

**Table S3.** Primers used in the study

Name	Sequence (5' → 3')	Features
<b>Southern blot primers</b>		
GG1 GG2	GACTGGCTGCTATTGGGCGAAG AGAAGGCGATAGAAGGCGATG	Primers for PCR-based generation of Tn-specific probe, $\phi$ MycoMarT7 ( $\phi$ MMT7) transposon complementary sequence (NCBI reference sequence AF411123.1) location 1467-1978
<b>Complementation construct primers</b>		
NJ1	AAATTATAAGATAACCAGTTCCTGGTACC TCACCA	Forward primer for the generation of pML- <i>crt</i> , containing: PstI site (underlined) and <i>Mycobacterium kansasii</i> ( <i>Mk</i> ) genomic complementary sequence starting at NC_022663.1 (NCBI reference sequence) genomic location 2342585
NJ2	AAACAATTGGTTCATCGAGGAGCTTCGC ATCT	Reverse primer for the generation of pML- <i>crt</i> and pML- <i>crtR</i> , containing: MfeI site (underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2350376
NJ3	AAATTATAAGAGACCGGTGAAGCCGGTG AGG	Forward primer for the generation of pML- <i>crtR</i> , containing: PstI site (underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2349511
NJ46	AAATCATGAGCCCGCCTAATGGCGGGC TTTTTTTGAATTC	Forward primer for the generation of pML- <i>cco1</i> <sup>Mk-part</sup> - <i>ccoR</i> <sup>Mk</sup> containing: BspHI site (underlined), <i>tttA</i> <sup>+</sup> transcriptional terminator from pML1335 (bold) [81], EcoRI site (bold and underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4262321
NJ47	AAACAATTGGGCCGGCCCTTGGGGTTG CGGTTTCATC	Reverse primer for the generation of pML- <i>cco1</i> <sup>Mk-part</sup> - <i>ccoR</i> <sup>Mk</sup> containing: MfeI site (underlined), FseI site (bold and underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4263914
NJ112	TGGCGGGCTTTTTTTTCTTAAGGATAACC AGTTCCTGGTACCTCACCA	Forward primer for the generation of pML- <i>crtE</i> , and for the generation of the carotenoid biosynthesis (CRT) locus promoter P1 fragment of pML- <i>crtI</i> , pML- <i>crtB</i> , pML- <i>crtYc</i> and pML- <i>crtYd</i> , containing: AflII site (underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2342585
NJ102	GGCTCGCCAAGAAGCGGAATCGACGGT GGGT	Reverse primer for the generation of the CRT locus promoter P1 fragment of pML- <i>crtI</i> , pML- <i>crtB</i> , pML- <i>crtYc</i> and pML- <i>crtYd</i> containing: <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2343093
NJ101	AAACAATTGTCATGCGGTGCGCTCCACG	Reverse primer for the generation of pML- <i>crtE</i> containing: MfeI site (underlined), <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2344128
NJ103	GCTACCGATCACCCACCGTCGATTCCG CTTCTTGGCGAGCCATGCGGACCATCC AGGGAC	Forward primer for the generation of pML- <i>crtI</i> containing: <i>Mk</i> genomic complementary sequence from NC_022663.1 genomic location 2343053 to 2343096 (3')

		of P1 promoter, bold) fused to sequence starting at NC_022663.1 genomic location 2344145 (5' of <i>crtI</i> )
NJ104	<b>CATTAGGCGGGCTCAATTGTCATGACAT</b> CTGCGCTTTACGTCGA	Reverse primer for the generation of pML- <i>crtI</i> containing: pML1335Δ <i>xyIE</i> sequence (bold), MfeI site (underlined), <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2345683
NJ105	<b>ATCACCCACCGTCGATTCCGCTTCTTG</b> <b>CGAGCCATG</b> ATTACACAACGAGTTAGACG CCGC	Forward primer for the generation of pML- <i>crtB</i> containing: <i>Mk</i> genomic complementary sequence from NC_022663.1 genomic location 2343060 to 2343096 (3' of P1 promoter, bold) fused to sequence starting at NC_022663.1 genomic location 2345683 (5' of <i>crtB</i> )
NJ106	<b>CATTAGGCGGGCTCAATTGTCATGCGG</b> CACCCGGCTC	Reverse primer for the generation of pML- <i>crtB</i> containing: pML1335Δ <i>xyIE</i> sequence (bold), MfeI site (underlined), <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2346642
NJ107	<b>GATCACCCACCGTCGATTCCGCTTCTTG</b> <b>GCGAGCCATG</b> AACGATCACTGGCAATA CCTGC	Forward primer for the generation of pML- <i>crtYc</i> containing: <i>Mk</i> genomic complementary sequence from NC_022663.1 genomic location 2343059 to 2343096 (3' of P1 promoter, bold) fused to sequence starting at 2346642 (5' of <i>crtYc</i> )
NJ108	<b>CATTAGGCGGGCTCAATTGTCATCGGC</b> GGGTGAGCCG	Reverse primer for the generation of pML- <i>crtYc</i> containing: pML1335Δ <i>xyIE</i> sequence (bold), MfeI site (underlined), <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2346963
NJ109	<b>ACCGATCACCCACCGTCGATTCCGCTT</b> <b>CTTGCGAGCCATG</b> ACCGGGCTGGGAT ATACGC	Forward primer for the generation of pML- <i>crtYd</i> containing: <i>Mk</i> genomic complementary sequence from NC_022663.1 genomic location 2343056 to 2343096 (3' of P1 promoter, bold) fused to sequence starting at 2346962 (5' of <i>crtYd</i> )
NJ110	<b>CATTAGGCGGGCTCAATTGTCATCGACG</b> CGCTCGCGG	Reverse primer for the generation of pML- <i>crtYd</i> containing: pML1335Δ <i>xyIE</i> sequence (bold), MfeI site (underlined), <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2347270
NJ147	<b>AAACTTAAGGAAGGAGATATACAT</b> ATGA CCGGGCTGGGATATACGC	Forward primer for the generation of pML <sup>myc</sup> - <i>crtYd</i> , containing: AflII site (underlined), ribosome binding site (bold) and spacer (bold, underlined) from pSE100 and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2346959
NJ145	<b>AAACCTGCAGGTCATCGACGCGCTCGC</b> GG	Reverse primer for the generation of pML <sup>myc</sup> - <i>crtYd</i> , containing: SbfI site (underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 2347270
NJ64	<b>AAACTTAAGGAAGGAGATATACAT</b> GTGA CGACAATGCGAACTGCC	Forward primer for the generation of pML <sup>myc</sup> - <i>cco1<sup>Mk</sup></i> , containing: AflII site (underlined), ribosome binding site (bold) and spacer (bold, underlined) from pSE100 and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 (NCBI reference sequence) genomic location 4263096
NJ65	<b>AAACCTGCAGGTC</b> AAGGAGTCGCCGGT GC	Reverse primer for the generation of pML <sup>myc</sup> - <i>cco1<sup>Mk</sup></i> , containing: SbfI site (underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4264616
NJ66	<b>AAACTTAAGGAAGGAGATATACAT</b> ATGA CCACCGCACAAAGCC	Forward primer for the generation of pML <sup>myc</sup> - <i>cco1<sup>Mt</sup></i> , containing: AflII site (underlined), ribosome binding site (bold) and spacer (bold, underlined) from pSE100 and

		<i>Mycobacterium tuberculosis</i> ( <i>Mt</i> ) genomic complementary sequence starting at NC_000962.3 (NCBI reference sequence) genomic location 750000
NJ67	AAACCTGCAGGTCAGGTGGTCGGCGCC CA	Reverse primer for the generation of pML <sup>myc-cco1<sup>Mt</sup></sup> , containing: SbfI site (underlined) and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 751505
NJ111	AAACTTAAGTCAGCGGGTTTGCAGGGCA TCGGGT	Forward primer for the generation of pML-cco1 <sup>Mk</sup> , containing: AflII site (underlined), stop codon (bold) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4262909
NJ78	AAACAATTGTCAAGGAGTCGCCGGTGCC C	Reverse primer for the generation of pML-cco1 <sup>Mk</sup> containing: MfeI site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_022663.1 genomic location 4264616
NJ79	AAACTTAAGCCTTGCCATGTTCTCACCA GGGCG	Forward primer for the generation of pML-cco1 <sup>Mt</sup> , containing: AflII site (underlined) and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 749930
NJ80	AAACAATTGTCAGGTGGTCGGCGCCCA G	Reverse primer for the generation of pML-cco1 <sup>Mt</sup> containing: MfeI site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 751505
NJ56	AAATCATGAGCCCGCCTAATGGCGGGC TTTTTTTCTTAAGTCAACCGAAAGCGGA GCGC	Forward primer for the generation of pML-ccoR <sup>Mk</sup> containing: BspHI site (underlined), <i>ttrpA</i> + transcriptional terminator from pML1335 (bold), AflII site (bold, underlined) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4262322
NJ57	AAACAATTGGGCGGCCCTTCGCATTGTC GTCACGGCCGA	Reverse primer for the generation of pML-ccoR <sup>Mk</sup> containing: MfeI site (underlined), FseI site (bold) and <i>Mk</i> genomic complementary sequence starting at NC_022663.1 genomic location 4263111
NJ58	AAATCATGAGCCCGCCTAATGGCGGGC TTTTTTTCTTAAGTCAAGTAGCTCTGCC CAGC	Forward primer for the generation of pML-ccoR <sup>Mt</sup> containing: BspHI site (bold), <i>ttrpA</i> + transcriptional terminator from pML1335 (underlined), AflII site (bold and underlined) and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 749235
NJ59	AAACAATTGGGCGGCCGGCGGCTTGT GCGGTGGTCAT	Reverse primer for the generation of pML-ccoR <sup>Mt</sup> containing: MfeI site (underlined), FseI site (bold) and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 750020
NJ119	AAACTTAAGCCGCAGACGCCTCGAAGCC T	Forward primer for the generation of pML- <i>fnr1</i> , containing: AflII site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 4704636
NJ121	AAACAATTGTTAGATGTCGACCACACAAT CGCCCGAGGCA	Reverse primer for the generation of pML- <i>fnr1</i> containing: MfeI site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 4706005
NJ122	AAACTTAAGGCCGGATGGGGATCTGTCA ATCGT	Forward primer for the generation of pML- <i>desA3</i> , containing: AflII site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 4705860

NJ120	AAACAATTGTCAGGCCGCCAGGTCCGCT	Reverse primer for the generation of pML- <i>desA3</i> containing: MfeI site (underlined), and <i>Mt</i> genomic complementary sequence starting at NC_000962.3 genomic location 4707355
<b>Plasmid rescue method primers</b>		
CB2	GAGATAGGGTTGAGTGTGTTCC	Reverse (relative to the kanamycin-resistance cassette ( $Km^R$ )), outward-facing sequencing primer starting at $\phi$ MMT7 transposon complementary sequence (NCBI reference sequence AF411123.1) location 84; see Budell et al. 2020 [38]
SR2b	CGCTTCCTCGTGCTTTACGGTATCGC	Forward (relative to $Km^R$ ), outward-facing sequencing primer starting at $\phi$ MMT7 transposon complementary sequence (AF411123.1) location 1914; see Budell et al. 2020 [38]
<b>AP-PCR method primers</b>		
TR-1	GGATCTGGTCCGCGCACATT	Reverse, transposon primer for the first round of arbitrarily primed PCR (AP-PCR), starting at $\phi$ MMT7 transposon complementary sequence (AF411123.1) location 240
ARB-1b	GCGCTCACAGCAGTCGAGTCGAGANNN NNNNNNN <b>CGGCG</b>	Forward, arbitrary primer for the first round of AP-PCR, containing an adapter sequence, a stretch of random nucleotides (N) and a pentameric sequence common in the <i>Mk</i> genome (bold)
TR-2	CCAATAGGCCGAAATCGGCAAATCCCT	Reverse, transposon primer for the second round of AP-PCR, starting at $\phi$ MMT7 transposon complementary sequence (AF411123.1) location 133; This primer is also used for sequencing of PCR products.
ARB-2	GCGCTCACAGCAGTCGAGTCGAGA	Forward primer for the second round of AP-PCR, binding to the adapter sequence of the PCR fragment created with primer ARB-1b in the first round of AP-PCR
<b>Locus-targeted PCR method primers</b>		
NJ1	AAATTATAAGATAACCAGTTCCTGGTACC TCACCA	Forward (NJ1) and reverse (NJ37) primers for fragment 1 of the <i>Mk</i> carotenoid biosynthesis locus (CRT locus), NC_022663.1 genomic complementary sequence location 2342585 – 2344674, yielding a 2099 bp fragment
NJ37	ATGGCGTCCCAGTTGCGGAAT	
NJ8	GGCTGTACCGCACCGAAAT	Forward (NJ8) and reverse (NJ38) primers for fragment number 2 of the <i>Mk</i> CRT locus, NC_022663.1 genomic complementary sequence location 2344554 – 2346653, yielding a 2100 bp fragment
NJ38	CCAGTGATCGTTCATGCGGCA	
NJ13	GACGATTTGAGGTGTTCACTCA	Forward (NJ13) and reverse (NJ39) primers for fragment number 3 of the <i>Mk</i> CRT, NC_022663.1 genomic complementary sequence location 2346499 – 2348334, yielding a 1836 bp fragment
NJ39	CCGTACGTTGAGCGCATCGA	
NJ17	TCGCGGTCGTGGTCTTTT	Forward (NJ17) and reverse (NJ2) primers for fragment number 3 of the <i>Mk</i> CRT, NC_022663.1 genomic complementary sequence location 2348240 – 2350376, yielding a 2146 bp fragment
NJ2	AAACAATTGGTTCATCGAGGAGCTTCGC ATCT	

NJ156	GGCCCAGCACGCAATCACCAGGA	Forward (NJ156) and reverse (NJ157) primers for a fragment of the <i>Mk</i> CRT locus containing <i>MKAN_RS10035</i> and <i>MKAN_RS10040</i> ( <i>crtYc</i> and <i>crtYd</i> ), NC_022663.1 genomic complementary sequence location 2346366 – 2347539, yielding a 1174 bp fragment
NJ157	GCCACTGTCCGAGTTCACCAGCAGCA	
RT-qPCR primers		
NJ81	CCAGCCCCGTACTCTCGACGA	Forward (NJ81) and reverse (CB11, see Budell et al. 2020 [38]) RT-qPCR primers for <i>MKAN_RS24220</i> ( <i>sigA</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 5589256 – 5589392, yielding a 133-bp amplicon
CB11	ATCCAGGTAGTCGCGCAGGACT	
NJ82	CTGCGGACCGTCCTCGACAT	Forward (NJ82) and reverse (NJ83) RT-qPCR primers for <i>MKAN_RS10020</i> ( <i>crtE</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2343625 – 2343758, yielding a 133-bp amplicon
NJ83	ATCGGCACCGTAGAGCCCGA	
NJ148	CTCGACGTTGTCACCCCGCT	Forward (NJ148) and reverse (NJ149) RT-qPCR primers for <i>MKAN_RS10025</i> ( <i>crtI</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2345402 – 2345530, yielding a 129-bp amplicon
NJ149	ATTGTCGACGCCGCGAACCA	
NJ150	GGCAACCGCTTTGACGCTGTACT	Forward (NJ150) and reverse (NJ151) RT-qPCR primers for <i>MKAN_RS10030</i> ( <i>crtB</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2346450 - 2346584, yielding a 135-bp amplicon
NJ151	CAGGCTTGGATCAGTCCGGCT	
NJ152	GTGGCCGTTCCGTTTCGGGT	Forward (NJ152) and reverse (NJ153) RT-qPCR primers for <i>MKAN_RS10035</i> ( <i>crtYc</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2346843 - 2346954, yielding a 112-bp amplicon
NJ153	GGGTGAGCCGATCGAGAATCGT	
NJ154	AAACGACGGGCCTGCGGTT	Forward (NJ154) and reverse (NJ155) RT-qPCR primers for <i>MKAN_RS10040</i> ( <i>crtYd</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2347158 - 2347277, yielding a 120-bp amplicon
NJ155	GGCGACTTCATCGACGCGCT	
NJ84	ACCTCACGTTTGCCGGTGGT	Forward (NJ84) and reverse (NJ85) RT-qPCR primers for <i>MKAN_RS10045</i> ( <i>mmpL1</i> ), amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2348923 - 2349052, yielding a 130-bp amplicon
NJ85	AAGCCGCTGTGAGGGAAAGCA	

NJ86	GCAGCATGGAGTTGGCTCACGA	Forward (NJ86) and reverse (NJ87) RT-qPCR primers for <i>MKAN_RS10050 (crtR)</i> , amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2350031 - 2350168, yielding a 138-bp amplicon
NJ87	CAAATGCAGACATCCCCGCGATCA	
NJ89	TCGCTACGGTGAACTGCGCT	Forward (NJ89) and reverse (NJ88) RT-qPCR primers for <i>MKAN_RS10055 (fni)</i> , amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 2350482 - 2350593, yielding a 134-bp amplicon
NJ88	CGTCCATCCCGGTGCGGATA	
NJ91	CCATCGAGCGGCCGCATATGT	Forward (NJ91) and reverse (NJ90) RT-qPCR primers for <i>MKAN_RS18575 (ccoR<sup>Mk</sup>)</i> , amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 4262594 - 4262724, yielding a 131-bp amplicon
NJ90	ATGCGCGAAGCTGGGATGGT	
NJ92	CCGCTCAACGCCTACACCGA	Forward (NJ92) and reverse (NJ93) RT-qPCR primers for <i>MKAN_RS18580 (cco1<sup>Mk</sup>)</i> , amplifying <i>Mk</i> NC_022663.1 genomic complementary sequence location 4264038 - 4264165, yielding a 128-bp amplicon
NJ93	CGATCCAGTGACGGACGGGTGT	