Serine Integrase Directional Recombination (SIDR) for rapid metabolic pathway assembly and modification

Supplementary Information

Sean D. Colloms[1]*, Christine A. Merrick[1], Femi J. Olorunniji[1], W. Marshall Stark[1], Maggie C. M. Smith[2], Anne Osbourn[3], Jay D. Keasling[4,5,6], and Susan J. Rosser[1]*

[1]Institute of Molecular, Cell and Systems Biology,
University of Glasgow,
Bower Building,
University Ave,
Glasgow G12 8QQ,
Scotland UK.

[2] Department of Biology, University of York, Wentworth Way, YO10 5DD, UK.

[3] Department of Metabolic Biology, The John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK.

[4] Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, California 94608, USA.

[5]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA

[6]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

* Corresponding authors
Telephone: 0141-330-6236
email: Sean.Colloms@glasgow.ac.uk / Susan.Rosser@glasgow.ac.uk

Supplementary Figures

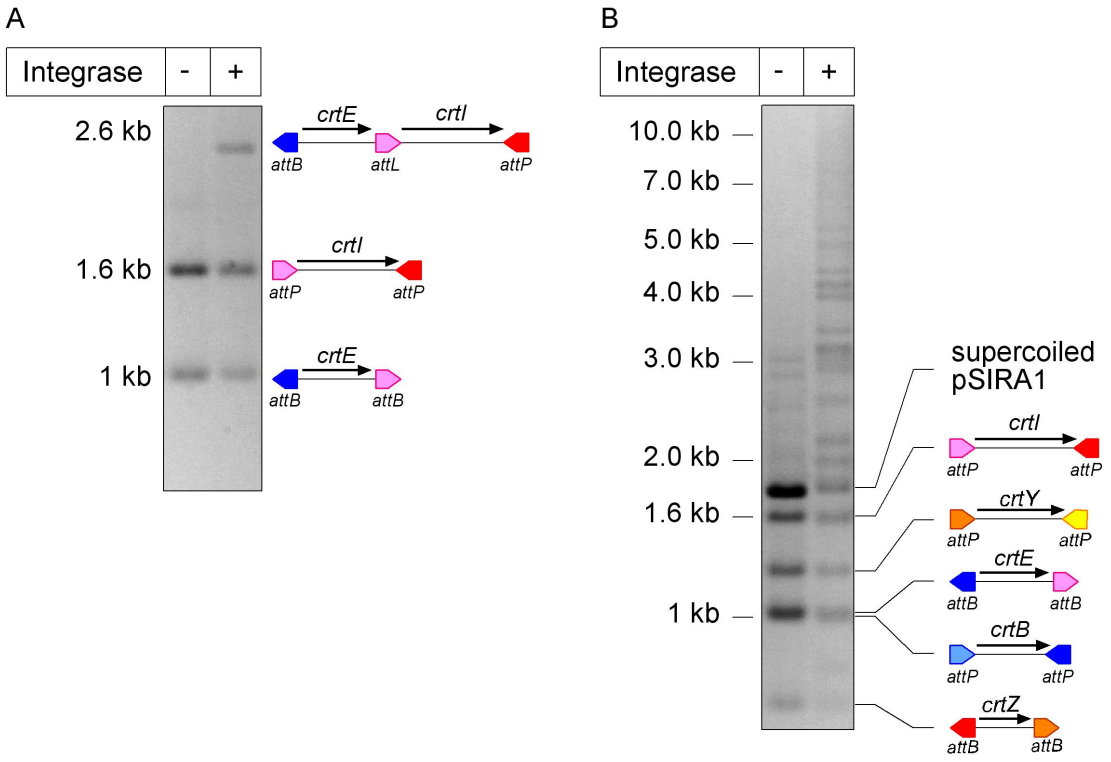
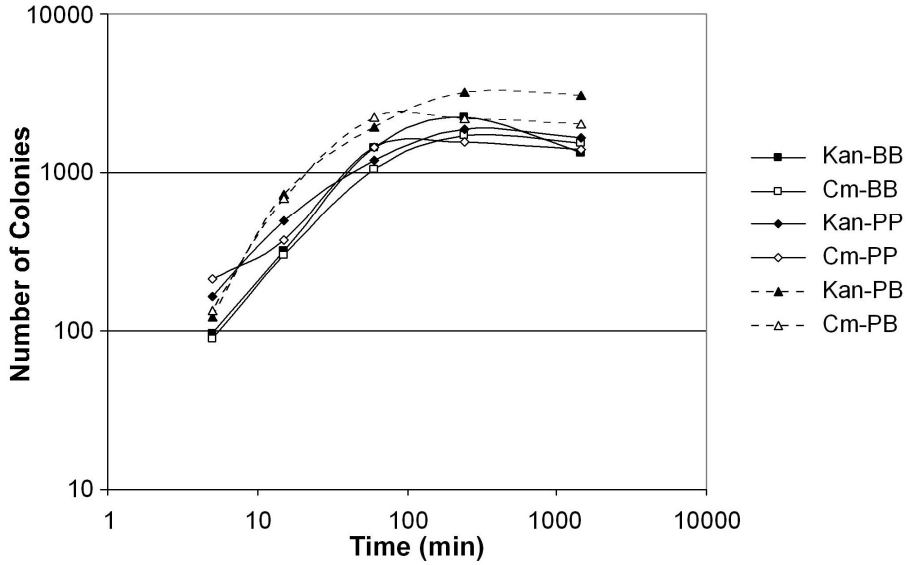
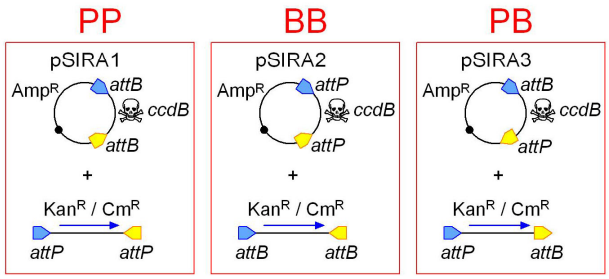


Figure S1. (A) Recombination specifically between fragments with matching *attP* and *attB* sites. A 1.0 kb *crtE* fragment with $attB^{CT}$ and $attB^{GT}$ sites and a 1.6 kb *crtI* fragment with $attP^{GT}$ and $attP^{CA}$ sites were incubated in the presence or absence of 200 nM ϕC31 integrase. The 2.6 kb product formed by recombination between $attB^{GT}$ and $attP^{GT}$ is indicated. **(B)** SIRA reaction to join the five zeaxanthin biosynthetic genes *crtB*, *crtE*, *crtI*, *crtY* and *crtZ* together and insert them into pSIRA1. Equimolar amounts of the indicated PCR products were incubated with pSIRA1 with or without 200nM integrase. All reactions contained ~5 nM of each PCR product and were incubated for four hours at 30°C in integrase reaction buffer with 10% ethylene glycol. Reactions were stopped by heating to 75°C for 10 minutes, treated with SDS and protease K and run on a 1.2% agarose Tris-Acetate EDTA (TAE) gel. The gel was stained with ethidium bromide and photographed on a Biorad GelDoc apparatus. Images are shown in reverse contrast.

A





Number of ampicillin resistant colonies

Time (min)	Kan-BB	Cm-BB	Kan-PP	Cm-PP	Kan-PB	Cm-PB
No Integrase	0	0	0	0	0	0
5	97	89	164	212	122	134
15	320	298	496	374	734	694
60	1448	1060	1200	1448	1944	2260
240	2232	1712	1896	1560	3240	2224
1440	1344	1544	1672	1400	3096	2048

Number of ampicillin resistant colonies (no insert)

Time (min)	pSIRA1	pSIRA2	pSIRA3
No Integrase	0	0	0
240	0	0	59
1440	0	0	68

B

Fraction of colonies correctly assembled (resistant / total)

TIME	Kan-BB	Cm-BB	Kan-PP	Cm-PP	Kan-BP	Cm-BP
5	20/20	20/20	20/20	20/20	22/22	19/20
15	20/20	21/21	20/20	20/20	20/20	20/20
60	20/20	22/22	20/20	20/20	20/20	22/22
240	20/20	20/20	20/20	20/20	64/66	64/66
1440	20/20	20/20	20/20	20/20	44/46	46/46

Figure S2. Time course of SIRA reactions to insert single linear DNA fragments into plasmid vectors. DNA fragments, containing a chloramphenicol resistance (Cm^R) or a kanamycin resistance (Kan^R) gene, were produced by PCR with pSW23 or pSW29 as template. The primers used incorporated $attB^{TT}$ upstream and $attB^{TC}$ downstream (BB), $attP^{TT}$ upstream and $attP^{TC}$ downstream (PP), or $attP^{TT}$ upstream and $attB^{TC}$ downstream (PB) of the resistance gene. Reactions (60 μl at 30°C) contained ~ 5 nM linear DNA fragment and ~5 nM pSIRA1 (PP), pSIRA2 (BB) or pSIRA3 (PB), and were set up in integrase reaction buffer without ethylene glycol. Recombination was initiated by the addition of 6 μl of 2 μM ϕC31 integrase. Samples (10 μl) were withdrawn after 5 minutes, 15 minutes, one hour, 4 hours and 24 hours and heated at 75°C for 10 minutes to inactivate the recombinase. A 2 μl aliquot from each time-point was transformed into chemically competent TOP10 cells (6×10^6 tranformants / μg pUC19 DNA) and transformants were selected on LB-agar plates containing ampicillin. Control reactions were carried out with no integrase. **(A)** The number of ampicillin-resistant colonies obtained for each reaction are plotted against time in the graph and shown in the tables below. **(B)** Randomly chosen transformant colonies were streaked onto one plate containing ampicillin and another plate containing chloramphenicol or kanamycin as appropriate. The table shows the fraction of colonies expressing the expected antibiotic resistance gene (number of chloramphenicol- or kanamycin-resistant colonies / number of ampicillin-resistant colonies).

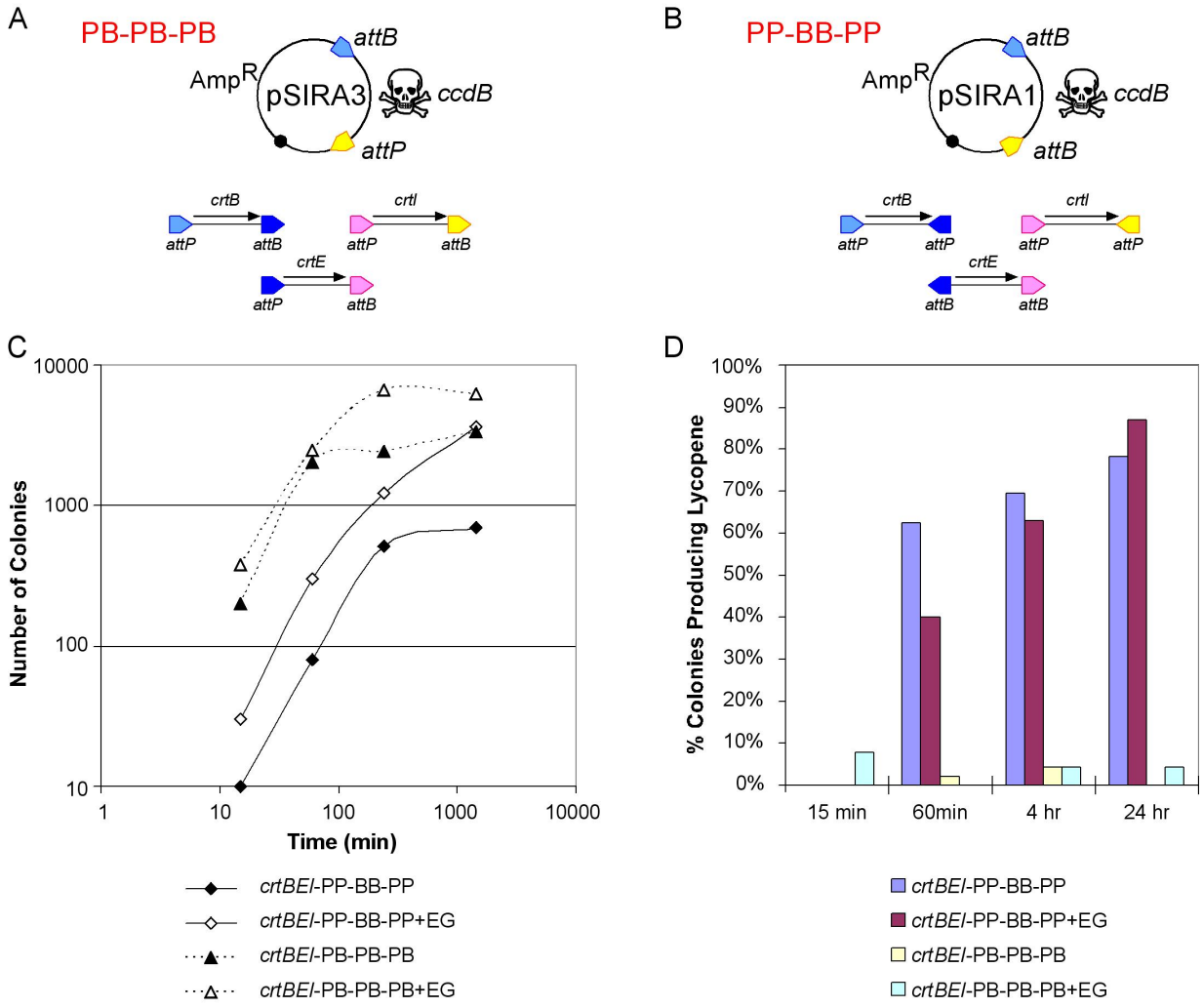


Figure S3. Time course of three-fragment SIRA reactions to assemble the *crtB-crtE-crtI* lycopene biosynthetic pathway. Assembly reactions were carried out with **(A)** pSIRA3 and linear PCR products, each with an upstream *attP* and a downstream *attB* (PB-PB-PB) or **(B)** with pSIRA1 and linear PCR products, each with either two *attP* or two *attB* sites (PP-BB-PP). Reactions (60 µl at 30°C) contained 5 nM of each linear DNA fragment and 3 nM SIRA vector and were in integrase reaction buffer with or without 5% ethylene glycol (EG). Recombination was initiated by the addition of 6 µl of 2 µM ϕC31 integrase. Samples (10 µl) were withdrawn after 15 minutes, one hour, 4 hours and 24 hours and heated at 75°C for 10 minutes to stop the reaction. A 2 µl aliquot from each time-point was transformed into chemically competent TOP10 cells (10^6 transformants / µg pUC19) and transformants were selected on LB-agar plates containing ampicillin. **(C)** Graph showing the number of ampicillin-resistant transformants obtained, plotted against reaction time. **(D)** Randomly chosen transformant colonies were streaked on plates containing ampicillin and grown overnight at 37°C. The histogram shows the percentage of colonies that were correctly assembled and therefore produced lycopene.

Table S1 Oligonucleotides.

S1.1 Oligonucleotides used for plasmid construction

attP-RX-TT-top	AATTCAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGTT
attP-RX-TT-bot	CTAGAACGCCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACTG
attB-RX-TT-top	AATTCGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCT
attB-RX-TT-bot	CTAGAGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCACG
attP-PS-TC-top	GAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCGTA
attP-PS-TC-bot	CTAGTACGCCCCCAACTGAGAGAACTCGAAGGTTACCCCAGTTGGGGCACTCTGCA
attB-PS-TC-top	GGTGCGGGTGCCAGGGCGTGCCCTCGGGCTCCCCGGGCGCGTACTCCA
attB-PS-TC-bot	CTAGTGGAGTACGCGCCCGGGGAGCCCGAGGGCACGCCCTGGCACCCGCACCTGCA
attP-SP-TC-top	CTAGAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCGTCTGCA
attP-SP-TC-bot	GACGCCCCCAACTGAGAGAACTCGAAGGTTACCCCAGTTGGGGCACT
pL-Eco-top	ACTGACCAAGCTTGCTCTAGA
pL-Eco-bot	AATTTCTAGAGCAAGCTTGGTCAGTGCGTCCTGCTGATGTGCTCA
pL-tet-top	AATTCTTAAGTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATCAGCAGGACGC
pL-tet-bot	GTATCTCTATCACTGATAGGGATGTCAATCTCTATCACTGATAGGGACTTAAG
pL-lac-top	AATTCTTAAGAATTGTGAGCGGATAACAATTGACATTGTGAGCGGATAACAAGATACTGAGCACATCAGCAGGACGC
pL-lac-bot	GTATCTTGTTATCCGCTCACAATGTCAATTGTTATCCGCTCACAATTCTTAAG

Table S1.2 PCR primers for initial isolation of *crt* genes

crtB-EU-for	**AGGAGGA**TTACAAA**ATG**AATAATCCGTCGTTACTCAATCATGCGG
crtB-EU-rev	**TTA**GAGCGGGCGCTGCCAG
crtE-EU-for	**AGGAGG**ATTACAAA**ATG**ACGGTCTGCGCAAAAAAACACG
crtE-EU-rev	**TTA**ACTGACGGCAGCGAGTTTTTTGTC
crtI-EU-for	**AGGAGGA**TTACAAA**ATG**AAACCAACTACGGTAATTGGTGCAGG
crtI-EU-rev	**TTA**TATCAGATCCTCCAGCATCAAACCTGC
crtY-EU-for	**AGGAGGA**TTACAAA**ATG**CAACCGCATTATGATCTGATTCTCG
crtY-EU-rev	**TTA**ACGATGAGTCGTCATAATGGCTTGC

Table S1.3 PCR primers incorporating *attP* and *attB* sequences

attB-TT-antibiotic-resistance-F	GAATTCGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCAAGAGGTTCCAACTTTCACCAT
attB-TC-antibiotic-resistance-R	CCATGGTGCGGGTGCCAGGGCGTGCCCTCGGGCTCCCCGGGCGCGTACTCCTTTCTAGGCACCAATAACTGC
attP-TT-antibiotic-resistance-F	TCTAGAAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGTAAGAGGTTCCAACTTTCACCAT
attP-TC-antibiotic-resistance-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCGTTTTCTAGGCACCAATAACTGC
attB-TC-antibiotic-resistance-R	ACTAGTGGAGTACGCGCCCGGGGAGCCCGAGGGCACGCCCTGGCACCCGCACTTTCTAGGCACCAATAACTGC
attP-TT-crtB-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGT**AGGAGGA**TTACAAA**ATG**AATAATCCGTCG
attP-CT-crtB-R	AGCAAATTCAGTGCCCCAACTGGGGTAACCTCTGAGTTCTCTCAGTTGGGGGCGT**TTA**GAGCGGGCGCTGCCAG
attB-CT-crtB-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCGCAC**TTA**GAGCGGGCGCTGCCAG
attB-CT-crtE-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCGCAC**AGGAGGA**TTACAAA**ATG**ACGGTCTGC
attP-CT-crtE-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTCTGAGTTCTCTCAGTTGGGGGCGT**AGGAGGA**TTACAAA**ATG**ACGGTCTGC
attB-GT-crtE-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCACGGGCACGCCCTGGCACCCGCAC**TTA**ACTGACGGCAGCGAGTTTTTTGTC

attP-GT-crtI-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTGTGAGTTCTCTCAGTTGGGGGCG TAGGAGGATTACAAAATGAAACCAACTACG
attP-CT-crtI-R	AGCGAATTCAGTGCCCCAACTGGGGTAACCTCTGAGTTCTCTCAGTTGGGGGCG TTTATATCAGATCCTCCAGCATCAAACCTGC
attP-CA-crtI-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTCAGAGTTCTCTCAGTTGGGGGCG TTTATATCAGATCCTCCAGCATCAAACCTGC
attP-TC-crtI-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCG TTTATATCAGATCCTCCAGCATCAAACCTGC
attB-CC-crtI-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCGGGGGCACGCCCTGGCACCCG CACTTATATCAGATCCTCCAGCATCAAACCTGC
attB-TC-crtI-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCGAGGGCACGCCCTGGCACCCG CACTTATATCAGATCCTCCAGCATCAAACCTGC
attP-CC-crtY-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTCCGAGTTCTCTCAGTTGGGGGCG TAGGAGGATTACAAAATGCAACCGC
attP-TC-crtY-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCG TTTAACGATGAGTCGTCATAATGGCTTGC
attB-CA-crtZ-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCTGGGGCACGCCCTGGCACCCGC ACAGGAGGATTACATTATGTTGTGGATTTGGAATGC
attB-CC-crtZ-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCGGGGGCACGCCCTGGCACCCG CACCCTTACTTCCCGGATGC
attP-TT-crt*-F	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGT TCGCCCTTAGGAGGATTACA
attP-CT-crt*-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTCTGAGTTCTCTCAGTTGGGGGCG TGCTGGAATTCGCCCTTTTA
attB-CT-crt*-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCG CACTCGCCCTTAGGAGGATTACA
attB-GT-crt*-R	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCACGGGCACGCCCTGGCACCCGC ACGCTGGAATTCGCCCTTTTA
attP-GT-crt*-F	AGCACTAGTAGTGCCCCAACTGGGGTAACCTGTGAGTTCTCTCAGTTGGGGGCG TTCGCCCTTAGGAGGATTACA
attP-TC-crt*-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCG TGCTGGAATTCGCCCTTTTA
attR-GT-BB-F	AGCACTAGTAGTGCCCCAACTGGGGTAACCTGTGGGCTCCCCGGGCGCGTACTC CCGCTAAGGATGATTTCTGGA
attR-CA-BB-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTCAGGGCTCCCCGGGCGCGTACTC CGGTGACACCTTGCCCTTTT
attB-CT-idi-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCG CACAGGAGGATTACAAAATGCAAACGGAACACGTCAT
attB-CC-idi-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCGGGGGCACGCCCTGGCACCCG CACTTATTTAAGCTGGGTAAATGCAGATA
attP-CC-dxs-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTCCGAGTTCTCTCAGTTGGGGGCG TAGGAGGATTACCTGATGAGTTTTGATATTGCC
attP-CA-dxs-R	GCACTAGTAGTGCCCCAACTGGGGTAACCTCAGAGTTCTCTCAGTTGGGGGCGT GGGATTATGCCAGCCAGG

attP-TT-vioA-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGT **AGGAGGA**TTACAAA**ATG**AAGCATTCTTCCGATATCTGC
attP-CT-vioA-R	AGCAATTCAGTGCCCCAACTGGGGTAACCTCTGAGTTCTCTCAGTTGGGGGCGT**T TA**CGCGGCGATGCGCTGCAGCAG
attB-CT-vioB-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCG CAC**AGGAGGA**TTACAAA**ATG**AGCATTCTGGATTTTCCACGC
attB-GT-vioB-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCACGGGCACGCCCTGGCACCCGC AC**TTA**GGCCTCTCTAGAAAGCTTTCC
attP-GT-vioC-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTGTGAGTTCTCTCAGTTGGGGGCG T**AGGAGGA**TTACAAA**ATG**AAAAGAGCAATCATAGTCGGA
attP-CA-vioC-R	GCACTAGTAGTGCCCCAACTGGGGTAACCTCAGAGTTCTCTCAGTTGGGGGCGT **TTA**GTTGACCCTCCCTATCTTGTAC
attB-CA-vioD-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCTGGGGCACGCCCTGGCACCCGC AC**AGGAGGA**TTACAAA**ATG**AAGATTCTGGTCATCGGCGCG
attB-CC-vioD-R	AGCACTAGTGGAGTACGCGCCCGGGGAGCCCGGGGGCACGCCCTGGCACCCG CAC**TTA**GCGTTGCAGCGCGTAGCGCAG
attP-CC-vioE-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTCCGAGTTCTCTCAGTTGGGGGCG T**AGGAGGA**TTACAAA**ATG**GAAAACCGGGAACCGCCGCTG
attP-TC-vioE-R	AGCACTAGTAGTGCCCCAACTGGGGTAACCTTCGAGTTCTCTCAGTTGGGGGCG T**TTA**GCGCTTGGCGGCGAAGACGGC
D-attP-TT-vioA-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGT **ARRARRA**TTAAAAA**ATG**AAGCATTCTTCCGATATCTGC
D-attB-CT-vioB-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCAGGGGCACGCCCTGGCACCCG CAC**ARRARRA**TTAAAAT**ATG**AGCATTCTGGATTTTCCACGC
D-attP-GT-vioC-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTGTGAGTTCTCTCAGTTGGGGGCG T**ARRARRA**TTAAAAAATGAAAAGAGCAATCATAGTCGGA
D-attB-CA-vioD-F	AGCTCTAGAGGAGTACGCGCCCGGGGAGCCCTGGGGCACGCCCTGGCACCCGC AC**ARRARRA**TTACAAAATGAAGATTCTGGTCATCGGCGCG
D-attP-CC-vioE-F	AGCTCTAGAAGTGCCCCAACTGGGGTAACCTCCGAGTTCTCTCAGTTGGGGGCG T**ARRARRA**TTAAAAA**ATG**GAAAACCGGGAACCGCCGCTG

attP sequences are shown highlighted in yellow, *attB* sequences are highlighted in cyan. Bottom strand *attP* and *attB* sequences are underlined. Central dinucleotides (named according to their sequence on the top strand) are highlighted red. Ribosome binding sites (generally AGGAGGA) and start codons (ATG) in forward primers, and the reverse complement of stop codons (TTA) in reverse primers are shown underlined and bold.

Table S1.4 Sequencing Primers

VF2	TGCCACCTGACGTCTAAGAA
VR	ATTACCGCCTTTGAGTGAGC
crtB-out	CTGGAGCATGAAGGTCTGAA
crtE-out	GACGCTGGTCAATCTGTTAG
crtE-int-for	AACGTATTCGCCCCATGTT
crtI-int-for	GCCACCTCATCCATTTATAC
crtI-int-rev	CCACCCAGATCCTGAAACAG
crtI-3'-out	TCTGTGGAGCCCGTTCTTAC
crtI-out	GCATAACCGCGATAAAACCA
vioA-out	GTTCTGCCGCGACAGCGATA
vioB-out	ATACCCGCAGCTACGACGAC
vioC-out	ACACCCGCTACATGCATAGC
vioD-out-2	AGTCCGGCCACTTCTCCAT

Table S2 Summary of PCRs for assembly reactions

1 gene (*attP attP*)	**Cm^R into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-antibiotic-resistance-F	*attP*-TC-antibiotic-resistance-R	pSW23

1 gene (*attP attB*)	**Cm^R into pSIRA3**	
Forward Primer	Reverse Primer	Template
attP-TT-antibiotic-resistance-F	*attB*-TC-antibiotic-resistance-R	pSW23

1 gene (*attB attB*)	**Cm^R into pSIRA2**	
Forward Primer	Reverse Primer	Template
attB-TT-antibiotic-resistance-F	*attB*-TC-antibiotic-resistance-R	pSW23

1 gene (*attP attP*)	**Kan^R into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-antibiotic-resistance-F	*attP*-TC-antibiotic-resistance-R	pSW29

1 gene (*attP attB*)	**Kan^R into pSIRA3**	
Forward Primer	Reverse Primer	Template
attP-TT-antibiotic-resistance-F	*attB*-TC-AbR	pSW29

1 gene (attB attB)	**Kan^R into pSIRA2**	
Forward Primer	Reverse Primer	Template
attB-TT-antibiotic-resistance-F	attB-TC-antibiotic-resistance-R	pSW29

3 genes	***crtB, crtE, crtI* into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-crtB-F	attP-CT-crtB-R	pCM1
attB-CT-crtE-F	attB-GT-crtE-R	pCM2
attP-GT-crtI-F	attP-TC-crtI-R	pCM3

3 genes	***crtB, crtE, crtI* into pSIRA3**	
Forward Primer	Reverse Primer	Template
attP-TT-crtB-F	attB-CT-crtB-R	pCM1
attP-CT-crtE-F	attB-GT-crtE-R	pCM2
attP-GT-crtI-F	attB-TC-crtI-R	pCM3

4 genes	***crtB, crtE, crtI, crtY* into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-crtB-F	attP-CT-crtB-R	pCM1
attB-CT-crtE-F	attB-GT-crtE-R	pCM2
attP-GT-crtI-F	attB-CC-crtI-R	pCM3
attP-CC-crtY-F	attP-TC-crtY-R	pCM4

5 genes	***crtB, crtE, crtI, crtZ, crtY* into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-crtB-F	attP-CT-crtB-R	pCM1
attB-CT-crtE-F	attB-GT-crtE-R	pCM2
attP-GT-crtI-F	attP-CA-crtI-R	pCM3
attB-CA-crtZ-F	attB-CC-crtZ-R	*P. agglomerans* genomic DNA
attP-CC-crtY-F	attP-TC-crtY-R	pCM4

3 genes random order	***crtB, crtE, crtI, crtZ, crtY* into pSIRA1**	
Forward Primer	Reverse Primer	Template
attP-TT-crt*-F	attP-CT-crt*-R	pCM1
attB-CT-crt*-F	attB-GT-crt*-R	pCM1
attP-GT-crt*-F	attP-TC-crt*-R	pCM1
attP-TT-crt*-F	attP-CT-crt*-R	pCM2
attB-CT-crt*-F	attB-GT-crt*-R	pCM2
attP-GT-crt*-F	attP-TC-crt*-R	pCM2
attP-TT-crt*-F	attP-CT-crt*-R	pCM3
attP-CT-crt*-F	attB-GT-crt*-R	pCM3
attP-GT-crt*-F	attP-TC-crt*-R	pCM3

5 genes	***vioA vioB, vioC, vioD, vioE* into pSIRA4**	
Forward Primer	Reverse Primer	Template
attP-TT-vioA-F	attP-CT-vioA-R	*C. violaceum* genomic DNA
attB-CT-vioB-F	attB-GT-vioB-R	*C. violaceum* genomic DNA
attP-GT-vioC-F	attP-CA-vioC-R	*C. violaceum* genomic DNA
attB-CA-vioD-F	attB-CC-vioD-R	*C. violaceum* genomic DNA
attP-CC-vioE-F	attP-TC-vioE-R	*C. violaceum* genomic DNA

5 genes	***vioA vioB, vioC, vioD, vioE* degenerate RBS into pSIRA4**	
Forward Primer	Reverse Primer	Template
D-attP-TT-vioA-F	attP-CT-vioA-R	*C. violaceum* genomic DNA
D-attB-CT-vioB-F	attB-GT-vioB-R	*C. violaceum* genomic DNA
D-attP-GT-vioC-F	attP-CA-vioC-R	*C. violaceum* genomic DNA
D-attB-CA-vioD-F	attB-CC-vioD-R	*C. violaceum* genomic DNA
D-attP-CC-vioE-F	attP-TC-vioE-R	*C. violaceum* genomic DNA

***ccdB*-Cm^R cassette**	**into pSIRA1 *crtB-crtE-crtI-crtZ-crtY***	
Forward Primer	Reverse Primer	Template
attR-GT-BB-F	attR-CA-BB-R	p-Cm^R-ccdB

***crtI* mutant library**	**into pSIRA1 *crtB-crtE-ccdB-Cm^R-crtZ-crtY***	
Forward Primer	Reverse Primer	Template
attP-GT-crtI-F	attP-CA-crtI-R	pCM3

3 genes crtI-dxs-idi	**into pSIRA1 *crtB-crtE-ccdB-Cm^R-crtZ-crtY***	
Forward Primer	Reverse Primer	Template
attP-GT-crtI-F	attP-CT-crtI-R	pCM3
attB-CT-idi-F	attB-CC-idi-R	*E. coli* genomic DNA
attP-CC-dxs-F	attP-CA-dxs-R	*E. coli* genomic DNA