCCTCGACGCGGTCGAGCAGAAGCTCACGGTGCGGACTGACGAGGACGAG
GAAGTCGGATACGACTTCGACGAACTGGACGAGCTGACCCACGCCTACGCCGTCACGATCCACAGATCGCAGGGCAGCGAGTACCCGGCGGTGG
TGATCCCCGTCACTACCGGGGCTTGGATGATGCTCCAACGCAACCTGCTTTACACCGCGGTCACGAGGGCGAAACGCCTGGTCGTGCTGGTGGG
GTCGCGTCGGGCGATCGGGCAGGCCGTGCGGACGGTCTCCGCGGGTCGTCGCTTCACGGCTCTCGACCATCGGCTTGATCTGGGCGCAAAATAG
GCACTCCGTGCCACTTTTCGGCCCGATGGCCGACCCCGAGTGCACACCCCAGTGCCAAATGGGGGACAGTGGACGTAGTCAGGGCACCTCGAAG
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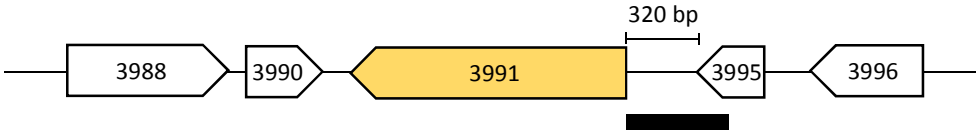
CAAGTCCGCGATCACCTTCCTCGACGGTGAGCAGGGCATCCTGCGTTACCGCGGGTATCCCATCGAGCAGATCGCCGAGCGCGGCTCCTTCCTG
GAGACCGCGTACCTGCTGATCAACGGCGAGCTGCCGACCGTCGACCAGCTCTCCGCGTTCCAGGGGCAGATCACCCAGCACACGCTGCTGCACG
AGGACGTCAAACGGTTCTACGACGGCTTCCCCCGGGACGCCCACCCGATGGCGATGCTCTCCTCGGTGGTCAGCGCGCTGTCGACGTTCTACCA
GGACAGCCACAACCCCTTCGACCCCGAGCAGCGCGACCTGTCCACGATCCGGCTGCTCGCCAAGCTGCCGACGATCGCGGCGTACGCGTACAAG
AAGTCCGTCGGCCAGCCGGTGGTCTACCCGCGCAACGACCTCGGCTACGTCGAGAACTTCCTGCGGATGACCTTCTCGGTCCCGGCCGAGGAGT
ACGAACTCGACCCGGTCGTGGTCAACGCGCTGGACAAGCTGCTCATCCTGCACGCCGACCACGAGCAGAACTGTTCGACGTCCACGGTCCGGCT
GGTCGGCTCCTCGCAGGCCAACCTGTTCGCGTCGATCTCCGCCGGCATCAGCGCGCTGTGGGGGCCGCTGCACGGTGGCGCGAACCAGTCGGTG
CTGGAGATGCTGGAGCGCATCAAGGCCGAGGGCGGCGACGTCGACTCCTTCATCCGCCGGGTGAAGAACAAGGAAGACGGCGTGAAGCTCATGG
GCTTCGGGCACCGCGTCTACAAGAACTTCGACCCGCGGGCGAAGATCATCAAGGCGGCGGCGCACGACGTCCTCTCGGCGCTCGGCAAGTCGGA
CGAGCTGCTGGACATCGCGCTCAAGCTGGAGGAGCACGCGCTCAGCGACGACTACTTCGTCTCGCGCAAGCTCTACCCGAACGTGGACTTCTAC
ACCGGGCTGATCTACCGGGCGATGGGCTTCCCGACCAGCATGTTCACCGTGCTGTTCGCGCTCGGCCGGCTGCCCGGCTGGATCGCCCAGTGGC
ACGAGATGATCAAGGAGCCCGGCTCCCGCATCGGTCGGCCGCGGCAGATCTACACCGGCGTCGTCACCCGCGACTACGTCCCGGTCGAAGCCCG

CTGA

rvr3991

Day	Gene ID	3991	3995	3996
		Median normalized RPKM		
1		871	142	64
2		846	536	43
3		714	5139	39

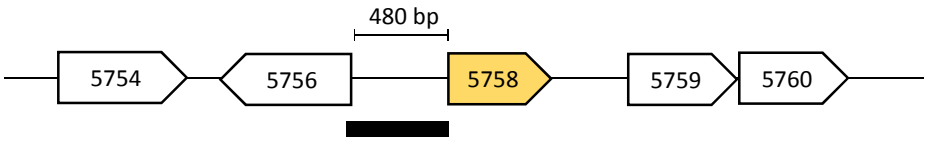


The *rvr3991* gene has a 320 bp gap after the upstream *rvr3995* gene. Based on RNA-seq data, these genes do not appear to be coordinately regulated, suggesting that the *rvr3991* gene is controlled independently.

GCTACGGCTTCATCGCGGTCGACGGTGGTGCGGATGTCTTCGTCCACTACAGCGCGATCCAGATGGACGGGTACCGCACCCTCGAAGAAGGCCA
GCGGGTCGAGTTCGAGATCTCGCAGGGTCAGAAGGGGCCGCAGGCGGACATGGTCCGTCTCGCCGGCTGACTTCACGGCGCGTTCGACCATCGA
CGCGACCTTTCACACGGAGGAGCCGCACCCCGCCCGGGGTGCGGCTCCCTCCTTTTCCGGCGCCCCGCCGGGGCGCCCCCGACCTGCGGGGCAA
CCCTGTTCACGACCGGACCCCCGGAGCGAACCGCTTGCACTCGCAGGGGGTGAGTGCTAATCATTGGCGTTAGCACTCTCCGCGTGAGAGTGAC
AGCAGGGCCGGGTCGGTGAGGGCCGTGCGGACGTCGGGAAGGAACCGGCGGCCGGCGGGCCGTCCGTCGCGGGCACCACACGGTCCCGAGCGCA
AACAACCCAGTCCGCGGAGGACACTTCACCATGGCCAAGATCATCGCGTTCGACGAGGAGGCACGGCGCGGCCTTGAGCGCGGGATGAACCAGC
TCGCCGACGCCGTCAAGGTCACCCTGGGCCCCAAGGGCCGGAACGTGGTCCTCGAGAAGAAGTGGGGCGCTCCCACGATCACCAACGATGGTGT
GTCCATCGCCAAGGAGATCGAACTCGAGGACCCGTACGAGAAGATCGGCGCCGAGCTGGTCAAGGAAGTCGCGAAGAAGACCGACGACGTCGCC
GGTGACGGCACGACGACCGCGACCGTCCTGGCCCAGGCCCTGGTCAAGGAGGGCCTGCGCAACGTCGCCGCCGGCGCCAACCCGATGGCCCTCA
AGCGCGGGATCGAGAAGGCCGTCGAGGCCGTCTCCGCCGCGCTGCTGGAGCAGGCGAAGGACGTGGAGACCAAGGAGCAGATCGCCTCCACCGC
CTCCATCTCCGCCGCCGACACCCAGATCGGCGAGCTGATCGCCGAGGCCATGGACAAGGTCGGCAAGGAAGGCGTCATCACCGTCGAGGAGTCG
CAGACCTTCGGGCTCGAACTCGAGCTCACCGAGGGCATGCGCTTCGACAAGGGCTTCATCTCCGCCTACTTCGCCACCGACCTGGAGCGGATGG
AGGCGTCGCTGGACGACCCCTACATCCTGATCGTCAACTCCAAGGTCTCCGCGGTGAAGGACCTGCTTCCGCTGCTGGAGAAGGTCATGCAGTC
GGGCAAGCCGCTGCTGATCATCGCCGAGGACGTCGAGGGCGAGGCCCTGTCGACCCTGGTGGTCAACAAGATCCGCGGCACCTTCAAGTCCGTC
GCCGTCAAGGCCCCGGGCTTCGGTGACCGCCGCAAGGCCATGCTCGGCGACATCGCCATCCTCACCGGTGGCACCGTCATCTCCGAGGAGGTCG
GCCTCAAGCTGGAGAACGCCGGTCTCGACCTGCTCGGCCGCGCCCGCAAGGTCGTCATCACCAAGGACGAGACCACCATCGTCGACGGTGCCGG
CGACACCGAGCAGGTCGGCGGCCGGGTCAACCAGATCCGTGCCGAGATCGAGAACAGCGACTCGGACTACGACCGCGAGAAGCTCCAGGAGCGC
CTGGCGAAGCTGGCCGGCGGCGTGGCCGTCATCAAGGCCGGTGCCGCCACCGAGGTCGAGCTCAAGGAGCGCAAGCACCGCATCGAGGACGCGG
TGCGCAACGCCAAGGCCGCCGTCGAGGAGGGCATCGTCGCCGGTGGCGGCGTGGCCCTGCTCCAGGCGACCGCCGTCTTCGAGAAGCTGGAGCT
GGAGGGCGACGAGGCGACCGGTGCGGCCGCCGTCAAGATCGCCCTGGAGGCCCCGCTGAAGCAGATCGCGGTCAACGCCGGCCTTGAGGGCGGT
GTCGTGGTGGAGAAGGTGCGCAGCCTCACCCCCGGCCACGGCCTCAACGCGGCGAGCGGCGAGTACGTCGACCTGGTCGCCGAGGGCATCATCG
ACCCGGCCAAGGTGACGCGCTCCGCGCTGCAGAACGCGGCCTCCATCGCCGCGCTCTTCCTCACCACCGAGGCGGTCATCGCCGACAAGCCCGA
GAAGGCGGCCCCCGCCGCTCCGGGTGGCATGCCCGGCGGTGACATGGACTTCTGA

rvr5758

Day	Gene ID	5756	5758
		Median normalized RPKM	
1		220	1162
2		134	391
3		199	4149



Because the gene upstream of the *rvr5758* gene is oriented in the opposite direction, the *rvr5758* gene must harbor its own promoter element.

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TGCTCTCGGCCGCGCACCGGCCCCGCCATTAGGGCGGGTTGATTAGCGACGCGCCCGGGGCGGGGCCGGTGCGCCTTTTCCATGATCGGGCAAA
ATTCGATCTTCGGAGGGCCGGTAACCGGCCATCGGCACGAACGGCGTAGCGAGGACGCACTCCTGCAGGAGGGACGAATCGGGACGTATCGCCC
CGTGTTCGGGGCCGCCGGGGGTCGTAACGGGCACGTTTCGCCCCCGGCGCATACCCGCTCCGACCTGCGGATACCCCGCCCCGGAGGTCCCCTC
CAGCACGTTCCGGGGGCGCTTGTCAAGCCCCGAGATATGCCCTGACCTGCGGAAACGCCATTCAGAAGACGCCGTTCCCGTGTTACCCTGGATA
GCCACGGAAGGGGTACCTGTCACATGACGTTCAAGGTTGGCGACACCGTGGTCTATCCCCATCACGGGGCCGCGCTGATCGAAGCGATCGAAAT
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GTCGGCGTGCGCGACGTGGTCGGTCAGGACGGTTTGGACCGGGTCTTCGAGGTGCTGCGCGCGCCGTACACCGAGGAGCCCACCAACTGGTCCC
GCCGCTACAAGGCGAATCTCGAGAAGCTCGCGTCCGGCGACGTGATCAAGGTCGCGGAAGTCGTGCGCGACCTGTGGCGCCGCGAGCGCGAGCG
CGGCCTGTCGGCGGGTGAGAAGCGCATGCTCGCCAAGGCGCGACAGATCCTGGTCAGCGAACTGGCACTGGCCGAGAACACCAACGAGGACAAG
GCCGAGACGCTGCTCGACGAGGTCCTCGCGTCGTAA

rvr6185

Day	Gene ID	6184	6185
		Median normalized RPKM	
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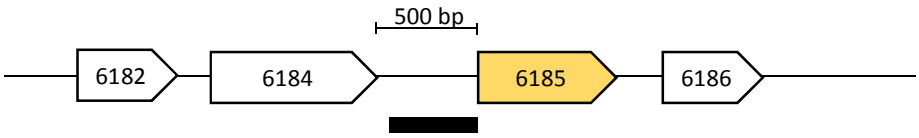
2		129	1557
3		127	25348

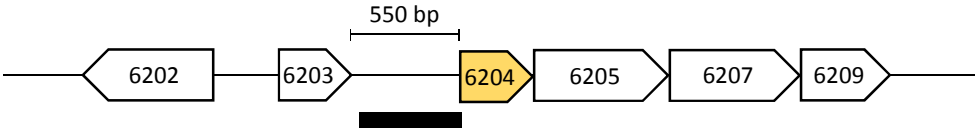


Similar to the rationale give for the *rvr3291* promoter, the *rvr6185* and *rvr6204* genes have relatively large gaps (500 bp and 550 bp) between themselves and their respective upstream genes, *rvr6184* and *rvr6203*. Based on RNA-seq data, the *rvr6184* and *rvr6203* genes appear to be weakly expressed. Therefore, we designed the gap sequences upstream of the *rvr6185* and *rvr6204* genes as putative promoters.

```
GTGCGCGGACCGGTACTCCGACCACGGTCTCAAGGTCCGTGTCACGGCGCCGCCGGCCTCGCAGACCACCGGCTTCTCCCGCCTGCTCGACCCG
ACCTCGTAGGCCGCCGGTCCGGCGCACCCCGGAGGCGATTTGCTCTGGGGCGGCATGTGCCCGTACCTTTACATCGAAGCCAAAGACCGCAGGT
TGTCGCCGTGCCCGGGAACGGGTGGGGCGGCCGAAGGATTCCGCTCGGTGCGGACGGCCCGCGTAGGTGATCGGAAGAAGATCTCCGGGAGTGC
GTCAAGTCCTCTCGTCGAGTTCCGCCCCGTGCGCCTGCGCCCGGGGCGTTTGTGTTCGTCGCGCCAGTCAAGTCCCCTTCGTCAACCGGGCGGT
CCGCAATCACCCGGAAGGAGGCCGAGACTCATGGCGAGGCCCGACAAGGCTGCCGCGGTTGCCGAGTTGACGGACAAGTTCCGCACCTCGAACG
CCGCGATGCTGACCGAGTACCGCGGTCTCACCGTGGCGCAGCTCAAGACGCTGCGCCGTTCGCTCGGTGAGAACGCCACGTACGCCGTGGTGAA
GAACACGCTGACCAAGATCGCGGCCAACGAGGCCGGGATCGACACGCTCGACGACCTGTTCGCAGGTCCGTCGGCCGTCGCCTTCGTGACCGGT
GACCCGGTCGAGGCGGCGAAGGGTCTGCGCGACTTCGCCAAGGACAACCCCGCTCTCGTCATCAAGGGCGGTGTCCTTGACGGCAAGGCGCTGT
CCGCCGACGAGATCAAGAAGCTTGCGGACCTCGAGTCCCGCGAGGTTCTGCTCGCCAAGCTGGCGGGCGCCATGAAGGCCAAGCAGTCCCAGGC
TGCCGCGCTCTTCCAGGCGCTCCCGTCGAAGTTCGTCCGCACCGCGGAGGCACTTCGGGCCAAGAAGGCCGAGGCGGAGCAGGGCGGTGCCGAG
TAA
```

rvr6204

Day	Gene ID	6203	6204
		Median normalized RPKM	
1		43	2102
2		17	472
3		16	3909

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TGGGACGGACGTACGGGAATCTTCCGGGAAGTGTCAGCGCGGCTCCGACCAGGCATCCGGTGGGTGGTATCTCCCACGCAAATCCCTGTCGCAG
GGCCTTTCCGCATGCGGAAAGGCATGTGCGAGGGAGGCGACACGCCCGACCGCGTGGGTCGGACAAGCGGATGCCAGAGCGTAAACAGAGACAA
GGACTACGGAGTAGCCATGGCGGGACAGAAGATCCGCATCCGGCTCAAGGCCTACGACCACGAGGTCATCGACTCCTCGGCGAAGAAGATCGTC
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ACAAGGACTCGCGCGAGCACTTCGAGATGCGCACGCACAAGCGCCTGATCGACATCCTCGACCCGACGCCCAAGACCGTTGACTCGTTGATGCG
CCTGGACCTGCCGGCCGGCGTCGACATCGAGATCAAGCTCTGA
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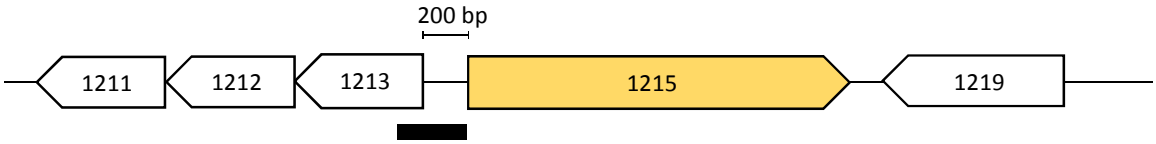
Promoter screening based on 2-D gel electrophoresis analysis

rvr1215



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TCCAGGCGTACGCCTCGACCGCCGGGGTCACCGTCGAGAGCCGGGACATCTCGCTGGCGGGTCGGATCATCGCCGGCTTCCCGGAGTACCTGGA
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ACCCTCGCGGTGCTGCCCGACAGCAGCTACGCGGGCGTCTACCAGGTCGTGCTGGACGACTGCCGCGCGCACGGCGCCTACGACCCGGCCACCA
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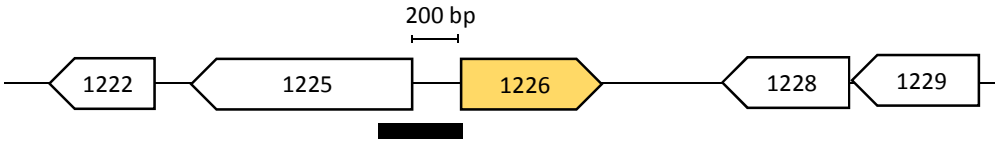
rvr1226



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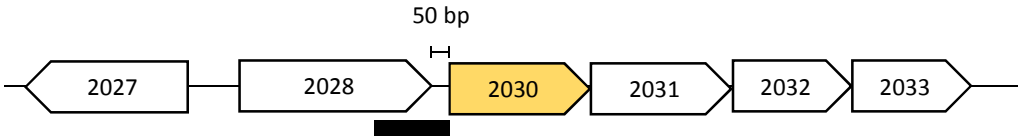
***rvr2030* Case I**

For *rvr2030* gene expression, a putative promoter region in the cording region of *RVR2028* gene was tested.

Day	Gene ID	2027	2028	2030
		Median normalized RPKM		
1		148	144	146

2	109	103	131
3	269	124	197



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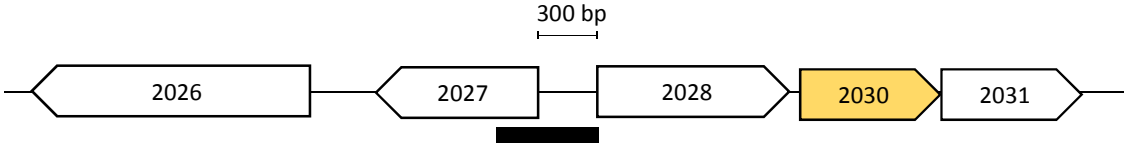
***rvr2030* Case II**

For *rvr2030* gene expression, a putative promoter region in the upstream of *RVR2028* gene was tested.

Day	Gene ID	2027	2028	2030
		Median normalized RPKM		
1		148	144	146
2		109	103	131
3		269	124	197



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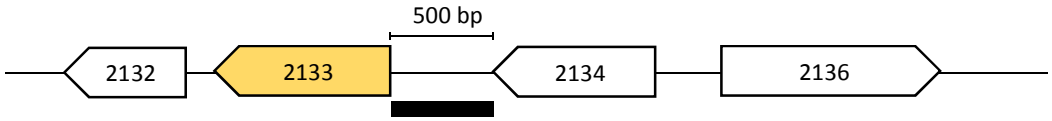
rvr2133

Day	Gene ID	2133	2134	2136
		Median normalized *RPKM		
1		1278	129	142
2		287	92	118
3		924	173	244



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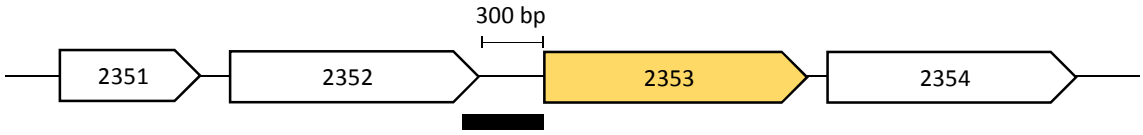
rvr2353

Day	Gene ID	2352	2353
		Median normalized RPKM	
1		134	904
2		115	168
3		65	1495



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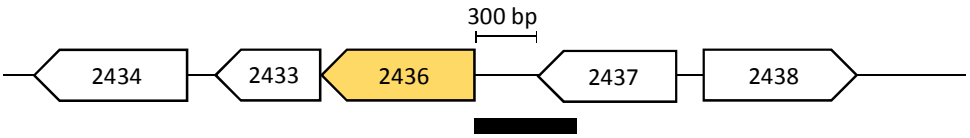
rvr2436

Day	Gene ID	2436	2437
		Median normalized RPKM	
1		210	106
2		131	89

3 274 147



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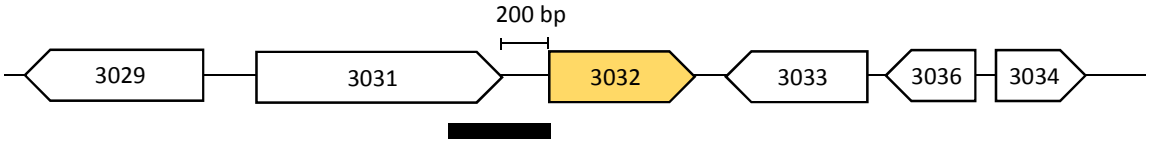
rvr3032

Day	Gene ID	3031	3032
		Median normalized RPKM	
1		96	198
2		132	214
3		85	435



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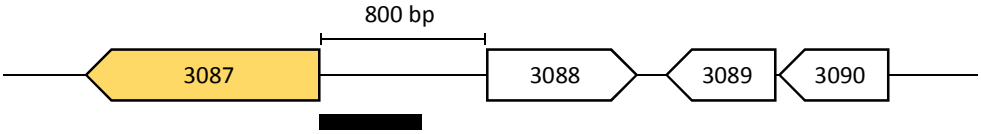
rvr3087



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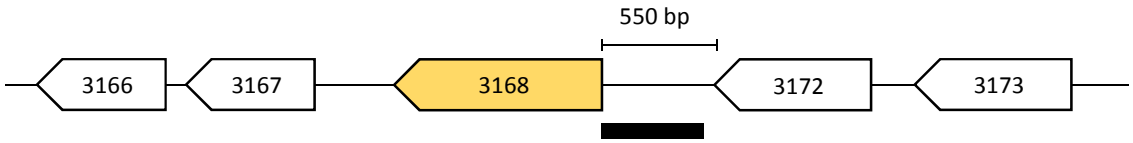
rvr3168

Day	Gene ID	3168	3172	3173
		Median normalized RPKM		
1		375	191	140
2		146	124	128
3		287	748	72



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rvr3493

Day	Gene ID	3493	3495	3497
		Median normalized RPKM		
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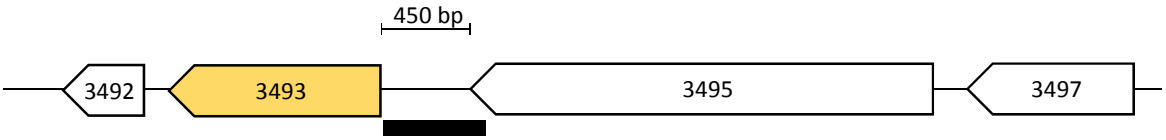
2		157	143	167
3		140	95	72



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rvr3650

Day	Gene ID	3650	3651	3653
		Median normalized *RPKM		
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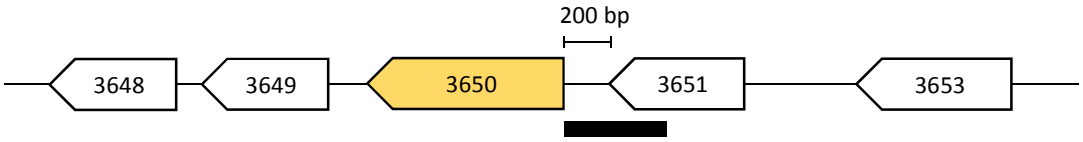
2		159	91	149
3		250	88	108



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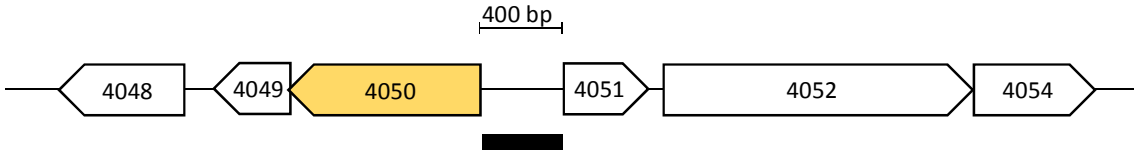
rvr4050



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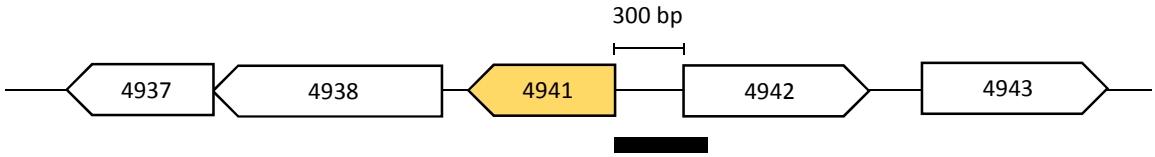
rvr4941



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rvr6279

Day	Gene ID	6277	6279	6281
		Median normalized RPKM		
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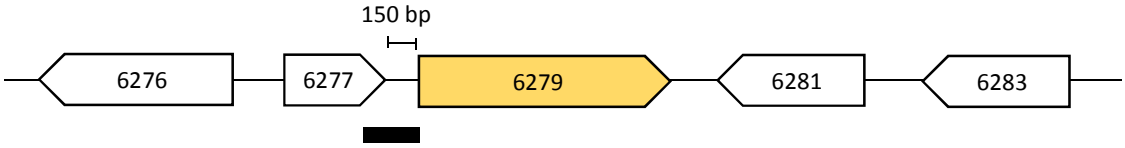
2		407	234	173
3		2911	223	117



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rvr6682

Day	Gene ID	6682	6683	6684
		Median normalized RPKM		
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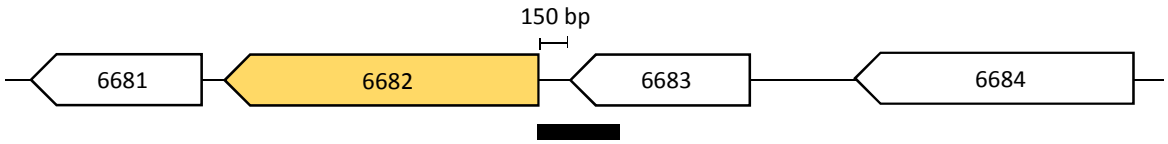
2		884	189	128
3		3051	1238	82



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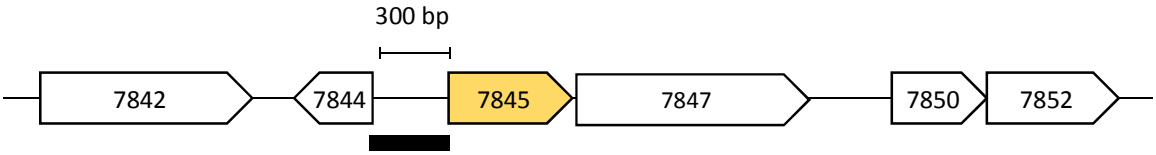
rvr7845



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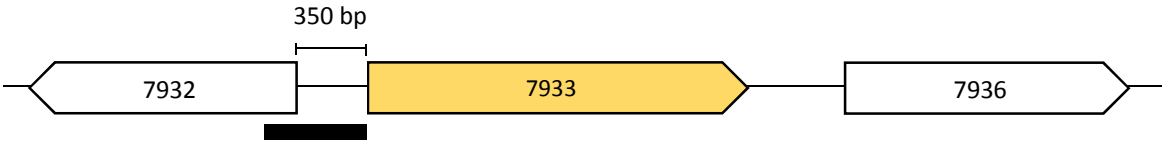
rvr7933



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rvr7936

Day	Gene ID	7933	7936
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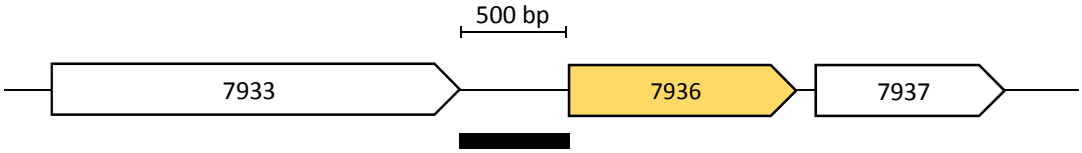
3		793	418



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rvr7941

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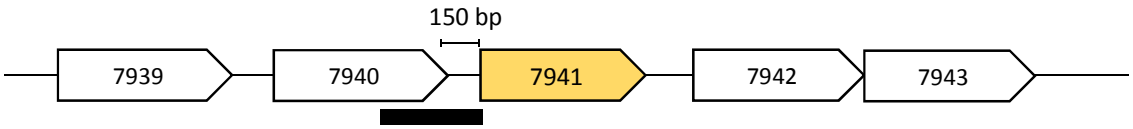

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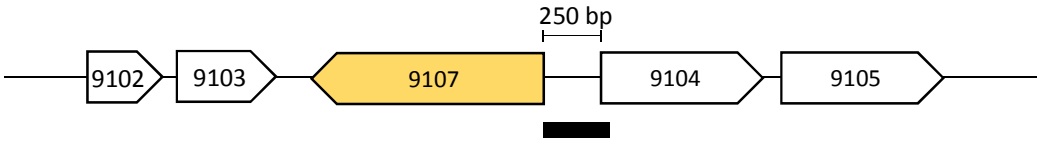
rvr9107



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The shaded sequence indicates coding region of gene. The thick bar and non-shaded sequence indicate putative promoter region examined in this study. Target genes selected by RNA-seq and 2-D gel electrophoresis analysis are shown in orange. The thin bar indicates gap sequence size (bp) between coding regions.

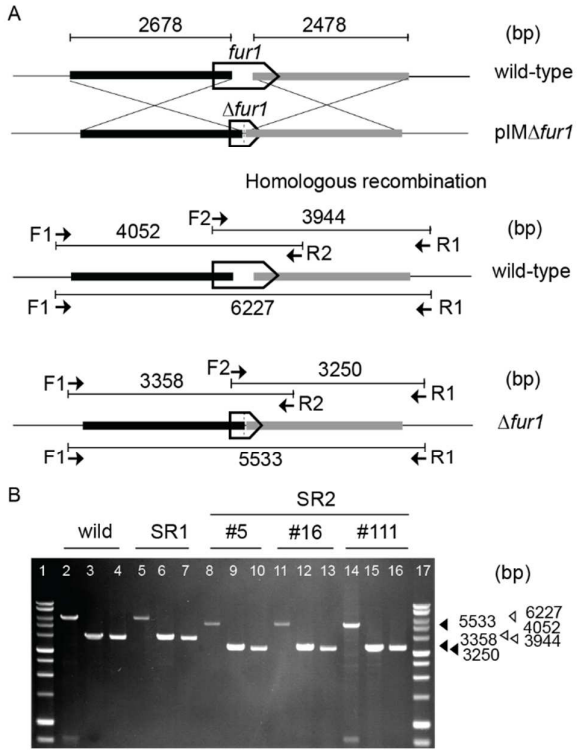

Figure S1. In-frame deletion of *fur1* gene. (A) Scheme describing gene deletion. The thick black bar shows upstream and downstream DNA regions targeted for homologous recombination. Numbers above the horizontal bars indicate the expected amplification size of the DNA, and the arrows indicate primer sites. Three sets of primers (F1-R1, F1-R2, and F2-R1) were used for gene amplification. The primers sequences are listed in Table S3. (B) Agarose gel electrophoresis (1%) analysis. DNA was amplified using genomic DNA from wild-type (lanes 2, 3, and 4), SR1 (*revC* and *revD* gene disruption in wild-type; lanes 5, 6, and 7), and SR2 (*fur1* gene deletion in SR1; lanes 8–16). Lanes 1 and 17 show the DNA ladder. Arrows indicate the expected size of DNA fragments amplified from the wild-type (open) and Δ*fur1* mutant (solid).

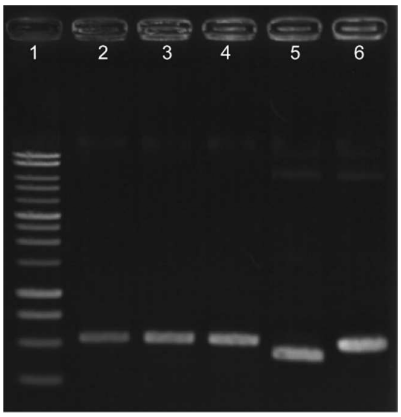

Figure S2. Confirmation of the promoter-*fur22* gene fragment in the transformed strain. DNA fragments were amplified using genomic DNA from SR1-12 (lane 2), SR1-15 (lane 3), SR1-18 (lane 4), SR1-19 (lane 5), and SR1-20 (lane 6). Lane 1 shows 1 kb DNA ladder. Forward primer was designed in the putative promoter sequence. The reverse primer was designed in the *fur22* gene. Primer sets used for PCR reaction and expected DNA size are listed in Table S3. PCR was performed using KOD-FX DNA polymerase (TOYOBO, Japan) and the following reaction conditions: 98°C for 1 min, followed by 30 cycles at 98°C for 10 s, 64°C for 30 s, and 72°C for 30 s with a final extension of 1 min. 1% agarose gel electrophoresis was performed to confirm the correct band size.

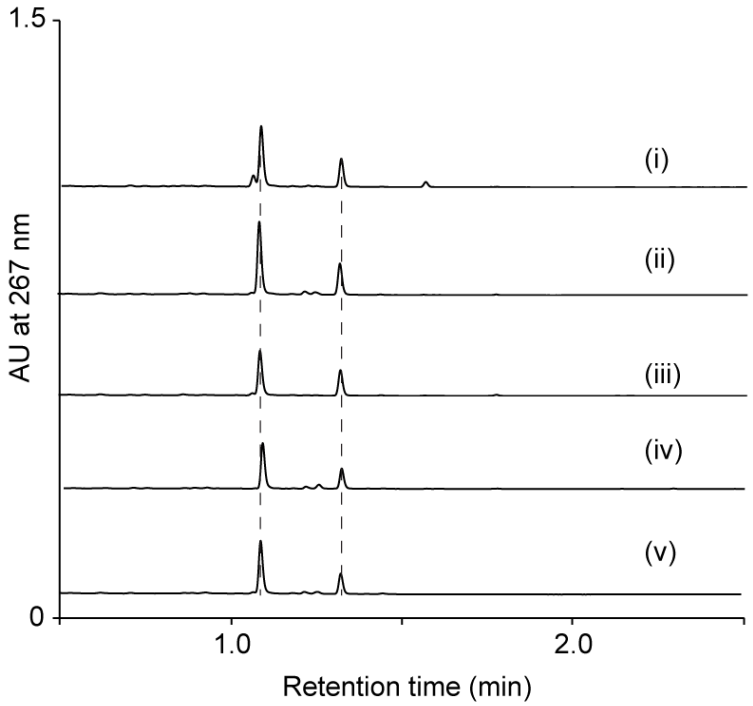

Figure S3. LC-MS analysis of FQs. The transformants harboring promoter-*fur22* cassettes (Table S2) were evaluated. The promoters were selected based on RNA-seq analysis and the following transformants were evaluated: SR1-12 (i), SR1-15 (ii), SR1-18 (iii), SR1-19 (iv), and SR1-20 (v). The chromatogram was produced at 267 nm [the absorption maximum of (**2**)]. All of the samples were extracted and analyzed in the same way as described in Methods. Based on UV and *m/z*, two peaks does not correspond to FQ derivatives.

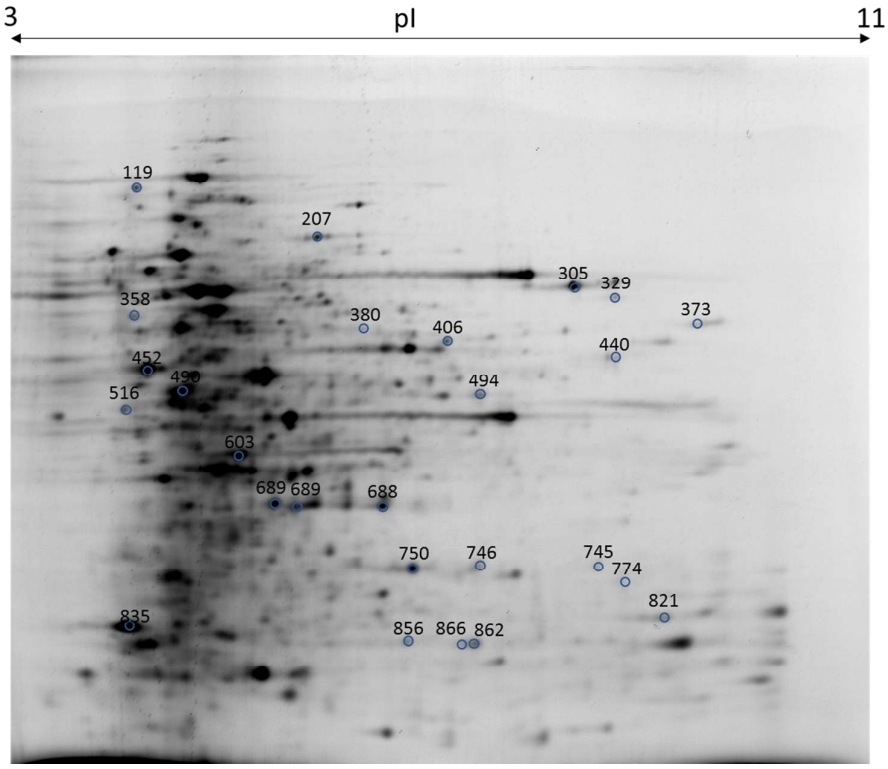

Figure S4. 2-D gel electrophoresis analysis. Proteins were extracted from SR1 strain at day 2, 3, 4, and 5 culture in RM-PM. Gel shown here is from day 2 analysis.

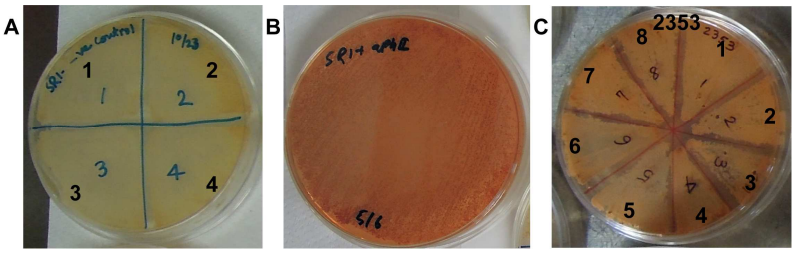

Figure S5. Reporter assay using the colour of FQ. (A) SR1-VC as vector control, (B) SR1-1 as positive control. (C) SR1-7 (clone #1-8) indicates the production of FQs.

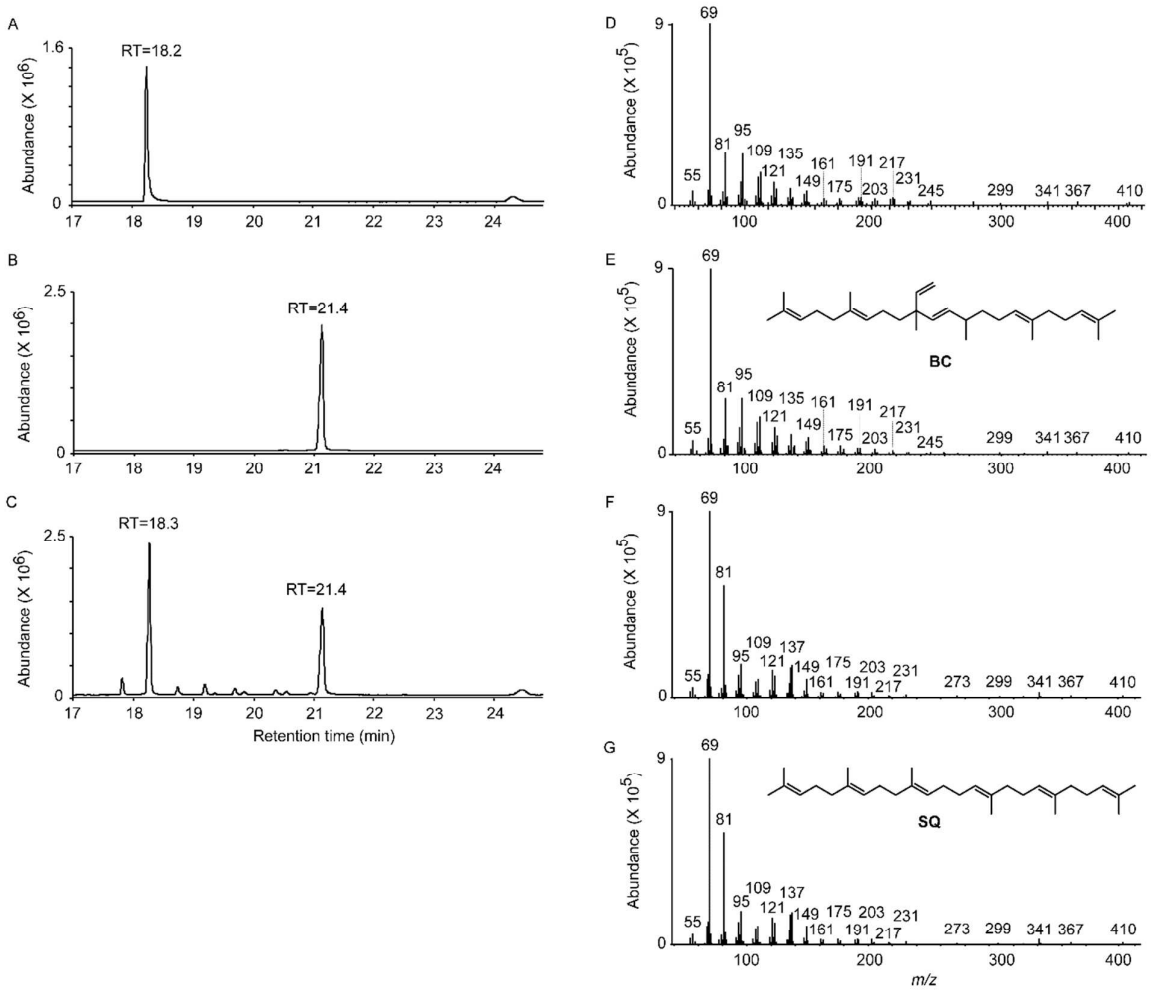

Figure S6. GC-MS analysis of BC and SQ. Total ion chromatogram of the standards for BC (A) and SQ (B), as well as *n*-hexane extracts from the SR2-4 strain (C). (D) Mass spectrum of the peak at the retention time of 18.3 min from *n*-hexane extracts from the SR2-4 strain. (E) Mass spectrum of the standard for BC. (F) Mass spectrum of the peak at the retention time of 21.4 min for *n*-hexane extracts from the SR2-4 strain. (G) Reference mass spectrum peak for SQ from the W9N11 library.

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