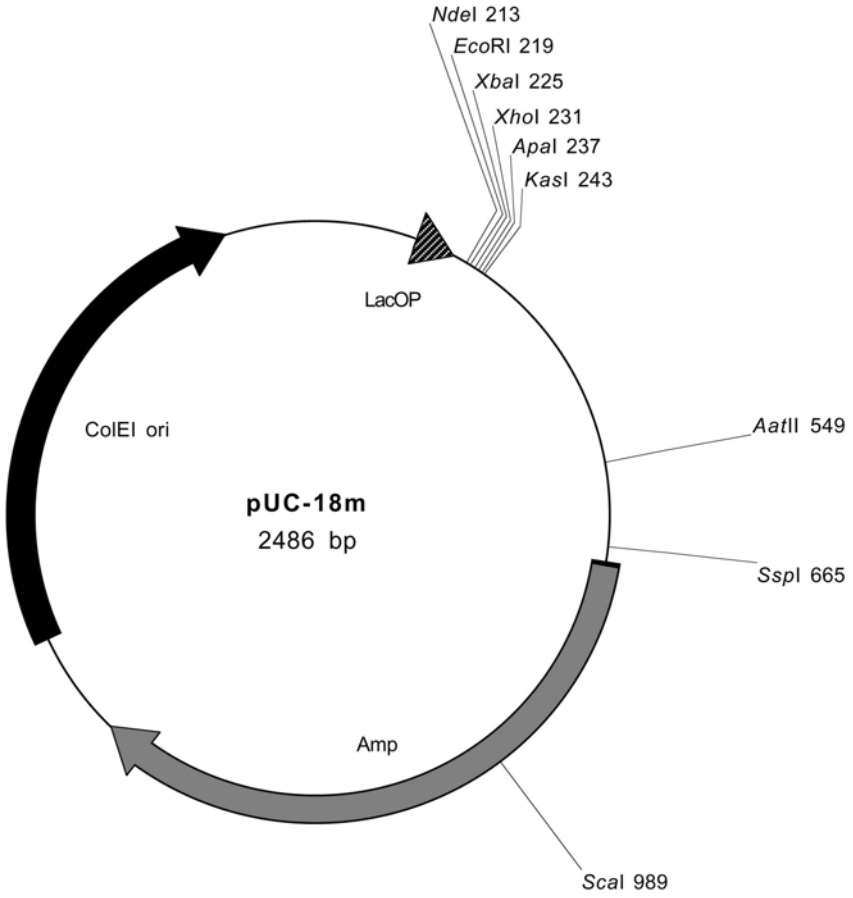
APPENDIX A

Plasmid Sequences and Maps

Plasmid name pUC18m (empty vector); 2486 bp

Construction
The *lacZ* gene from pUC18 was cut out and replaced by a *lac* promoter/operator and the multi-cloning site: *Nde*I-*Eco*RI-*Xba*I-*Xho*I-*Apa*I-*Ehe*I/*Kas*I.

Plasmid map



pUC18m sequence

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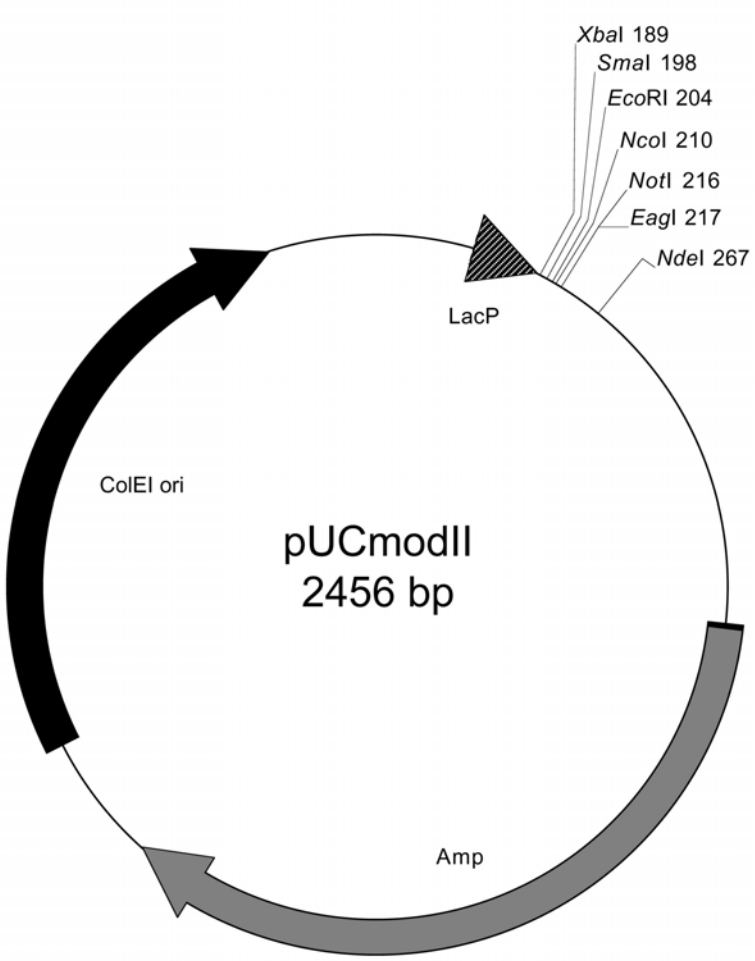
Plasmid name pUCmodII (empty vector); 2456 bp

Construction
Based on pUC19. The *lac* operator and multi cloning site from pUC19 were removed and replaced with multi cloning site: *Xba*I-*Sma*I-*Eco*RI-*Nco*I-*Not*I/*Eag*I (see plasmid map below).

Remarks
High copy number (several hundred). Since the *lac* operator has been removed, the genes cloned into the MCS are expressed constitutively in *E. coli*.

Plasmid map



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Plasmid name pACmod (empty vector); 4245 bp; medium copy

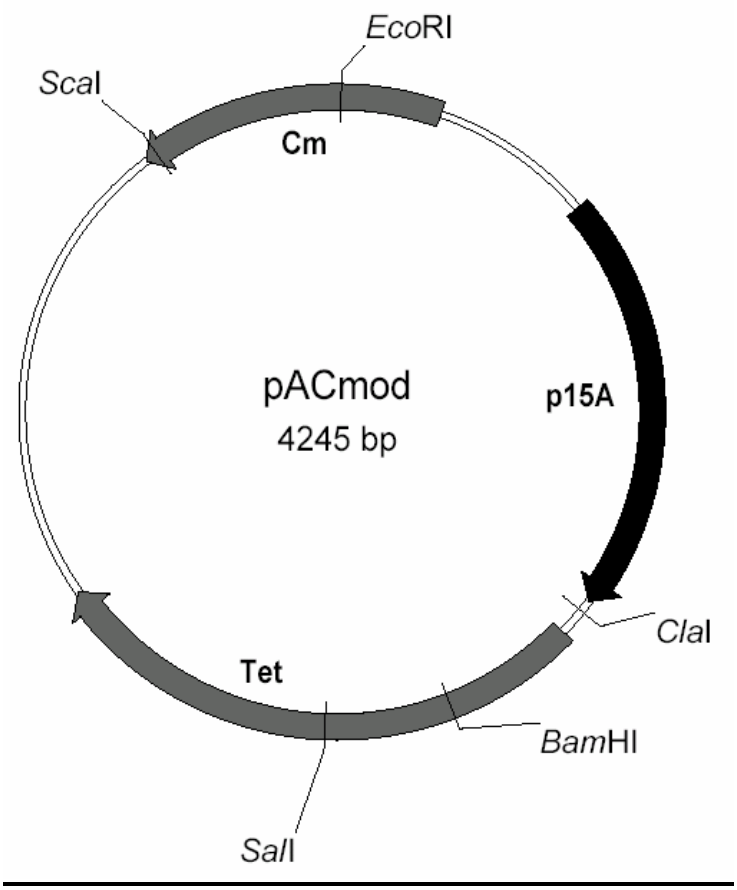
Construction

Identical to commercially available pACYC184 except the *Xba*I site in that plasmid (TCTAGA) has been mutated to TCAAGA. Although this mutation is in the p15A origin of replication, the plasmid seems fine.

Sequence landmarks

Chloramphenicol resistance gene 3805-219 (reverse orientation); tetracycline resistance gene 1581-2771; p15A origin 581-1493

Plasmid map



pACmod sequence

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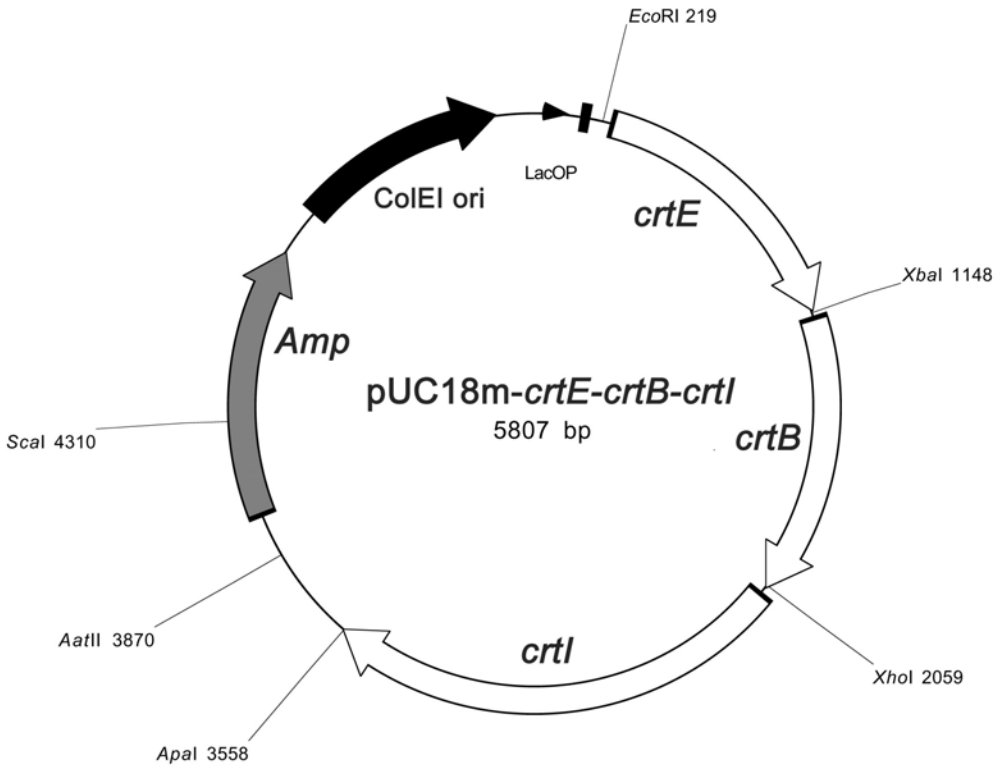
Plasmid name pUC18m-*crtE-crtB-crtI*; 5807 bp

Based on vector pUC18m.

Construction

All 3 genes are on a single operon, regulated by a single *lac* promoter and operator. Just upstream of each gene is an optimized Shine-Dalgarno site (AGGAGG) followed by 8 spacer nucleotides. The gene *crtE* from *Erwinia uredovora*, (GGPP synthase) is inserted between the *Eco*RI and *Xba*I sites. The gene *crtB* from *Erwinia uredovora* (C_{40} carotenoid synthase) is inserted between the *Xba*I and *Xho*I sites. The gene *crtI* from *Erwinia uredovora* (C_{40} carotenoid desaturase) is inserted between the *Xho*I and *Apa*I sites.

Plasmid map



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pUC18m-*crtE-crtB-crtI* sequence

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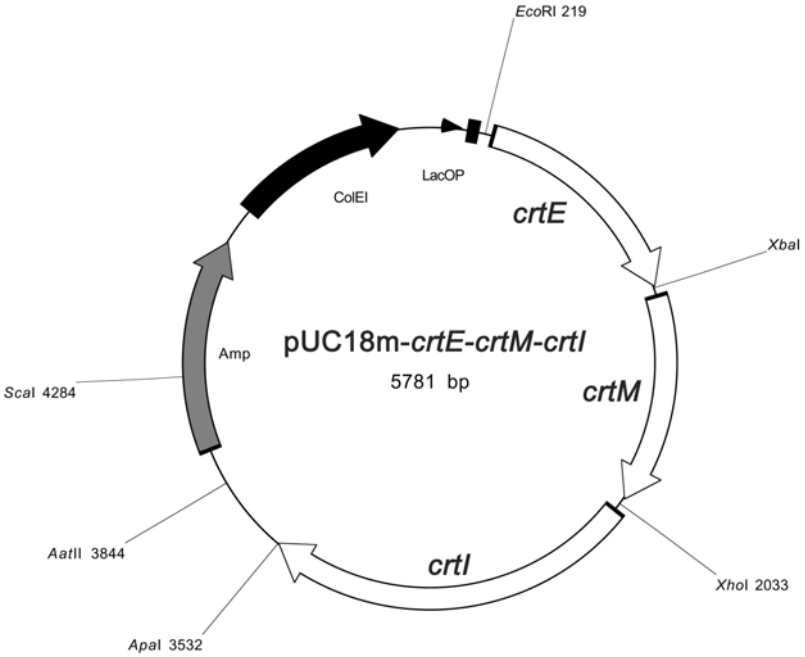
Plasmid name pUC18m-*crtE-crtM-crtI*; 5781 bp

Based on vector pUC18m

Construction

All 3 genes are on a single operon, regulated by a single *lac* promoter and operator. Just upstream of each gene is an optimized Shine-Dalgarno site (AGGAGG) followed by 8 spacer nucleotides. The gene *crtE* from *Erwinia uredovora* encoding a GGPP synthase is inserted between the *Eco*RI and *Xba*I sites. The gene *crtM* from *Staphylococcus aureus* encoding a C_{30} carotenoid synthase is inserted between the *Xba*I and *Xho*I sites. The gene *crtI* from *Erwinia uredovora* encoding a C_{40} carotenoid desaturase (4-step) is inserted between the *Xho*I and *Apa*I sites.

Plasmid map



pUC18m-*crtE-crtM-crtI* sequence

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Plasmid name pUC18m-*crtE-M*$_8$-*crtI*; 5781 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtE-crtM-crtI* except in the place of wild-type *crtM* is M_8, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid map
See pUC18m-*crtE-crtM-crtI* plasmid map.

Mutations
Point mutations in M_8 (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
T78A (F26L)
A345G (silent)

pUC-*crtE-M*$_8$-*crtI* sequence
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gcgcagcgagtcagtgagcgaggaagcggaaga

Plasmid name pUC18m-*crtE-M*$_9$-*crtI*; 5781 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtE-crtM-crtI* except in the place of wild-type *crtM* is *M*$_9$, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid map
See pUC18m-*crtE-crtM-crtI* plasmid map.

Mutations
Point mutations in *M*$_9$ (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
T77C (F26S)
T119C (I40T)
T135C (silent)
T141C (silent)
A850G (after stop codon)

pUC-*crtE-M*$_9$-*crtI* sequence
gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
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Plasmid name pUC18m-*crtE-M*$_{10}$*-crtI*; 5781 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtE-crtM-crtI* except in the place of wild-type *crtM* is M_{10}, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid map
See pUC18m-*crtE-crtM-crtI* plasmid map.

Mutations
Point mutations in M_{10} (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
A10G (M4V)
A35G (H12R)
A39G (silent)
T176C (F59S)
A242G (Q81R)
A539G (E180G)

pUC-*crtE-M*$_{10}$*-crtI* sequence
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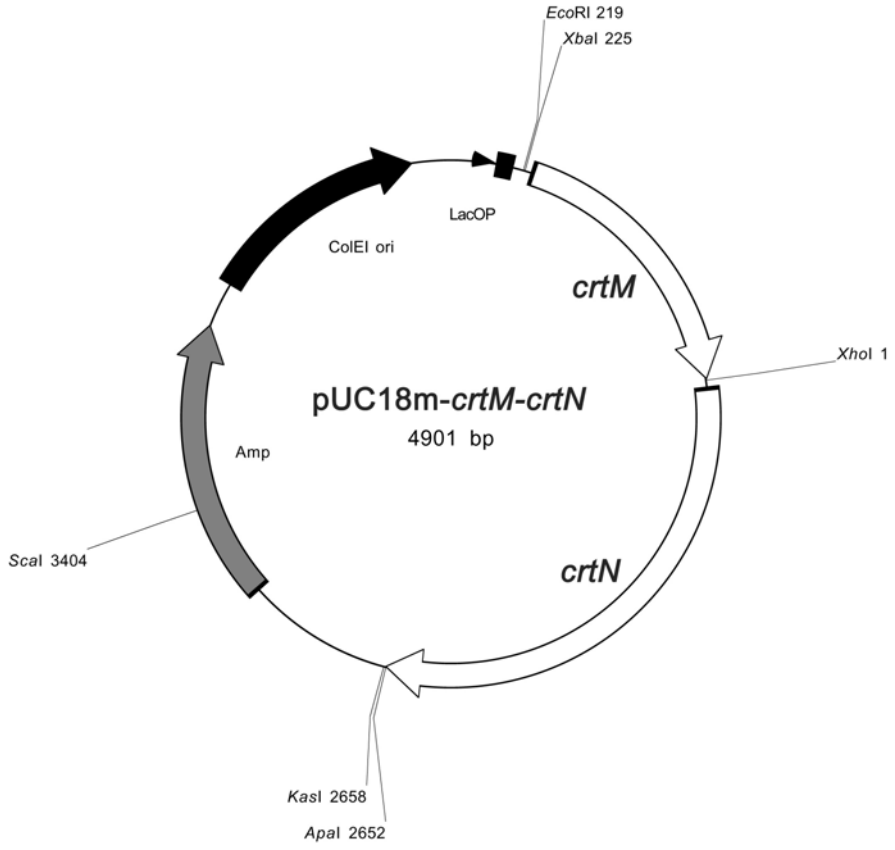
Plasmid name pUC18m-*crtM-crtN*; 4901 bp

Based on vector pUC18m

Construction

crtM, encoding the C_{30} carotenoid synthase from *Staphylococcus aureus* is inserted between the *Xba*I and *Xho*I sites. *crtN*, also from *S. aureus*, encoding a C_{30} carotenoid desaturase, is inserted between the *Xho*I and *Apa*I sites. Just upstream of each ORF is an optimized Shine-Dalgarno site (AGGAGG) followed by 8 spacer nucleotides. A single *lac* operator/promoter site regulates the entire operon, as shown in the plasmid map.

Plasmid map



pUC18m-*crtM-crtN* sequence

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gcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgagacgaaagggc
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agcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccct
gattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagc
gaggaagcggaaga

Plasmid name pUC18m-*M_8-crtN*; 4901 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtM-crtN* except in the place of wild-type *crtM* is *M_8*, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid Map
See pUC18m-*crtM-crtN* plasmid map.

Mutations
Point mutations in *M_8* (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
T78A (F26L)
A345G (silent)

pUC18m-*M_8-crtN* sequence
gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
cgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgt
tgtgtggaattgtgagcggataacaatttcacacaggaaacacatatgGAATTCTCTAGAaggaggattacaaaatga
caatgatgaatatgaattttaaatattgtcataaaatcatgaagaaacattcaaaaagcttttcttacgctt**A**gacttgttaccagaag
atcaaagaaaagcggtttgggcaatttatgctgtgtgtcgtaaaattgatgacagtatagatgtttatggcgatattcaatttttaaatc
aaataaaagaagatatacaatctattgaaaaatacccatatgaacatcatcactttcaaagtgatcgtagaatcatgatggcgcttca
gcatgttgcacaacataaaaatatcgcctttcaatctttttataatctcattgatactgtatataaagatca**G**cattttacaatgtttgaa
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gggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgca
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caggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctggccttttgctcacatgttc
tttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccga
gcgcagcgagtcagtgagcgaggaagcggaaga

Plasmid name pUC18m-*M_9-crtN*; 4901 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtM-crtN* except in the place of wild-type *crtM* is *M_9*, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid Map
See pUC18m-*crtM-crtN* plasmid map.

Mutations
Point mutations in *M_9* (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
T77C (F26S)
T119C (I40T)
T135C (silent)
T141C (silent)
A850G (after stop codon)

pUC18m-*M_9-crtN* sequence
gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
cgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgt
tgtgtggaattgtgagcggataacaatttcacacaggaaacacatatgGAATTCTCTAGAaggaggattacaaaatga
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atcaaagaaaagcggtttgggcaa**C**ttatgctgtgtgtcg**C**aaaat**C**gatgacagtatagatgtttatggcgatattcaatttttaa
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caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatc
gttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcg
caaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggacca
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actggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagac
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cgagcgcagcgagtcagtgagcgaggaagcggaaga

Plasmid name pUC18m-*M_{10}-crtN*; 4901 bp

Based on vector pUC18m

Construction
This plasmid is identical to pUC18m-*crtM-crtN* except in the place of wild-type *crtM* is *M_{10}*, a mutant of the C_{30} carotenoid synthase *crtM* from *Staphylococcus aureus* that is capable of synthesizing C_{40} carotenoids.

Plasmid Map
See pUC18m-*crtM-crtN* plasmid map.

Mutations
Point mutations in *M_{10}* (compared with wild-type *crtM*) are shown below in the plasmid sequence in bold, underlined capital letters and are summarized below (numbered according to their position in the *crtM* ORF; amino acid substitutions are listed in brackets).
A10G (M4V)
A35G (H12R)
A39G (silent)
T176C (F59S)
A242G (Q81R)
A539G (E180G)

pUC18m-*M_{10}-crtN* sequence
gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
cgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgt
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caatg**G**tgaatatgaattttaaatattgtc**G**taa**G**atcatgaagaaacattcaaaaagcttttcttacgcttttgacttgttaccaga
agatcaaagaaaagcggtttgggcaatttatgctgtgtgtcgtaaaattgatgacagtatagatgtttatggcgatattcaat**C**ttta
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gcttcagcatgttgcacaacataaaaatatcgcctttcaatctttttataatctcattgatactgtatataaagatcaacattttacaatgt
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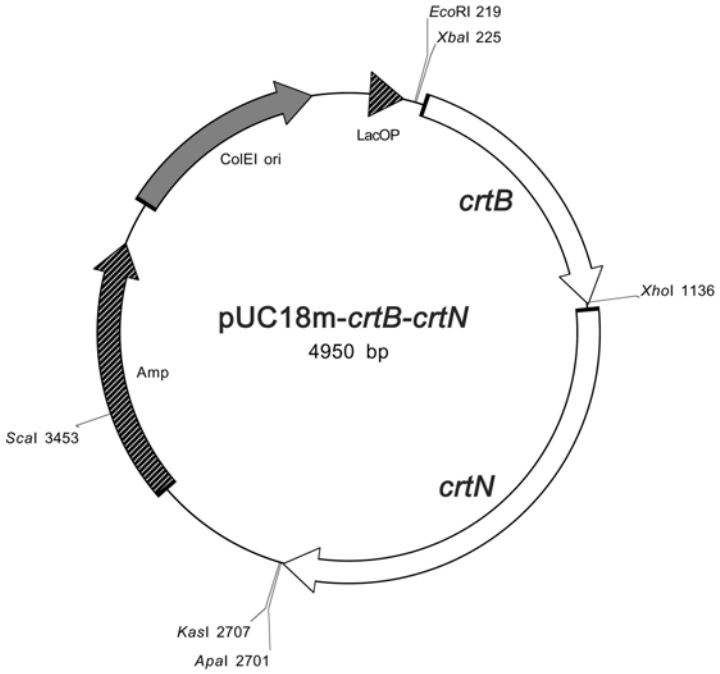
Plasmid name pUC18m-*crtB*-*crtN*; 4950 bp

Based on vector pUC18m

Construction

The gene *crtB* from *Erwinia uredovora* (C_{40} carotenoid synthase) is inserted between the *Xba*I and *Xho*I sites. The gene *crtN* from *Staphylococcus aureus* (C_{30} carotenoid desaturase) is inserted between the *Xho*I and *Apa*I sites. Just upstream of each ORF is an optimized Shine-Dalgarno site (AGGAGG) followed by 8 spacer nucleotides. A single *lac* operator/promoter site regulates the entire operon, as shown in the plasmid map. *E. coli* carrying this plasmid synthesize NO carotenoids, as CrtB accepts only GGPP as a substrate, and this is present only at low levels in *E. coli* that do not express an additional GGPP synthase.

Plasmid map



pUC18m-*crtB*-*crtN* sequence

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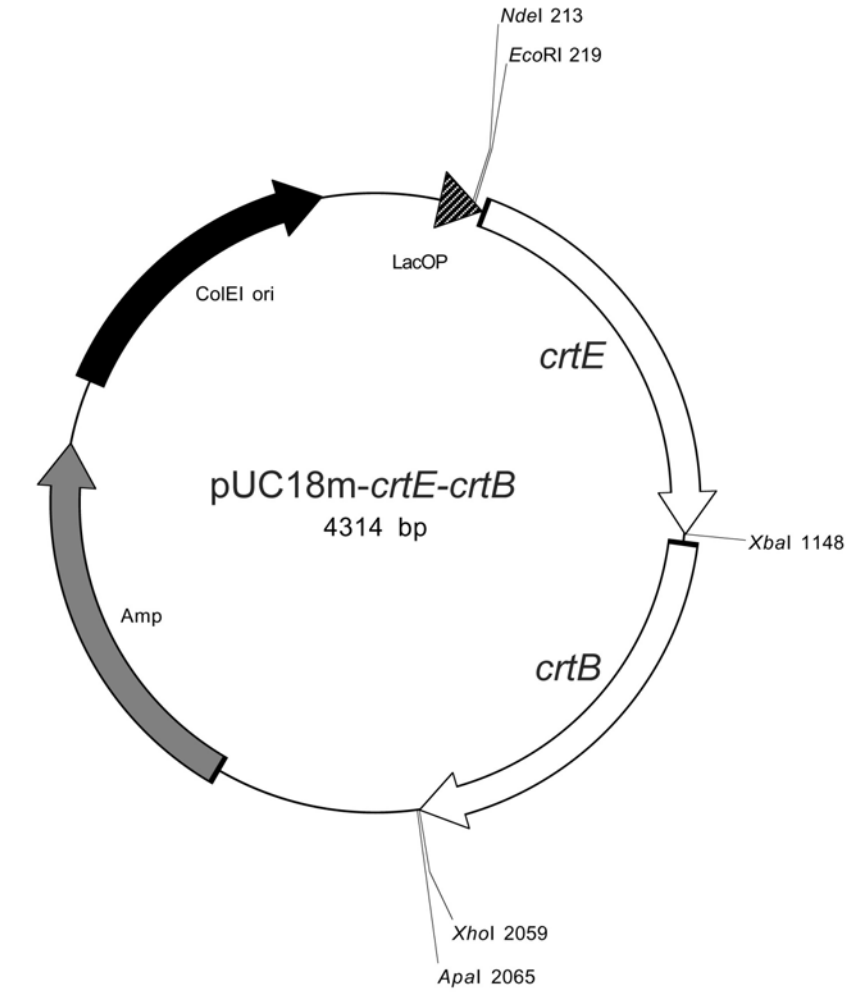
Plasmid name pUC18m-*crtE-crtB*; 4314 bp

Based on vector pUC18m

Construction

Both genes are on a single operon, regulated by a single *lac* promoter and operator. Just upstream of each gene is an optimized Shine-Dalgarno site (AGGAGG) followed by 8 spacer nucleotides. The gene *crtE* from *Erwinia uredovora* (GGPP synthase) is inserted between the *Eco*RI and *Xba*I sites. The gene *crtB* from *Erwinia uredovora* (C_{40} carotenoid synthase) is inserted between the *Xba*I and *Xho*I sites.

Plasmid map



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Plasmid name pUC18m-*crtE*; 3409 bp

Based on vector pUC18m

Construction
Identical to pUC18m-*crtE-crtB* but without the *crtB* gene.

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Plasmid name pUC18m-*crtM*$_{F26A,W38A}$; 3365 bp

Based on vector pUC18m

Construction

The gene encoding the F26A, W38A double mutant of CrtM is inserted between the *Xba*I and *Xho*I sites of pUC18m. The codon mutations encoding the F26A and W38A amino acid substitutions are shown in bold, underlined capital letters in the sequence below.

pUC18m-*crtM*$_{F26A,W38A}$ sequence

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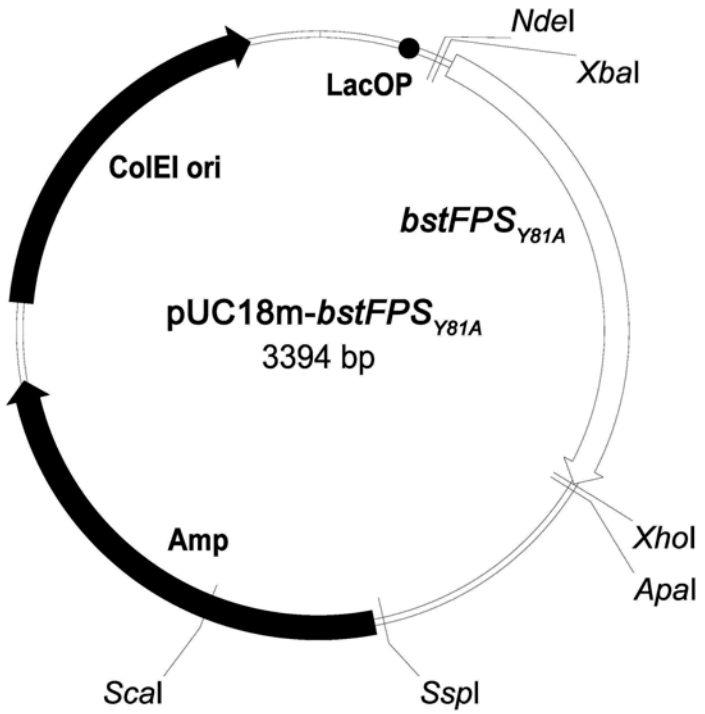
Plasmid name pUC18m-*bstFPS*$_{Y81A}$; 3394 bp

Based on vector pUC18m

Construction

The farnesyl diphosphate synthase gene from *Bacillus stearothermophilus* (with a codon mutation resulting in the amino acid substitution Y81A) is inserted in the *Xba*I and *Xho*I sites of the vector. This ORF is named *bstFPS*$_{Y81A}$ for short. Just upstream of the ORF is an optimized Shine-Dalgarno site (AGGAGG) followed by an 8-nucleotide spacer.

Plasmid map



pUC18m-*bstFPS*$_{Y81A}$ sequence

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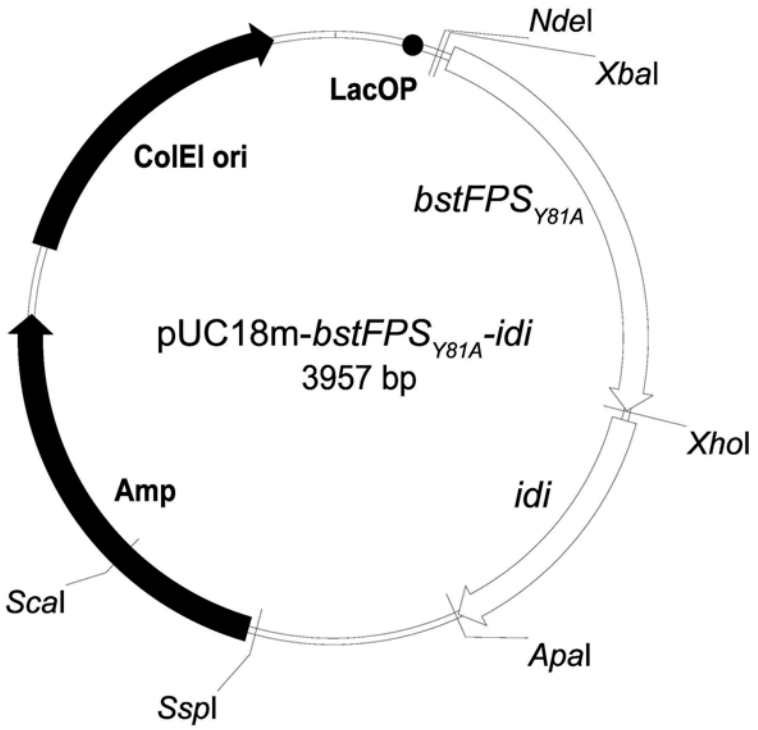
gaagcggaaga

Plasmid name pUC18m-*bstFPS*$_{Y81A}$-*idi*; 3957 bp

Based on vector pUC18m

Construction
Identical to pUC18m-*bstFPS*$_{Y81A}$ but with the addition of the *idi* gene encoding *E. coli* isopentenyl diphosphate isomerase between *Xho*I and *Apa*I sites.

Plasmid map



pUC18m-*bstFPS*$_{Y81A}$-*idi* sequence

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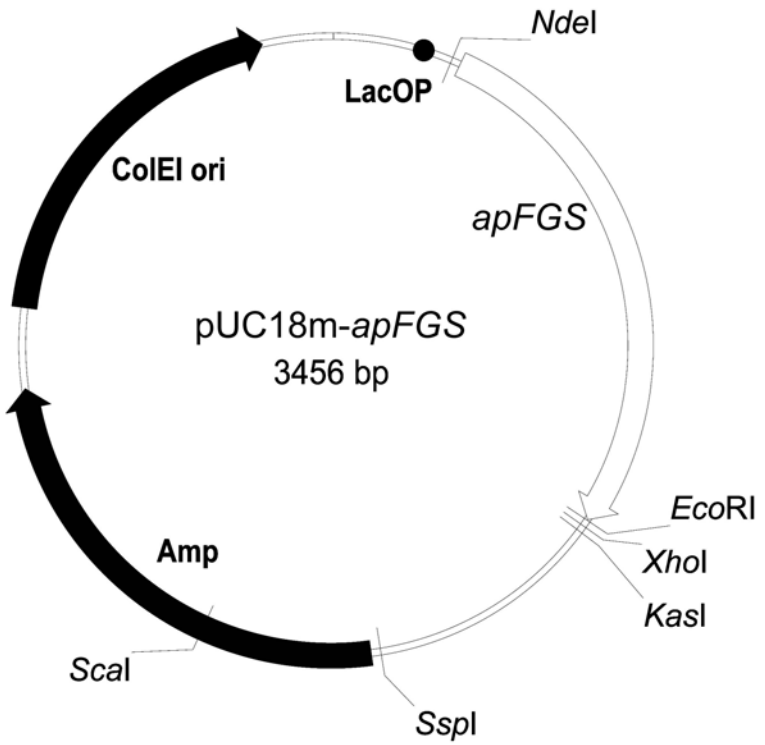
Plasmid name pUC18m-*apFGS*; 3456 bp

Based on vector pUC18m

Construction
The gene encoding farnesylgeranyl diphosphate (C_{25}PP) synthase from *Aeropyrum pernix* is inserted between the *Nde*I and *Eco*RI sites of pUC18m.

Plasmid map



pUC18m-*apFGS* sequence
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Plasmid name pUC18m-*apFGS-crtM*$_{F26A,W38A}$; 4335 bp

Based on vector pUC18m

Construction

Identical to pUC18m-*apFGS* but with the gene encoding the F26A, W38A double mutant of CrtM is inserted between the *Xba*I and *Xho*I sites. The codon mutations encoding the F26A and W38A amino acid substitutions are shown in bold, underlined capital letters in the sequence below.

pUC18m-*apFGS-crtM*$_{F26A,W38A}$ sequence

gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
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Plasmid name pUC18m-*hexPS*; 4629 bp

Based on vector pUC18m

Construction

The operon from *Micrococcus luteus* strain B-P 26 encoding the two polypeptide components of hexaprenyl diphosphate (C_{30}PP) synthase (*hexPS*) is inserted between the *Eco*RI and *Xba*I sites of pUC18m.

pUC18m-*hexPS* sequence

gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
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cgagcgcagcgagtcagtgagcgaggaagcggaaga

Plasmid name pUC18m-*hexPS-crtM-crtN*; 7044 bp

Based on vector pUC18m

Construction

Identical to plasmid pUC18m-*crtM-crtN* but with the additional operon from *Micrococcus luteus* strain B-P 26 encoding the two polypeptide components of hexaprenyl diphosphate (C_{30}PP) synthase (*hexPS*) inserted between the *Eco*RI and *Xba*I sites.

pUC18m-*hexPS-crtM-crtN* sequence

gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcggg
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Plasmid name pUC18m-*hexPS-crtB-crtI*; 7027 bp

Based on vector pUC18m

Construction

Identical to plasmid pUC18m-*crtE-crtB-crtI* but with the operon from *Micrococcus luteus* strain B-P 26 encoding the two polypeptide components of hexaprenyl diphosphate (C_{30}PP) synthase (*hexPS*) in place of *crtE* between the *Eco*RI and *Xba*I sites.

pUC18m-*hexPS-crtB-crtI* sequence

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gaggaagcggaaga

Plasmid name pUC18m-*hexPS-crtB*; 5534 bp

Based on vector pUC18m

Construction
Identical to plasmid pUC18m-*hexPS-crtB-crtI* but lacking *crtI*.

pUC18m-*hexPS-crtB* sequence
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aggaagcggaaga

Plasmid name pUC18m-*hexPS-crtM*$_{F26L}$; 5508 bp

Based on vector pUC18m

Construction

Identical to plasmid pUC18m-*hexPS-crtB* but with the gene *crtM*$_{F26L}$ encoding the F26L mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* in place of *crtB* between the *Xba*I and *Xho*I sites. The codon mutation encoding the F26L amino acid substitution in *crtM*$_{F26L}$ is shown in bold, underlined capital letters in the sequence below.

pUC18m-*hexPS-crtM*$_{F26L}$ sequence

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gagtcagtgagcgaggaagcggaaga

Plasmid name pUC18m-*hexPS-crtM*$_{F26A,W38A}$; 5508 bp

Based on vector pUC18m

Construction

Identical to plasmid pUC18m-*hexPS-crtB* but with the gene *crtM*$_{F26A,W38A}$ encoding the F26A, W38A mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* in place of *crtB* between the *Xba*I and *Xho*I sites. The codon mutations encoding the F26A and W38A amino acid substitutions in *crtM*$_{F26A,W38A}$ are shown in bold, underlined capital letters in the sequence below.

pUC18m-*hexPS-crtM*$_{F26A,W38A}$ sequence

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gcgagtcagtgagcgaggaagcggaaga

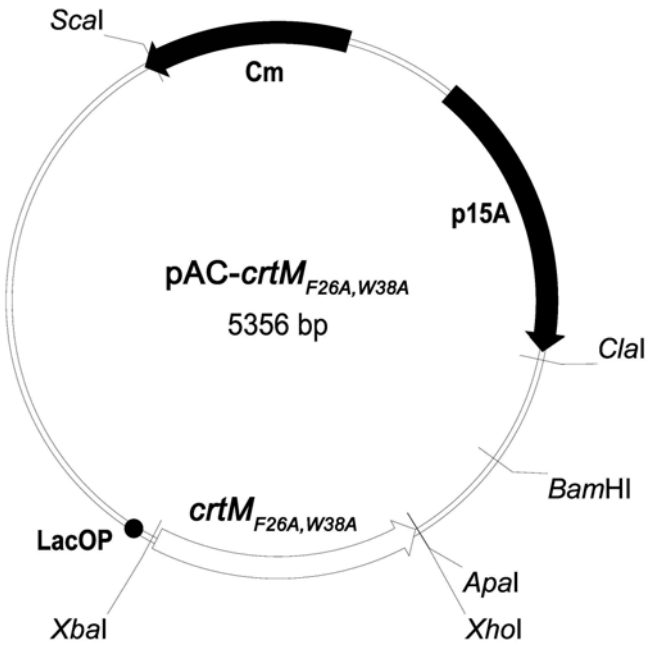
Plasmid name pAC-*crtM*$_{F26A,W38A}$; 5356 bp

Based on vector pACmod

Construction

A fragment containing a *lac* promoter/operator followed by an optimized Shine-Dalgarno sequence, 8 spacer nucleotides, and then *crtM*$_{F26A,W38A}$ (encoding the F26A, W38A variant of the C_{30} carotenoid synthase CrtM from *S. aureus*) was inserted in the *Sal*I site of pACmod (disrupting the tetracycline resistance gene on the plasmid backbone). The resulting plasmid was sequenced to confirm the direction of the insertion, which is as shown on the plasmid map. *crtM*$_{F26A,W38A}$ is directly flanked by unique *Xba*I and *Xho*I restriction sites. The codon mutations encoding the F26A and W38A amino acid substitutions are shown in bold, underlined capital letters in the sequence below. Note that the sequence shows the reverse strand of *crtM*$_{F26A,W38A}$.

Plasmid map



pAC-*crtM*$_{F26A,W38A}$ sequence

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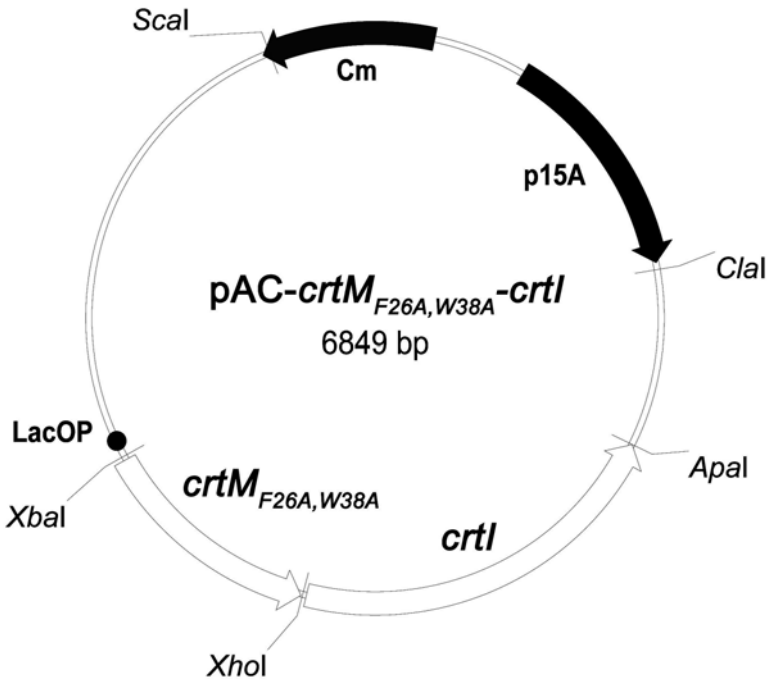
Plasmid name pAC-*crtM*$_{F26A,W38A}$-*crtI*; 6849 bp

Based on vector pACmod

Construction

Derived from pAC-*crtM*$_{F26A,W38A}$ but with the *crtI* gene (C_{40} carotenoid desaturase) from *Erwinia uredovora* inserted after the mutant allele of *crtM*. *crtI* is flanked by unique *Xho*I and *Apa*I restriction sites, which were used to insert it into pAC-*crtM*$_{F26A,W38A}$ with known orientation (see plasmid map). Each gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides. The codon mutations encoding the F26A and W38A amino acid substitutions in *crtM*$_{F26A,W38A}$ are shown in bold, underlined capital letters in the sequence below. Note that the sequence shows the reverse strand of *crtM*$_{F26A,W38A}$ and *crtI*.

Plasmid map



pAC-*crtM*$_{F26A,W38A}$-*crtI* sequence

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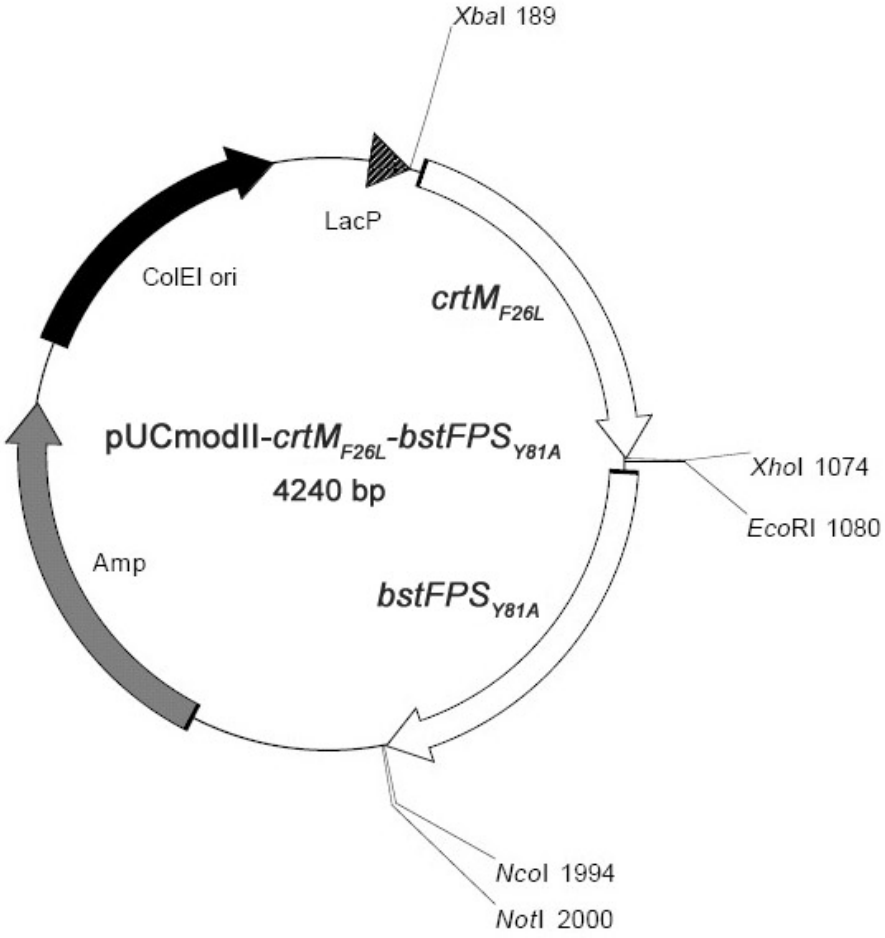
Plasmid name pUCmodII-*crtM*$_{F26L}$-*bstFPS*$_{Y81A}$; 4240 bp

Based on vector pUCmodII

Construction

The gene encoding the F26L mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* (*crtM*$_{F26L}$) is cloned between *Xba*I and *Xho*I sites. The gene encoding the Y81A mutant of the farnesyl diphosphate synthase from *Bacillus stearothermophilus* (*bstFPS*$_{Y81A}$) is cloned between *Eco*RI and *Nco*I sites. The genes are part of a single operon under the control of a single *lac* promoter (no operator). Each gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides. The codon mutation encoding the F26L amino acid substitution in *crtM*$_{F26L}$ is shown in bold, underlined capital letters in the sequence below.

Plasmid map



pUCmodII-*crtM*$_{F26L}$-*bstFPS*$_{Y81A}$ sequence

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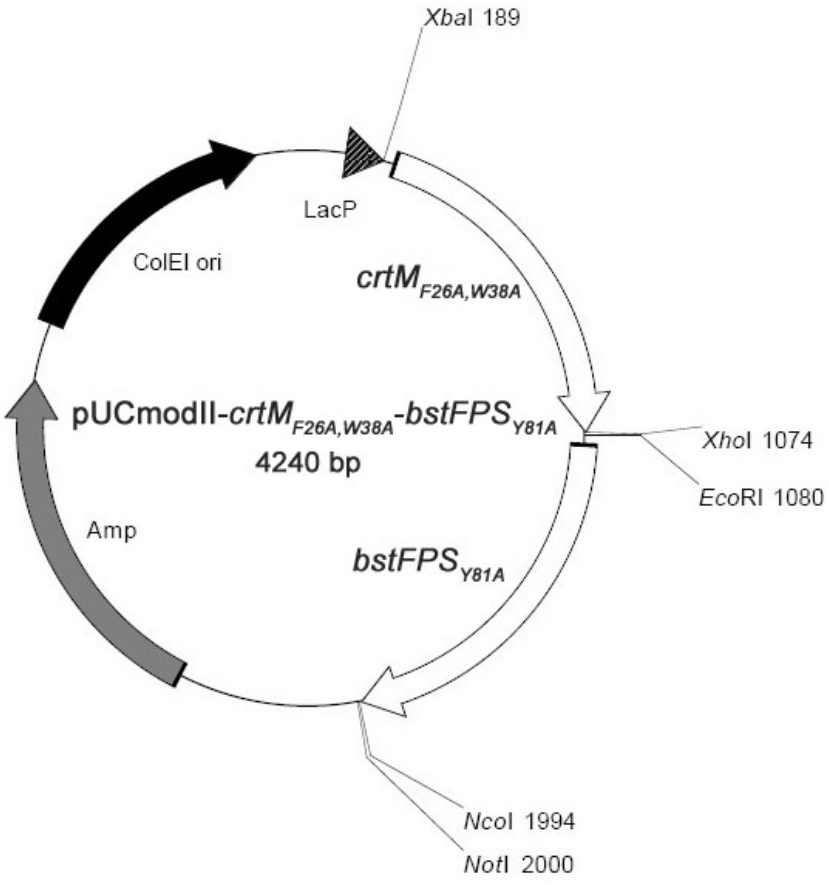
Plasmid name pUCmodII-*crtM*$_{F26A,W38A}$-*bstFPS*$_{Y81A}$; 4240 bp

Based on vector pUCmodII

Construction

The gene encoding the F26A, W38A double mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* (*crtM*$_{F26A,W38A}$) is cloned between *Xba*I and *Xho*I sites. The gene encoding the Y81A mutant of the farnesyl diphosphate synthase from *Bacillus stearothermophilus* (*bstFPS*$_{Y81A}$) is cloned between *Eco*RI and *Nco*I sites. The genes are part of a single operon under the control of a single *lac* promoter (no operator). Each gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides. The codon mutations encoding the F26A and W38A amino acid substitutions in *crtM*$_{F26A,W38A}$ are shown in bold, underlined capital letters in the sequence below.

Plasmid map



pUCmodII-*crtM*$_{F26A,W38A}$-*bstFPS*$_{Y81A}$ sequence

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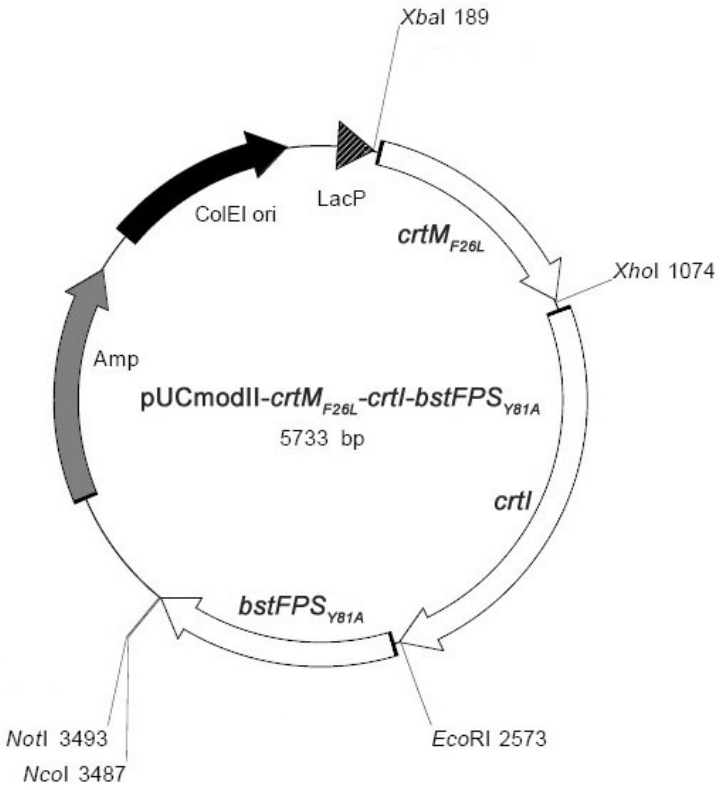
Plasmid name pUCmodII-*crtM*$_{F26L}$-*crtI*-*bstFPS*$_{Y81A}$; 5733 bp

Based on vector pUCmodII

Construction

The gene encoding the F26L mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* (*crtM*$_{F26L}$) is cloned between *Xba*I and *Xho*I sites. The gene encoding the C_{40} carotenoid desaturase CrtI from *Erwinia uredovora* (*crtI*) is cloned between *Xho*I and *Eco*RI sites. The gene encoding the Y81A mutant of the farnesyl diphosphate synthase from *Bacillus stearothermophilus* (*bstFPS*$_{Y81A}$) is cloned between *Eco*RI and *Nco*I sites. The genes are part of a single operon under the control of a single *lac* promoter (no operator). Each gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides. The codon mutation encoding the F26L amino acid substitution in *crtM*$_{F26L}$ is shown in bold, underlined capital letters in the sequence below.

Plasmid map



pUCmodII-*crtM*$_{F26L}$-*crtI*-*bstFPS*$_{Y81A}$ sequence

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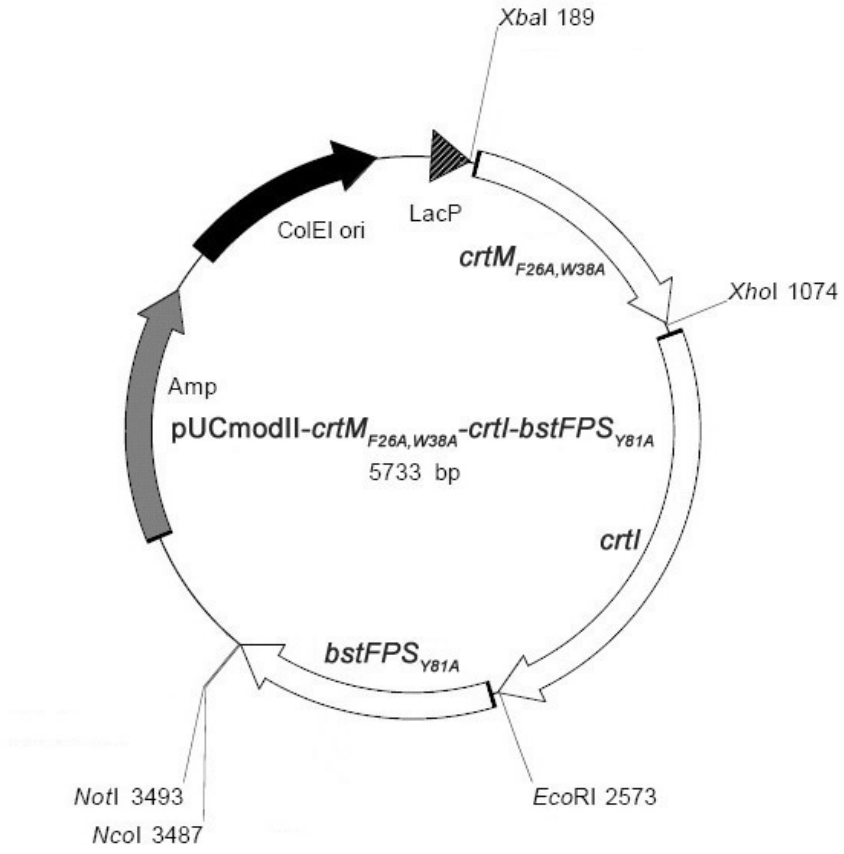
Plasmid name pUCmodII-*crtM*$_{F26A,W38A}$-*crtI-bstFPS*$_{Y81A}$; 5733 bp

Based on vector pUCmodII

Construction

The gene encoding the F26A, W38A double mutant of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* (*crtM*$_{F26A,W38A}$) is cloned between *Xba*I and *Xho*I sites. The gene encoding the C_{40} carotenoid desaturase CrtI from *Erwinia uredovora* (*crtI*) is cloned between *Xho*I and *Eco*RI sites. The gene encoding the Y81A mutant of the farnesyl diphosphate synthase from *Bacillus stearothermophilus* (*bstFPS*$_{Y81A}$) is cloned between *Eco*RI and *Nco*I sites. The genes are part of a single operon under the control of a single *lac* promoter (no operator). Each gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides. The codon mutations encoding the F26A and W38A amino acid substitutions in *crtM*$_{F26A,W38A}$ are shown in bold, underlined capital letters in the sequence below.

Plasmid map



pUCmodII-*crtM*$_{F26A,W38A}$-*crtI-bstFPS*$_{Y81A}$ sequence

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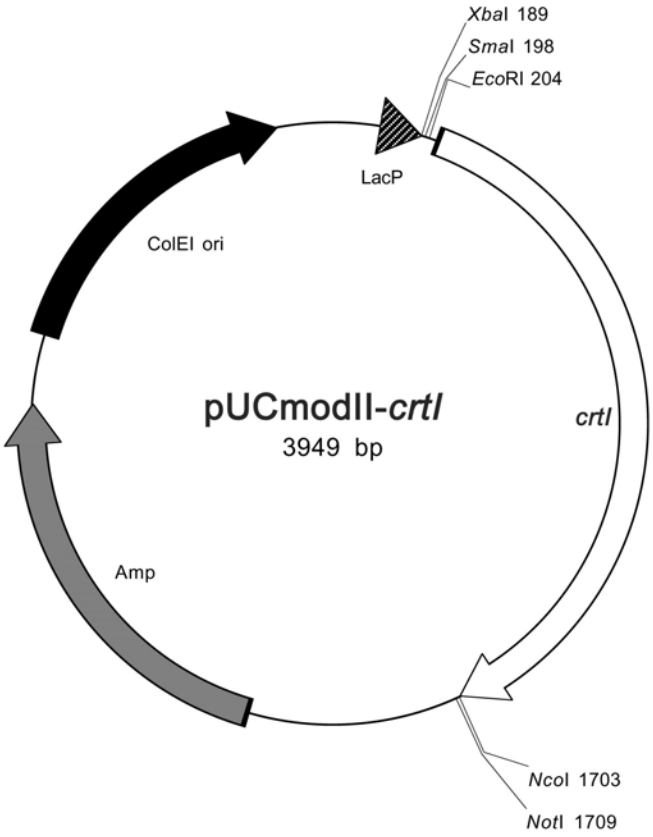
Plasmid name pUCmodII-*crtI*; 3949 bp

Based on vector pUCmodII

Construction

The gene encoding the C_{40} carotenoid desaturase CrtI from *Erwinia uredovora* (*crtI*) is cloned between the *Eco*RI and *Nco*I restriction sites of pUCmodII. The gene is preceded by a Shine-Dalgarno ribosomal binding sequence (AGGAGG) followed by 8 spacer nucleotides.

Plasmid map



pUCmodII-*crtI* sequence

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