

Table S1: Genotype of strains and plasmids used in this study.

Gene				Strain			Plasmid		
DVU no.	name	Size (nt)	Predicted function	DvH	JW9021	JW9063	pMO9116	pMO9117	pMO9118
DVU0846	<i>apsB</i>	504	adenylylsulphate reductase beta subunit	+	+	+	-	-	-
DVU0847	<i>apsA</i>	1995	adenylylsulphate reductase alpha subunit	+	+	+	-	-	-
DVU0848	<i>qmoA</i>	1239	Quinone-interacting membrane-bound oxidoreductase A	+	-	+	+	+	-
DVU0849	<i>qmoB</i>	2277	Quinone-interacting membrane-bound oxidoreductase B	+	-	+	+	+	-
DVU0850	<i>qmoC</i>	1155	Quinone-interacting membrane-bound oxidoreductase C	+	-	+	+	+	-
DVU0851	hypothetical	747	Hypothetical	+	-	-	-	+	+

Table S2: Primers used for PCR amplification, Southern/Northern probe generation, and sequencing.

Primer name	Primer sequence	Application
DVU0848-51-1	TCAGGCCAACCTCTGGGC	For deletion of <i>qmoABC</i> -DVU0851, upstream of DVU0848, forward
DVU0848-51-2	AAGACTGTAGCCGTACCTCGAATCTA <sup>a'</sup> CCTTGGTATCCTCCCTACGTGTTTTGG	Complement of common sequence-1 and for deletion of <i>qmoABC</i> -DVU0851, upstream of DVU0848, reverse
DVU0848-51-3	AATCCGCTCACTAAGTTCATAGACCG <sup>c</sup> TCGCCCCCGTCTTCATGC	Common sequence -2 and downstream of DVU0851 forward, for pMO9020 and pMO9062
DVU0848-51-4b	GTAGCTTGCGCGAATCTGCTTCA	For deletion of <i>qmoABC</i> -DUV0851, downstream of DVU0851, reverse, for pMO9020 and pMO9062
bc0059f	TAGATTCGAGGTACGGCTACAGTCTT <sup>a</sup> CTCTTTCTAAGTGAGTCGAG <sup>b</sup> CCCAGAGTCCCGCTCAG	Common sequence-1, barcode sequence-1, and for amplification of Kan <sup>r</sup> for pMO9020, forward
bc0059r	CGGTCTATGAACCTAGTGAGCGGATT <sup>c'</sup> CACCTGAGAAGACGTAGTAC <sup>d'</sup> GAGGTAGCTTGCAGTGGGCT	Complement of common-sequence-1, complement of barcode sequence-2, and for amplification of Kan <sup>r</sup> for pMO9020, reverse
RDVU0850-281F	CACTTCGCAACATGGCCTACCAG	For sequencing of complementing plasmids pMO9040 and pMO9042 and amplification of Southern probe for confirmation of JW9063, forward
DVU0851-2	AAGACTGTAGCCGTACCTCGAATCTA <sup>a'</sup> TTCGGCCTCCTGATTCTCTTTCCGG	Complement of common sequence-1 and for deletion of DVU0851, upstream of DVU0851, and amplification of Southern probe for confirmation of JW9063, reverse
bc0082f	TAGATTCGAGGTACGGCTACAGTCTT <sup>a</sup> GTGTCGACTCCGACGGTAT <sup>e</sup> CCCAGAGTCCCGCTCAG	Common sequence-1, barcode sequence-1, and for amplification of Kan <sup>r</sup> for pMO9062, forward
bc0082r	CGGTCTATGAACCTAGTGAGCGGATT <sup>c'</sup> GCGTTAATGTGAGCTACGA <sup>f</sup> GAGGTAGCTTGCAGTGGGCT	Complement of common sequence-1, complement of barcode sequence-2, and for amplification of Kan <sup>r</sup> for pMO9062, reverse
TOPO8-F	AACGACGGCCAGTCTTAAGC	For colony PCR screen and sequencing of deletion cassettes pMO9020 and pMO9062, forward
TOPO8-R	AGACACGGGCCAGAGCTG	For colony PCR screen and sequencing of deletion cassettes pMO9020 and pMO9062 (reverse); for sequencing of complementing plasmid pMO9074 (forward)
DVU0847-4F	TGGCGTGGGTACCTACTACACC	For sequencing of deletion cassette pMO9020 and amplification of <i>apsA</i> from cDNA, forward
DVU0847-4R	GGTGTAGTAGGTACCCACGCCA	For sequencing of deletion cassette pMO9020, amplification of Southern probe for confirmation of JW9021, and to obtain Northern blot probe of <i>apsA</i> , reverse
DVU0848-2F	TGAGACCAGCCTTGACTAC	For sequencing of deletion cassettes pMO9020 and pMO9062, forward
DVU0848-2R	GTAGTCCAAGGCTGGTGTCTCA	For sequencing of deletion cassette pMO9020 and amplification of <i>qmoABC</i> -DVU0851 for pMO9040, reverse
Km_int_Fwd_revcomp	CTCATCCTGTCTCTTGATCAGATCT	For sequencing of deletion cassettes pMO9020 and pMO9063, forward
Km_int_Rev_revcomp	CTACCCGTGATATTGCTGAAGAG	For sequencing of deletion cassettes pMO9020 and pMO9063, reverse
KO0851-1F	CATCAGGACGCAAACCGAGAATGC	For sequencing of deletion cassette pMO9062, forward
KO0851-2R	TTCGCCAAGCCGATCATGGC	For sequencing of deletion cassette pMO9062, reverse

qmoA-SLIC-RBS-F	AGGTTGGGAAGCCCTGCAA TGCAGTCCCAGGAGGTACCAT ATGTCGAACTCCATACTCGTCGTCG	Cloning RBS <sup>S</sup> into pMO9040 and pMO9042
DVU0851-SLIC-RBS-F	AGGTTGGGAAGCCCTGCAA TGCAGTCCCAGGAGGTACCAT ATGTCTGACGAAGGTCGTAGAATGTTCAA	Cloning RBS into pMO9074
pMO9075-SLIC-R2	TTGCAGGGCTTCCCAACCT	Cloning RBS into pMO9040, pMO9042, and pMO9074
DVU0850-1155R	CTAACGACGACCCATTGCGCG	For sequencing of deletion cassette pMO9062 and sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0847-3F	TGCTCGGTTGCACTCCG	For amplification of Southern probe for confirmation of JW9021 and Northern blot probe of <i>apsA</i> , forward
DVU0848-Pkan-F	CCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGA AGCCCTGCAA ATGTCGAACTCCATACTCGTCGTCG	For amplification of <i>qmoABC</i> DVU0851 with <i>aph(3')-IIp<sup>h</sup></i> for pMO9040 and pMO9042, forward
TOPO8-EcoRV-up	GCGAATTCGACCCAGCTTTCTTGT	For colony PCR screen and sequencing of complementing plasmids pMO9040 and pMO9042, forward
TOPO8-EcoRV-dw	ATTTCTGTCCTGGCTGGTCTAGAGG	For colony PCR screen and sequencing of complementing plasmids pMO9040 and pMO9042, reverse
DVU0848-252F	GGAGAAGGGCAATTACACCGTCTC	For colony PCR screen of pMO9040 and pMO9042 and sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0849-208R	TACCGCAGAGCAGGACACC	For colony PCR screen of pMO9040 and pMO9042 and sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0848-F	AATCTCGGTGCTGGCAAGATCG	For sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0848-1F	ATGTCGAACTCCATACTCGTCGTCG	For sequencing of complementing plasmids pMO9116 and pMO9117 and amplification of <i>qmoA</i> from cDNA, forward
DVU0846-51-1F	ATGGCCAGCAGAATTGGCGT	For sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0846-51-1R	ACGCCAATTCTGCTGGCCAT	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0846-51-R	CTAGAAGCCGAAGCCGTCGTC	For sequencing of complementing plasmids pMO9116, pMO9117, and pMO9118, reverse
DVU0849-740F	ATGAAGAGATCGCCGTTGGTGC	For sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0849-1371F	CATCATGAACTTCGAATACCGTCAGGG	For sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0849-2001F	ATGGGTTGCCGACGCCATGTC	For sequencing of complementing plasmids pMO9116 and pMO9117, forward
DVU0849-2021R	GACATGGCGTCGGCAACCCAT	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0849-1397R	CCCTGACGGTATTCGAAGTTCATGATG	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0849-761R	GCACCAACGGCGATCTTTCAT	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0850-570R	AGGCTTGAACGACGCAAGCAG	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0850-354R	GGATACCGAAGAGGATGGGCAGG	For sequencing of complementing plasmids pMO9116 and pMO9117, reverse
DVU0851-1F	ATGTCTGACGAAGGTCGTAGAATGTTCAA	For sequencing of complementing plasmids pMO9117 and pMO9118, forward
Kan-Tx-F	CCGGAATTGCCAGCTGGG	For amplification of Kan <sup>r</sup> to obtain pMO9071, forward
Kan-Tx-R	GCATGCGTTTAACTACGTAAGGAGTACTGCGCAGATCGG CATCAGAAGAACTCGTCAAGAAGGCGAT	For introduction of multicloning site and amplification of Kan <sup>r</sup> to obtain pMO9071, reverse
DVU0851-1F-EcoRV	GATATC ATGTCTGACGAAGGTCGTAGAATGTTCAA	For amplification of DVU0851 with EcoRI cloning site to construct pMO9074, forward
DVU0851-R-SphI	GCATGC CTAGAAGCCGAAGCCGTCGTC	For amplification of DVU0851 with SphI cloning site to construct pMO9074, reverse

pBG1-2199F	GCTGAAAGCGAGAAGAGCGCAC	For sequencing of complementing plasmid pMO9118, reverse
DVU0847-R2	TTCGGGGATGATCTGGTAGTAGGC	For amplification of <i>apsA</i> from cDNA, reverse
DVU0848-R	CGATCTTGCCAGCACCGAGATT	For amplification of <i>qmoA</i> from cDNA, reverse

a – common sequence-1, common sequence in marker exchange deletions constructed in the Wall lab.

a' – reverse complement of common sequence-1.

b – barcode-1, unique to JW9021.

c – common sequence -2, common sequence in marker exchange deletions constructed in the Wall lab.

c' – reverse complement of common sequence-2.

d' – reverse complement of barcode-2, unique to JW9021.

e – barcode-1, unique to JW9063.

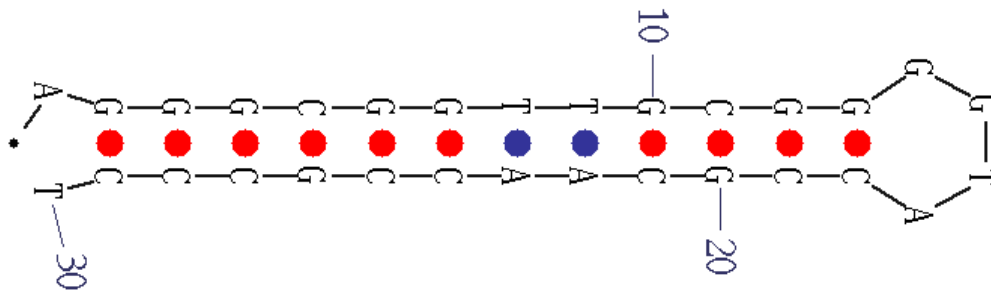
f' – reverse complement barcode-2, unique to JW9063.

g – RBS, ribosomal binding site (TGCAGTCCCAGGAGGTACCAT)

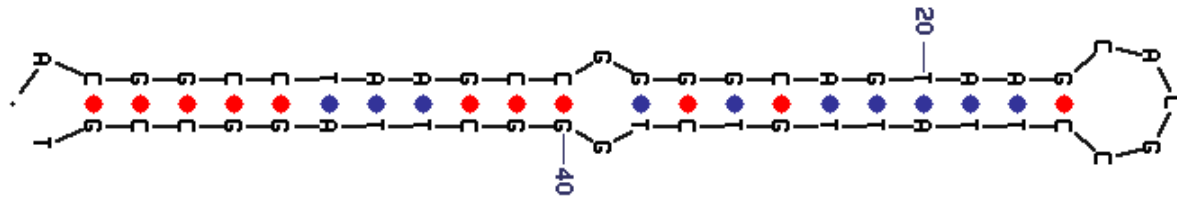
h - *aph(3')-I<sub>p</sub>* – promoter for the *aph(3')-II* gene

Figure S1. Stable hairpin structures located in the intergenic region between *apsA* and *qmoA* (Figs. S1A and S1B) and between *qmoC* and DVU0851 (Fig. S1C) in *D. vulgaris*. Melting temperature and hairpin predictions were determined using the mFold application on the IDT website, <http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/>.

A) Possible hairpin structure for bases 11 through 40 of the *apsA-qmoA* intergenic region, melting temperature of 84.2 °C.



B) Possible hairpin structure for bases 57 through 107 of the *apsA-qmoA* intergenic region, melting temperature of 64.6 °C.



C) Possible hairpin structure for bases 11 through 34 of the *qmoC-DVU0851* intergenic region, melting temperature of 84.2 °C.

