

Sfr_RpuS -MLSGSVILASAFAYLLLLFAVASYGDRRASRSKIA--SKGRPLVYALSLAIYCTSWTYFGGVLAAEHGLEFTGIYIGPILMFTLGMPLIRRIIVRLAKTEKLSVADFVAARYGKN
Sfr_CrbS --MPGWVLSVSTAYVVIIFLIAWTDHRGTGRRRLAVPSEWLAALGYALTIAINYTSWSFYGSVGRALS-GFDFLSIYVGPTLVLFVGGRLIARIVTVAKSQNITSIADFIAARYGKS
Pfl_CrbS MTLSSGLIAAVALAYMAIMFAIAFYGDRRRAP---LP-PRVRAMVYSLSLAVYCTSWTFFGAVGQAAEQWAFLLPYLGPVLLLVLPAPVWLQKMILISKQENITSIADFIAARYGKS

Sfr_RpuS PAVAAIVALISLVGAIPYIALQLKAVSSSVAAMIDTSDYGIGAGENFI~~DLPLLVTLFACFAIVFGTRHDATEHQDGLILAIAMESVVKLVAMLTAGIYIVFVLFDGPANLAAAQ~~
Sfr_CrbS QALAAVLTMAALLAVLPYIALQLKAVGTSFDILTSPVAAPGGTRPIRGDSTLAVAASMAALFAIMFGVRHIIHASEHHRGLMAAIAFESLVKLSAFVIVALFIVFGMFDGLGDLIARTQA
Pfl_CrbS QSLAVVVALICLVGLVPYIALQLKGIVLGVNLLIGAGADTTGTRA--QDTALIVSLVLALFTTVFGTRNLDATEHHRGMVLAIAFESLVKLSAFVAVGAFVTVGLYDGFGLFSQAIL

Sfr_RpuS SPAVLSALEYQTPVARWILLIVLS~~SFGIIMLP~~PRQFHVTVVENRTENELRTAGILFPLYLIAINLFLVPIAIGILTFSGSGDADLYLLALPLAGDVPFITLLTFIGGFSAAATAMVIVA
Sfr_CrbS DPRMESLLAPDFADPTWISNTIIAASIFLCLPHMFHVAVVENQSLAQARAAAWLYPAYLIVFSVLMVPIAMAGVARFADTVNPDTFVISLPLAADAPLMALVAFLGGSAAATGMVIVA
Pfl_CrbS APRLEEWKETVNWPSMVVQTGVAMMAIICLPRQFHVTVVENIDPQDLRLAKWVFPAYLILAAALFVVPIALGKMMPLPGSVLPDSYVISLPMAEHPALAVLAFIGGSAATGMVIVA

Sfr_RpuS SVALSIMISNDIVMPVFLRQRLGTRGTLQESMAGTLNIRRTAIFAVLLLGYSYRSADMSAGLASGLLSFVAISQMAPALLGGLVWRQANARGAIGMVSGLVWAYALFLPSLGG
Sfr_CrbS SVALSTMLCNDVIVPLLLRSRLVGRSGAIWSPSALLLVRRSVAGILLAYMMNRLVDQAYPLTVIGLLSFVAIAQFGPAFLGGLLWRRKAGAGAVAGISIGFAFWIYTLTLLPSIAP
Pfl_CrbS SIALSTMVSNMMLPWLLRRSSAERPFVEF--RHWMLSVRRVSIVIILLAYVSYRLLGSTASLATIGQIAFAAVTQLAPAMLGALYWKQANRRGVFAGLAAGTFLWFYTLVLPVTA

Sfr_RpuS PDNSHVAS--TI--LSFLLPFTDLFSGPQSDPLVNASALSLLVNVFAYVLGSLTRTPKPLERFQAGVITRRSRTERAFRGRKTKVTVRDLKTTIARYMGDERMQRSFHTYEQQSGR
Sfr_CrbS LVPADVALLVANGPFGGLAW-LRPQSLFGVAGLDPISHATLWSLGANLLVFAAVSLADRSPERLQAEFAEGTPAP-PPLASLPLVTRLDLKTFAARFVGAERSASAFEDFVEQRRR
Pfl_CrbS SLGWSLS----LFPGLTW-MHSHPLG--LSVTSLTGLTVFSLAGNFTLFVWWSMLSRTRVSEHWQAGRFIQEISQ-RASARSMLSVQISDLLSLAARFVGEERAQQSFIRFAYRQKG

Sfr_RpuS WLDENASADMALVHFSEQLLGSAGSSSARLVSLVLQRMDDPSSDTAWLLDQASEALQHNDQMLQALSQMDQGIQVFDNASNLIINWRRFRELDDLPEAAGQVGFPLADIVAILAR
Sfr_CrbS --RNDGLADTEAMRFTENLIAGALGAASARVMAAALESRLSRKAAGVGLDEASQALHFNRLKLLQGALSVPGICVFDANLAEAWNARFLTLLDLPRDLIRVGLPLAELVDFNRE
Pfl_CrbS GFNPQNQADNDWIAHTERLLAGVLGASSTRAVVKAIEGREMQLEDVVRIADEASEVLQFNRLALQGAENITQGISVVDQSLKLVAWNRRYLELFNYPDGLISVGRPIADIIRYNAE

Sfr_RpuS RGDIRKDEEKTIVANFLALD---KPFLL-LGNGTRIVEVRTNAMPDKGIVTTYTDITPRVAADMALKQANETLELVAERTSELTRVNRELGEARAVAEDANIGKTRFFAAAGHDIL
Sfr_CrbS RGEYDAEDLKALLVNR-DLATQSWPYVYERKPDGMVLEIAYDRMAAGGYISTYTDVTERHRAAGLRANEELERRVQERTQALEQ-----AKAEAERANIGKTRFLAAASHDLL
Pfl_CrbS RGLCGPGEAEVHVARRLHWMRQGRAHTSERLFPNGRVEILIGNPMPGGGFVMSFTDITAFREAEQALTEANEGLEQRTERTHELSQLNVALTDAKGVAESASQSKTRFLAAVSHDLM

Sfr_RpuS PLNAARLYSSSLVQERLG-----DSDNKALVQNDISSLESVEAILGAVLDISRLDTGAMKPRQAVPLNDLLRRIETDFAPMARAKDIAFTVMPTSLVVRSDPNLLRRVVQNL
Sfr_CrbS QPLNAARLYLAALDESRLKPDSAEKDLRTEERALAKNAAAALSSTERLLDELIDISSYSGAVRAQPVDFSVGSLLAQLELEFSALARQRLTLKVVASTLSVRTDPQLLRRVLQNL
Pfl_CrbS QPLNAARLFSAALSHQ-----NDGLSSEARQLVQHLDSLSRSAEDLISDLIDISRLNKGINPQRPVFLNELFDTLGAEFKALAEQGLRFLRGSRRLRVDSIDIKLLRRLQNF

Sfr_RpuS VSNAIKYTLEGKVLGVGRQHGTATIEVLDSGIGIPASKFRTVFKEFARLDEGAR-TASGLGLGLSIVDRISRVLNHPVGLQSKPGKGTGFKVSVPLDTSATEPARPQVAAATKTSEA
Sfr_CrbS LSNAIYRTPRGRVLLGCRRRGDSLRIEVRDGTIGIAPERQOEIIEEFRLDTGEE-REKSGSLGLAIVERICRLDLPLEVRSRPGQGTCTFWTVPRGTAGAAATVSEKS-ATPSVAS
Pfl_CrbS LTNAFRYA-DGPVLLGVRRRKGECLLEVWDRGPGIPQDKQKQVIFEEFKRLDSHQTRAEGKGLGLGLAIADGLCRVLDHRLSVRSWPGKGSVFSVRVPLARNQATPLVKTPQ-ETGLP-

Sfr_RpuS LTGLAVVCIDNEPKILEGMALLGGWGC~~AVTTVESV~~AACARMGPGNLPARPDAIVADYHLD-GTGIEAIAAIRALWQESIPALMVTADRTPEVRGAERDGVSLQHKPVRPAALRAW
Sfr_CrbS HTALTVLCVDNDEAIRAGLSALLSRWGHRIVASDDVTAL-ANC--SGQVPDLALVDYHLD-GTTGVEVLGRRLKHWGREVRGLVITADRSEAVGAKAKALACEIMAKPVKPAALRRY
Pfl_CrbS LSGAQVLCVDNEESILIGMRSLLTRWGC~~EVW~~TATDQQAALLA--EGVRQALALVDYHLDHGETGTLMGWLRAQLAEPIGVVISADGRPEMVAEVAAGLDYLAKPVKPAALRAL

Sfr_RpuS LTQLAAAGRTAAE-----
Sfr_CrbS LNAVGLQKGAGLQRDGEEVHEHAHRHG
Pfl_CrbS LSRHLPL-----

Figure S1. Alignment of NGR_RS10960 (RpuS) and NGR_RS12300 (CrbS) from *S. fredii* NGR234 with CrbS from *P. fluorescens* SBW25. Relevant features are marked as follows: Red, Transmembrane regions of the SLC5 domain; Light green, STAC domain; Orange, PAS domain; Brown, DHp domain; Dark green, CA domain; Light Blue, REC Domain. Underlined bases indicate coiled coils.

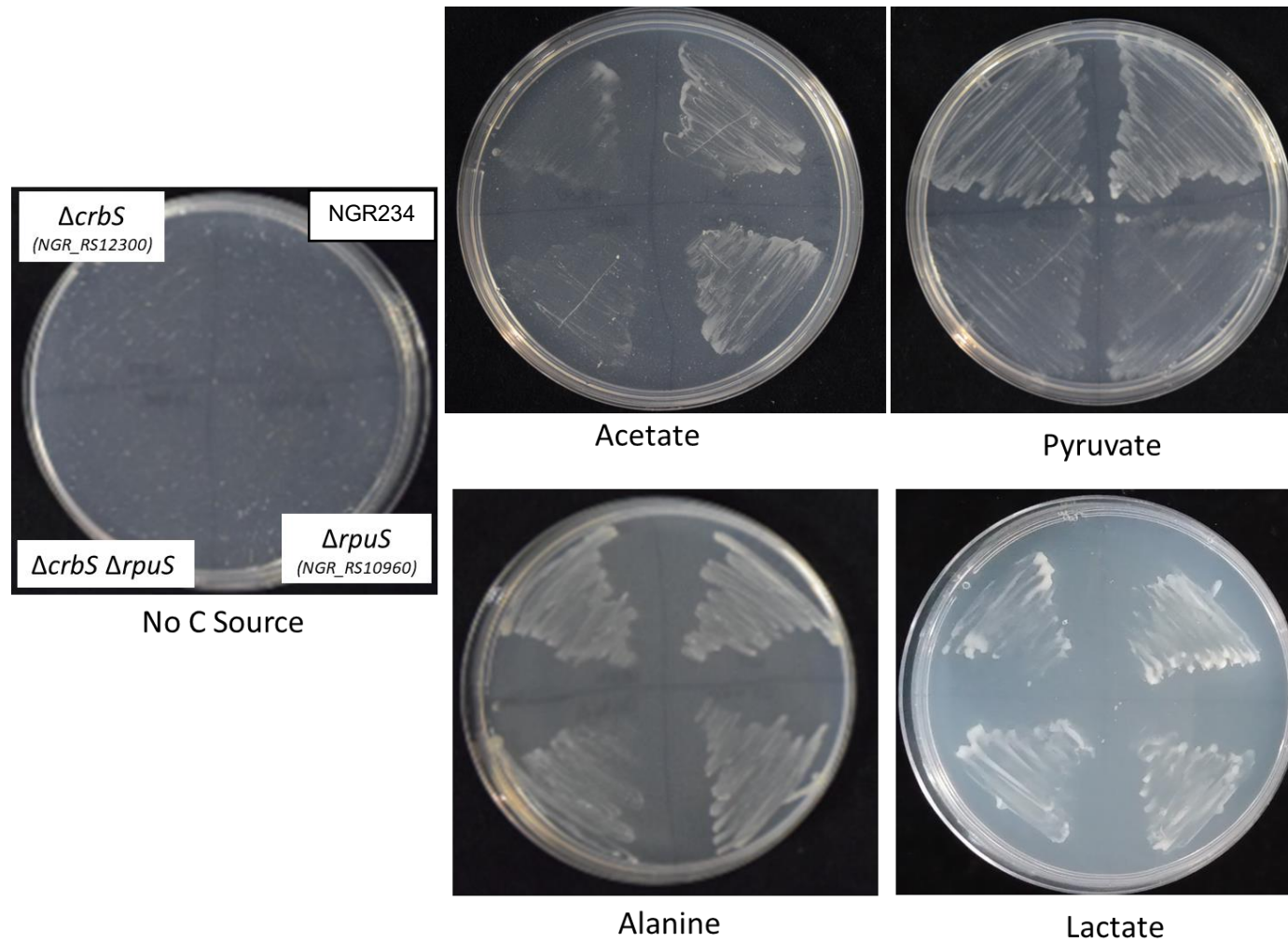


Figure S2. Growth of different *S. fredii* NGR234 strains in minimal media complemented with acetate, pyruvate or alanine as sole carbon source.

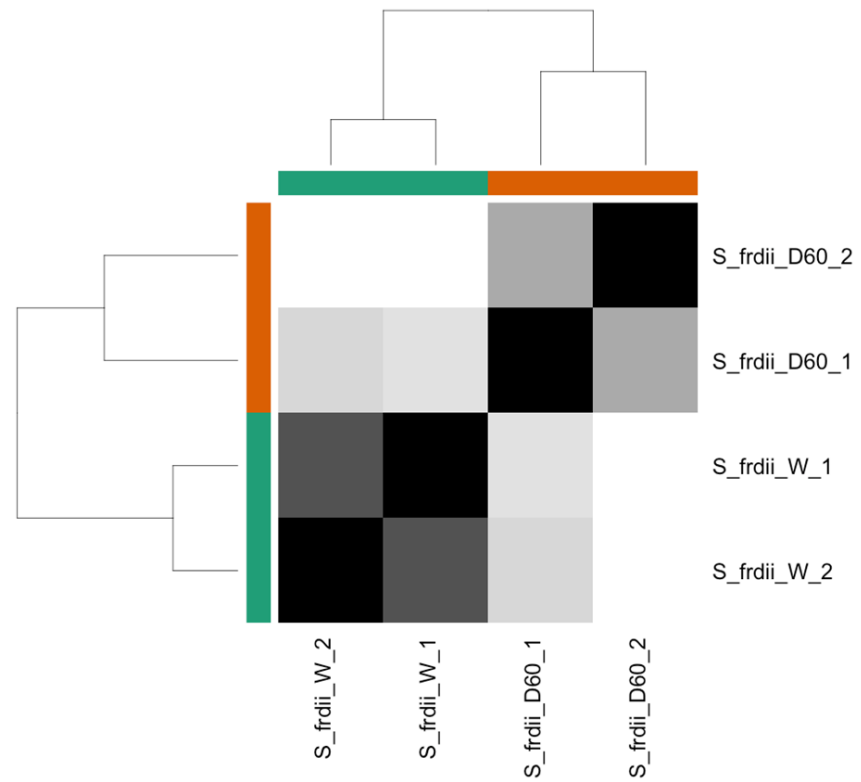


Figure S3. Global samples distance between RNAseq results from *S. fredii* NGR234 (S_frdii_W) and $\Delta rpuS$ strains (S_frdii_D60)

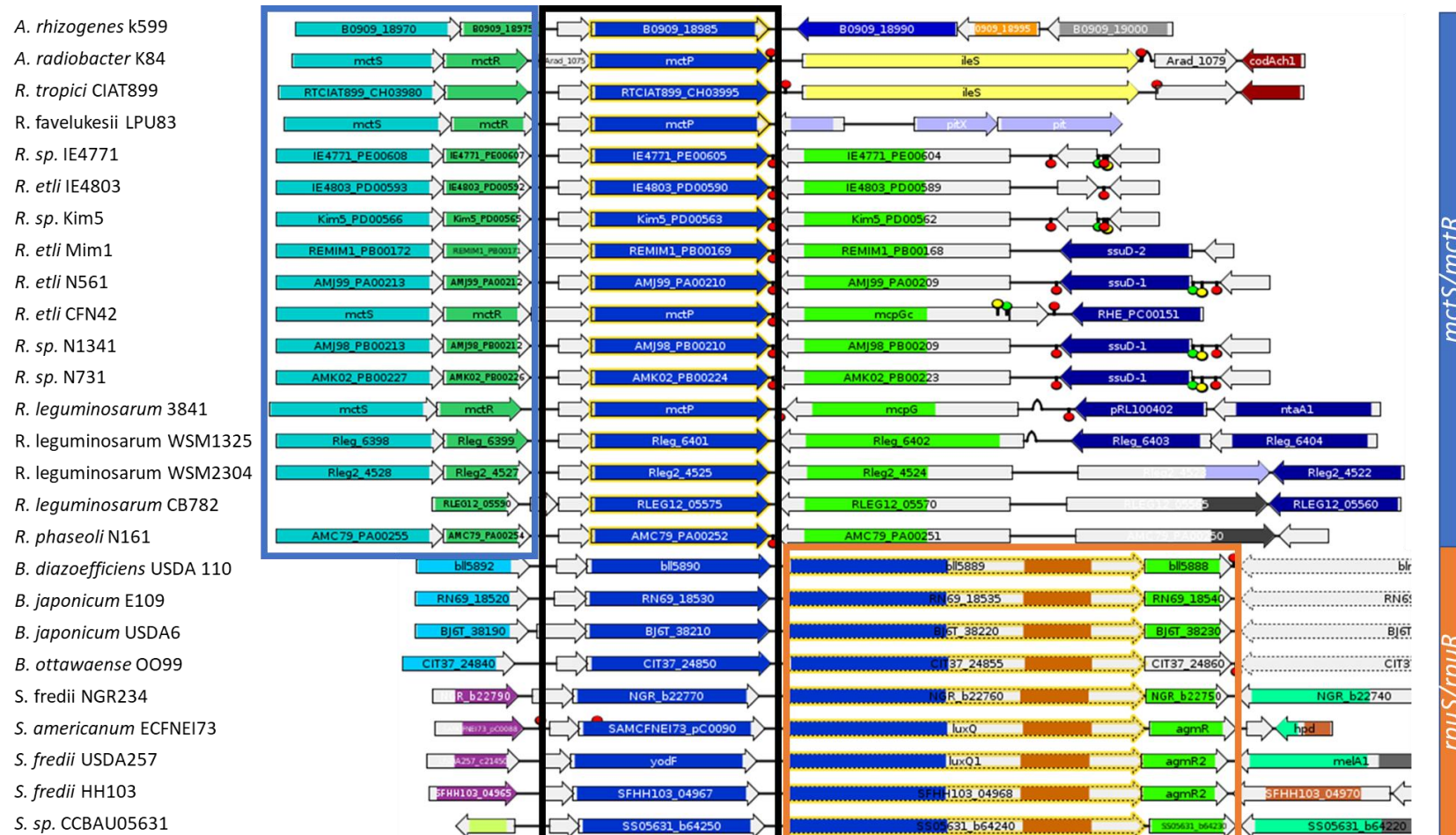


Figure S4. Genetic context of *mctP* (Blue arrows, black box) in different Rhizobaceae neighbored by a *mctS/mctR* system (Blue box) or a *rpuS/rpuR* system (Orange box)

Primer/Usage	Sequence
Deletion of <i>rpuS</i>	
22760Au	CGACGCTGTTCAAGACGCTGTTC
22760Al	GGCTGCCTGGACCATGGCACCTCGCTGCCCCAACTG
22760Bu	GGGCAGCGAGGTGCCATGGTCCAGGCAGCCTTGCG
22760Bl	GCGCGGCTATCGCGGCTATG
del22760Af	TGACTTAAGCTTCCTTCTCGGCGATCGCCATC
del22760AR	TGACTTGAATTCGCCGCACTGAAGAAGCATCTCAG
Deletion of <i>rpuR</i>	
22750u	TTGTAATAAGCTTAATCTGCTCTCGAACGCCATAC
22750l	TTGTAATAATTCCTATTTGCTCGACGACAAGGG
22750delF	CGGGTGATCATCCGCGATG
22750delR	TTCTCGATGGTGCTGCACTGAA
Deletion of <i>mctP</i>	
mctPu	TTGTAATAAGCTTCGATACGCGCCGTTACGC
mctpl	TTGTAATAATTCATGATGGCGAAGAGCGCCATCG
mctPdelF	TAAGTTTGAGGGGCCGGC
mctPdelR	TTAGCCGTCATGACGCACGCTC
Deletion of <i>NGR_RS10970</i>	
Dufu	TTGTAATAAGCTTCCTCGACAAGAACAAGCGGG
Dufl	TTGTAATAATTCATACATGAAGGCGGCAAGCG
DufdelF	ATGACGGCTAACATCGACATGTCTG
DufdelR	GTTATCCTCCCGCGATGTCTG
Deletion of <i>crbS</i>	
1850Au	TTTACGCGCCTAGAGCGGGATGA
1850Al	AAATTCCGCGTCCCGGCCTCACTCCTCGACGGTGAG
1850Bu	GTCGAGGAGTGAGGCCGGGACGCGGAATTTGCC
1850Bl	TCGTCGAGGCCGGCGATT
del1850AF	TGACTTAAGCTTCCCGCATCCCGCTCTAACCTAT
del1850AR	TGACTTCCCGGGGATGCTTCTCGATCTCACCATGCC
Deletion of <i>crbR</i>	
1840u	TTGTAATAAGCTTTTTTCGATCGTCGACCGCATC
1840l	TTGTAATAATCAATGAAGCCGTCGGCGAGAT
1840del1	GATGACGCTCCCTCCCAAGAG
1840del2	TAAGCGGTAACAACGCGGACG
Cloning of <i>rpuS</i> in pSRKgm	
pSRKRpusF	CGCATCGTCATCGCGGATGAAAGCTTATCGATACCGTCGA
pSRKRpusR	AGGACGACCCAACCGGGCATATGCTGTTTCCTGTGTGAAA
RpuSpSRKF	TTTCACACAGGAAACAGCATATGCCCGGTTGGGTGTCCT
RpuSpSRKR	TCGACGGTATCGATAAGCTTTCATCCGCGATGACGATGCG

Cloning of <i>rpvR</i> in pSRKgm	
22750NdeI	TTGTA <u>CTCATATGGTCCAGGCAGCCTTG</u>
22750HindIII	TTGTA <u>CTAAGCTTCTATCCCGCATCGCGG</u>
Cloning of <i>crbS</i> in pSRKgm	
pSRKCrbsF	GACGGACGGCGGCGGAGTAGAAGCTTATCGATAACCGTCGA
pSRKCrbsR	ATGACCGAACCCGAAAGCATATGCTGTTTCCTGTGTGAAA
CrbSpSRKF	TTTCACACAGGAAACAGCATATGCTTTCGGGTTTCGGTCATTCT
CrbSpSRKR	TCGACGGTATCGATAAGCTTCTACTCCGCCGCCGTCCGTC
Cloning of <i>crbR</i> in pSRKgm	
1840NdeI	TTGTA <u>CTCATATGCCCGACATGACCATCAT</u>
1840HindIII	TTGTA <u>CTAAGCTTTTAAGCCGCCGCCACGCT</u>
<i>In-Cis</i> transcriptional fusions of the NGR_RS10970-mctP promoter	
prVMGsalF	CTTTAGGTCGACCACGGCTCGAACGAACCGGA
prVMGBamR	TAGACGGGATCCGTTATCCTCCCGCGATGTCTGGA
<i>In-trans</i> transcriptional fusions of the NGR_RS10970-mctP promoter	
pr53gBcuF	CTTTAG <u>ACTAGT</u> CACGGCTCGAACGAACCGGA
pr53gBamRb	TAGACGGGATCCGTTATCCTCCCGCGATGTCTG
Mapping of the NGR_RS10970-mctP promoter	
pr+18	GATCACGGATCCGTGTGTCTCTCGAATAGCTCCAAACGC
pr-20	GATCACGGATCCTAGCTCCAAACGCCGCGCC
pr-75	CTTTAG <u>ACTAGT</u> AAGGGGGCGCCGGAGC
pr-82	CTTTAG <u>ACTAGT</u> AATGAGCGAAAGTAGGGGGGC
pr-30	CTTTAG <u>ACTAGT</u> TTGGAGCTATTCGAGAGACACACGTG
pr-40	CTTTAG <u>ACTAGT</u> GGCGCGCGTTTGGAGCTA
Mutagenesis of the NGR_RS10970-mctP promoter to PrmctP-Mut2	
prMut2U	GCGCCGCGGCCTCCT TGCAAT TCGCTCCGGCGCCCC
prMut2L	GGGGCGCCGAGCGAA TTGCA GGAGGCCGCGGCGC

Table S1. Restriction sites are underlined. ***Bold Italics*** indicate introduced nucleotide changes for mutagenesis

	GenBank				UniProt	
<i>S. fredii</i> NGR234	Accession	(formerly)	Gene Name	(formerly)	Accession	Gene Name
<i>rpuS</i>	WP_015888335.1	YP_002824474.1	<i>NGR_RS10960</i>	<i>NGR_b22760</i>	C3KN91_SINFN	<i>NGR_b22760</i>
<i>rpuR</i>	WP_015888334.1	YP_002824473.1	<i>NGR_RS10955</i>	<i>NGR_b22750</i>	C3KN90_SINFN	<i>NGR_b22750</i>
<i>crbS</i>	WP_012706770.1	YP_002824738.1	<i>NGR_RS12300</i>	<i>NGR_c01850</i>	C3MFT4_SINFN	<i>NGR_c01850</i>
<i>crbR</i>	WP_012706769.1	YP_002824737.1	<i>NGR_RS12295</i>	<i>NGR_c01840</i>	C3MFT3_SINFN	<i>NGR_c01840</i>
<i>P. fluorescens</i> SBW25						
<i>crbS</i>	WP_017337284.1		<i>PFLU_RS21935</i>	<i>PFLU_4471</i>	C3K0X7_PSEFS	<i>PFLU_4471</i>
<i>crbR</i>	WP_012722526.1		<i>PFLU_RS05910</i>	<i>PFLU_1195</i>	C3KDG5_PSEFS	<i>PFLU_1195</i>

Table S2. Equivalencies of identifiers for the RpuS and CrbS genes and proteins from *S. fredii* NGR234 and *P. fluorescens* SBW25 among different databases