SUPPLEMENTARY TABLES

Table S1: Predicted positive (+) and negative (-) effects of codon usage, based on different mechanistic hypotheses for selection on codon use

Target of selection	**Impact of codon identity (effect on protein cost vs. benefit)**	**Effect of % rare codons**	**Importance of codon position**	**Effect of high expression on fitness**
mRNA structure / sequence	1. Inhibits translation initiation (insufficient benefit)	No general prediction	5′ end most sensitive	+
	2. Promotes rapid degradation (insufficient benefit)	No general prediction	No general prediction	+
	3. Binds ribosomes (insufficient benefit and excess cost)	No general prediction	Affinity to anti-SD	+ (gene) – (global)
Ribosomal pausing during translation of rare codons (due to tRNA limitation)	4. Slows translation & decreases protein (insufficient benefit)	–	No general prediction	+
	5. Sequesters ribosomes (excess cost)	–	Rare codons good at 5′ end	–
	6. Promotes accurate protein folding. Lack of ribosome pause leads to: (a) Low protein activity or stability (insufficient benefit) (b) Misfolding-induced toxicity (excess cost)	No general prediction	Rare codons preferred at domain boundaries	+ –
	7. Increases translation errors causing: (a) Low protein activity or stability (insufficient benefit) (b) Misfolding-induced toxicity (excess cost)	– –	Rare codons bad at conserved or active residues	+ –

Table S2: List of frequently and rarely used codons at conserved sites, for each amino acid across all *M. extorquens* AM1 protein coding genes. Conserved amino acid residues were identified from a gene-by-gene direct comparison of coding sequences with the closely related strain *M. extorquens* DM4. For each amino acid, the z score of a codon is a measure of its association with conserved rather than variable residues (z_{codon} = [frequency of codon at conserved sites – average frequency of codons at conserved sites] / standard deviation in the frequency of codons at conserved sites]; the p value shows the significance of the association; and the odds ratio indicates the odds of the association. For each amino acid, the codon most enriched at conserved residues (most frequent) is shown in bold and the most rarely used codon is shaded light gray. Other codons are found at intermediate frequencies within conserved sites across the genome and were disregarded while designing our synonymous alleles.

Amino acid	Codon	z score	p value	odds ratio	ln (odds ratio)
A	GCA	-5.43	2.78E-08	0.72	-0.33
A	GCT	-3.33	4.33E-04	0.81	-0.21
A	GCG	-2.01	2.23E-02	0.94	-0.06
A	**GCC**	5.60	1.06E-08	1.17	0.16
C	TGT	-1.39	8.26E-02	0.68	-0.38
C	**TGC**	1.39	8.26E-02	1.47	0.38
D	GAT	-0.99	1.60E-01	0.95	-0.05
D	**GAC**	0.99	1.60E-01	1.05	0.05
E	GAA	-4.09	2.19E-05	0.79	-0.24
E	**GAG**	4.09	2.19E-05	1.27	0.24
F	TTT	-4.91	4.64E-07	0.57	-0.55
F	**TTC**	4.91	4.64E-07	1.74	0.55
G	GGA	-4.30	8.53E-06	0.72	-0.33
G	GGG	-3.74	9.10E-05	0.83	-0.19
G	GGT	-2.36	9.04E-03	0.84	-0.17
G	**GGC**	6.73	8.63E-12	1.34	0.29
H	CAC	-0.62	2.67E-01	0.94	-0.06
H	**CAT**	0.62	2.67E-01	1.06	0.06
I	ATA	-9.79	6.05E-23	0.17	-1.80
I	ATT	-3.47	2.65E-04	0.70	-0.36
I	**ATC**	6.90	2.69E-12	1.87	0.63
K	AAA	-5.68	6.61E-09	0.53	-0.64
K	**AAG**	5.68	6.61E-09	1.89	0.64

L	TTG	-8.01	5.90E-16	0.57	-0.56
L	CTA	-2.45	7.15E-03	0.67	-0.40
L	CTT	-4.71	1.23E-06	0.70	-0.36
L	TTA	-0.55	2.92E-01	0.84	-0.17
L	CTC	2.01	2.21E-02	1.08	0.08
L	**CTG**	4.26	1.04E-05	1.18	0.17
N	AAT	-1.53	6.25E-02	0.85	-0.17
N	**AAC**	1.53	6.25E-02	1.18	0.17
P	CCT	-5.22	8.73E-08	0.58	-0.54
P	CCA	-3.03	1.21E-03	0.69	-0.37
P	CCC	-4.22	1.25E-05	0.81	-0.21
P	**CCG**	7.16	4.09E-13	1.40	0.34
Q	CAA	-4.29	9.11E-06	0.68	-0.38
Q	**CAG**	4.29	9.11E-06	1.47	0.38
R	AGG	-23.13	1.27E-118	0.18	-1.69
R	AGA	-10.14	1.86E-24	0.22	-1.51
R	CGA	-3.29	4.99E-04	0.72	-0.33
R	CGT	-3.40	3.41E-04	0.77	-0.27
R	CGG	1.32	9.26E-02	1.06	0.06
R	**CGC**	10.93	4.03E-28	1.59	0.47
S	AGT	-7.17	3.70E-13	0.48	-0.73
S	TCT	-5.77	3.90E-09	0.50	-0.69
S	TCA	-1.81	3.51E-02	0.80	-0.23
S	TCC	-2.52	5.91E-03	0.89	-0.12
S	AGC	-1.70	4.44E-02	0.93	-0.08
S	**TCG**	8.76	9.74E-19	1.51	0.41
T	ACA	-7.32	1.26E-13	0.49	-0.72
T	ACT	-5.42	2.98E-08	0.55	-0.60
T	ACG	-1.18	1.18E-01	0.95	-0.05
T	**ACC**	5.52	1.72E-08	1.27	0.24
V	GTA	-2.20	1.38E-02	0.73	-0.31
V	GTT	-3.44	2.96E-04	0.76	-0.28
V	GTC	-2.72	3.26E-03	0.90	-0.11
V	**GTG**	4.85	6.21E-07	1.22	0.20
Y	TAT	-1.80	3.58E-02	0.80	-0.22
Y	**TAC**	1.80	3.58E-02	1.25	0.22

Table S3: Coding sequences of *fae* mutants with C-terminal FLAG epitope tag.

Key	
conserved residue	FLAG epitope tag
variable residue	rare codon
residue assigned randomly as conserved or variable	frequent codon
residue added / nucleotides changed for cloning	Codon changed for cloning

Position	**Amino Acid**	**WT***	**WT**	**AF**	**AR**	**RN**	**VA**	**CO**	**AC**
"-1"	–	CGA	CAT	CAT	CAT	CAT	CAT	CAT	CAT
1	M	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
2	A	GCA	GCA	GCC	GCA	GCA	GCC	GCA	GCC
3	K	AAA	AAA	AAG	AAA	AAA	AAG	AAA	AAG
4	I	ATC	ATC	ATC	ATA	ATA	ATC	ATA	ATC
5	T	ACC	ACC	ACC	ACA	ACC	ACC	ACA	ACC
6	K	AAG	AAG	AAG	AAA	AAG	AAG	AAA	AAG
7	V	GTT	GTT	GTG	GTA	GTG	GTG	GTA	GTG
8	Q	CAG	CAG	CAG	CAA	CAA	CAG	CAA	CAG
9	V	GTC	GTC	GTG	GTA	GTA	GTG	GTA	GTG
10	G	GGC	GGC	GGC	GGA	GGC	GGA	GGC	GGC
11	E	GAG	GAG	GAG	GAA	GAG	GAA	GAG	GAG
12	A	GCC	GCC	GCC	GCA	GCA	GCC	GCA	GCC
13	L	CTC	CTC	CTG	TTG	TTG	TTG	CTG	TTG
14	V	GTC	GTC	GTG	GTA	GTG	GTA	GTG	GTG
15	G	GGC	GGC	GGC	GGA	GGA	GGA	GGC	GGC
16	D	GAT	GAT	GAC	GAT	GAC	GAC	GAT	GAC
17	G	GGC	GGC	GGC	GGA	GGC	GGC	GGA	GGC
18	N	AAC	AAC	AAC	AAT	AAC	AAT	AAC	AAC
19	E	GAA	GAA	GAG	GAA	GAA	GAA	GAG	GAG
20	V	GTC	GTC	GTG	GTA	GTA	GTG	GTA	GTA
21	A	GCT	GCT	GCC	GCA	GCC	GCA	GCC	GCC
22	H	CAC	CAC	CAT	CAC	CAT	CAC	CAT	CAC
23	I	ATC	ATC	ATC	ATA	ATC	ATA	ATC	ATC
24	D	GAC	GAC	GAC	GAT	GAT	GAT	GAC	GAT
25	L	CTC	CTC	CTG	TTG	CTG	TTG	CTG	CTG
26	I	ATC	ATC	ATC	ATA	ATC	ATC	ATA	ATC
27	I	ATC	ATC	ATC	ATA	ATA	ATA	ATA	ATC
28	G	GGA	GGA	GGC	GGA	GGA	GGA	GGC	GGC
29	P	CCG	CCG	CCG	CCT	CCG	CCG	CCT	CCG
30	R	CGC	CGC	CGC	AGG	AGG	AGG	CGC	CGC
31	G	GGT	GGT	GGC	GGA	GGC	GGC	GGA	GGC
32	S	TCG	TCG	TCG	AGT	TCG	AGT	TCG	TCG
33	P	CCG	CCG	CCG	CCT	CCT	CCG	CCT	CCG
34	A	GCC	GCC	GCC	GCT	GCT	GCA	GCC	GCC
35	E	GAG	GAG	GAG	GAA	GAA	GAA	GAG	GAG
36	T	ACG	ACG	ACC	ACA	ACA	ACC	ACA	ACC
37	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
38	F	TTC	TTC	TTC	TTT	TTC	TTT	TTC	TTC

39	C	TGC	TGC	TGC	TGT	TGT	TGT	TGC	TGC
40	N	AAC	AAC	AAC	AAT	AAT	AAC	AAT	AAC
41	G	GGT	GGT	GGC	GGA	GGC	GGC	GGA	GGC
42	L	CTG	CTG	CTG	TTG	TTG	TTG	CTG	CTG
43	V	GTC	GTC	GTG	GTA	GTG	GTG	GTA	GTG
44	N	AAC	AAC	AAC	AAT	AAC	AAC	AAT	AAC
45	N	AAC	AAC	AAC	AAT	AAT	AAC	AAC	AAC
46	K	AAG	AAG	AAG	AAA	AAG	AAA	AAG	AAG
47	H	CAC	CAC	CAT	CAC	CAT	CAT	CAC	CAT
48	G	GGC	GGC	GGC	GGA	GGC	GGA	GGC	GGC
49	F	TTC	TTC	TTC	TTT	TTT	TTC	TTT	TTC
50	T	ACC	ACC	ACC	ACA	ACC	ACA	ACC	ACA
51	S	AGC	AGC	TCG	AGT	AGT	TCG	TCG	TCG
52	L	CTG	CTG	CTG	TTG	TTG	TTG	CTG	TTG
53	L	CTC	CTC	CTG	TTG	TTG	TTG	CTG	TTG
54	A	GCC	GCC	GCC	GCA	GCA	GCA	GCC	GCC
55	V	GTG	GTG	GTG	GTA	GTG	GTA	GTG	GTG
56	I	ATC	ATC	ATC	ATA	ATC	ATC	ATA	ATC
57	A	GCG	GCG	GCC	GCA	GCA	GCA	GCA	GCC
58	P	CCG	CCG	CCG	CCT	CCG	CCT	CCG	CCT
59	N	AAC	AAC	AAC	AAT	AAC	AAT	AAC	AAC
60	L	CTG	CTG	CTG	TTG	CTG	CTG	TTG	CTG
61	P	CCG	CCG	CCG	CCT	CCT	CCG	CCT	CCG
62	C	TGC	TGC	TGC	TGT	TGC	TGC	TGT	TGC
63	K	AAG	AAG	AAG	AAA	AAG	AAA	AAG	AAG
64	P	CCG	CCG	CCG	CCT	CCG	CCT	CCG	CCG
65	N	AAC	AAC	AAC	AAT	AAT	AAC	AAT	AAC
66	T	ACG	ACG	ACC	ACA	ACA	ACA	ACC	ACC
67	L	CTG	CTG	CTG	TTG	TTG	CTG	TTG	CTG
68	M	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
69	F	TTC	TTC	TTC	TTT	TTC	TTC	TTT	TTC
70	N	AAC	AAC	AAC	AAT	AAC	AAT	AAC	AAC
71	K	AAG	AAG	AAG	AAA	AAA	AAA	AAG	AAA
72	V	GTC	GTC	GTG	GTA	GTA	GTA	GTG	GTA
73	T	ACC	ACC	ACC	ACA	ACC	ACA	ACC	ACC
74	I	ATC	ATC	ATC	ATA	ATC	ATA	ATC	ATC
75	N	AAC	AAC	AAC	AAT	AAC	AAT	AAC	AAC
76	D	GAC	GAC	GAC	GAT	GAC	GAC	GAT	GAC
77	A	GCC	GCC	GCC	GCA	GCC	GCC	GCA	GCC
78	R	CGT	CGT	CGC	AGG	CGC	CGC	AGG	CGC
79	Q	CAG	CAG	CAG	CAA	CAA	CAA	CAG	CAG
80	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCA
81	V	GTC	GTC	GTG	GTA	GTG	GTA	GTA	GTA
82	Q	CAG	CAG	CAG	CAA	CAA	CAA	CAG	CAG
83	M	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
84	F	TTT	TTT	TTC	TTT	TTC	TTT	TTC	TTT
85	G	GGC	GGC	GGC	GGA	GGA	GGA	GGC	GGC
86	P	CCG	CCG	CCG	CCT	CCG	CCT	CCG	CCG
87	A	GCC	GCC	GCC	GCA	GCA	GCA	GCC	GCC
88	Q	CAG	CAG	CAG	CAA	CAG	CAA	CAG	CAA

89	H	CAT	CAT	CAT	CAC	CAC	CAT	CAC	CAT
90	G	GGC	GGC	GGC	GGA	GGA	GGC	GGA	GGC
91	V	GTC	GTC	GTG	GTA	GTG	GTA	GTG	GTG
92	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
93	M	ATG	ATG	ATG	ATG	ATG	ATG	ATG	ATG
94	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
95	V	GTG	GTG	GTG	GTA	GTA	GTA	GTG	GTG
96	Q	CAG	CAG	CAG	CAA	CAA	CAG	CAA	CAG
97	D	GAC	GAC	GAC	GAT	GAT	GAT	GAC	GAC
98	A	GCG	GCG	GCC	GCA	GCA	GCC	GCA	GCC
99	V	GTT	GTT	GTG	GTA	GTG	GTA	GTG	GTG
100	A	GCC	GCC	GCC	GCA	GCA	GCC	GCA	GCC
101	E	GAA	GAA	GAG	GAA	GAG	GAG	GAA	GAG
102	G	GGC	GGC	GGC	GGA	GGC	GGA	GGC	GGC
103	I	ATC	ATC	ATC	ATA	ATA	ATC	ATA	ATC
104	I	ATC	ATC	ATC	ATA	ATC	ATA	ATC	ATC
105	P	CCG	CCG	CCG	CCT	CCT	CCT	CCG	CCG
106	A	GCT	GCT	GCC	GCT	GCT	GCC	GCA	GCC
107	D	GAC	GAC	GAC	GAT	GAT	GAC	GAT	GAC
108	E	GAA	GAA	GAG	GAA	GAA	GAA	GAG	GAG
109	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
110	D	GAC	GAC	GAC	GAT	GAC	GAT	GAC	GAC
111	D	GAC	GAC	GAC	GAT	GAT	GAC	GAT	GAC
112	L	CTG	CTG	CTG	TTG	CTG	CTG	TTG	CTG
113	Y	TAC	TAC	TAC	TAT	TAT	TAC	TAT	TAC
114	V	GTG	GTG	GTG	GTA	GTA	GTG	GTA	GTG
115	L	CTG	CTG	CTG	TTG	CTG	CTG	TTG	CTG
116	V	GTC	GTC	GTG	GTA	GTA	GTA	GTG	GTG
117	G	GGC	GGC	GGC	GGA	GGA	GGC	GGA	GGC
118	V	GTG	GTG	GTG	GTA	GTG	GTA	GTG	GTG
119	F	TTC	TTC	TTC	TTT	TTT	TTC	TTT	TTT
120	I	ATC	ATC	ATC	ATA	ATA	ATA	ATC	ATC
121	H	CAC	CAC	CAT	CAC	CAC	CAC	CAC	CAT
122	W	TGG	TGG	TGG	TGG	TGG	TGG	TGG	TGG
123	E	GAA	GAA	GAG	GAA	GAA	GAG	GAA	GAG
124	A	GCG	GCG	GCC	GCA	GCA	GCA	GCC	GCC
125	A	GCC	GCC	GCC	GCA	GCA	GCC	GCA	GCC
126	D	GAC	GAC	GAC	GAT	GAT	GAT	GAT	GAC
127	D	GAC	GAC	GAC	GAT	GAC	GAT	GAC	GAC
128	A	GCC	GCC	GCC	GCA	GCA	GCC	GCA	GCC
129	K	AAG	AAG	AAG	AAA	AAG	AAA	AAG	AAG
130	I	ATC	ATC	ATC	ATA	ATA	ATA	ATC	ATC
131	Q	CAG	CAG	CAG	CAA	CAG	CAA	CAG	CAG
132	K	AAG	AAG	AAG	AAA	AAA	AAG	AAA	AAG
133	Y	TAC	TAC	TAC	TAT	TAC	TAC	TAC	TAC
134	N	AAC	AAC	AAC	AAT	AAC	AAT	AAC	AAC
135	Y	TAC	TAC	TAC	TAT	TAC	TAT	TAC	TAC
136	E	GAG	GAG	GAG	GAA	GAA	GAG	GAA	GAG
137	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
138	T	ACC	ACC	ACC	ACA	ACA	ACA	ACC	ACC

139	K	AAG	AAG	AAG	AAA	AAG	AAA	AAG	AAG
140	L	CTT	CTT	CTG	TTG	CTG	CTG	TTG	CTG
141	S	TCG	TCG	TCG	AGT	TCG	AGT	TCG	TCG
142	I	ATC	ATC	ATC	ATA	ATA	ATC	ATA	ATC
143	Q	CAG	CAG	CAG	CAA	CAG	CAG	CAA	CAG
144	R	CGC	CGC	CGC	AGG	CGC	AGG	CGC	CGC
145	A	GCC	GCC	GCC	GCA	GCC	GCA	GCC	GCC
146	V	GTC	GTC	GTG	GTA	GTA	GTG	GTA	GTG
147	N	AAC	AAC	AAC	AAT	AAT	AAC	AAT	AAC
148	G	GGC	GGC	GGC	GGA	GGA	GGC	GGA	GGC
149	E	GAG	GAG	GAG	GAA	GAG	GAA	GAA	GAG
150	P	CCG	CCG	CCG	CCT	CCG	CCG	CCG	CCG
151	K	AAG	AAG	AAG	AAA	AAA	AAG	AAA	AAG
152	A	GCT	GCT	GCC	GCA	GCA	GCC	GCA	GCC
153	S	TCG	TCG	TCG	AGT	AGT	TCG	AGT	TCG
154	V	GTT	GTT	GTG	GTA	GTA	GTG	GTA	GTG
155	V	GTC	GTC	GTG	GTA	GTA	GTG	GTA	GTG
156	T	ACG	ACG	ACC	ACA	ACA	ACC	ACA	ACC
157	E	GAG	GAG	GAG	GAA	GAG	GAG	GAA	GAG
158	Q	CAG	CAG	CAG	CAA	CAG	CAG	CAA	CAG
159	R	CGT	CGT	CGC	AGG	AGG	CGC	AGG	CGC
160	K	AAG	AAG	AAG	AAA	AAA	AAG	AAA	AAG
161	S	TCG	TCG	TCG	AGT	TCG	TCG	AGT	TCG
162	A	GCG	GCG	GCC	GCA	GCC	GCC	GCA	GCC
163	T	ACC	ACC	ACC	ACA	ACC	ACC	ACA	ACC
164	H	CAC	CAC	CAT	CAC	CAC	CAC	CAT	CAC
165	P	CCC	CCC	CCG	CCT	CCT	CCG	CCT	CCT
166	F	TTC	TTC	TTC	TTT	TTT	TTT	TTC	TTT
167	A	GCC	GCC	GCC	GCA	GCC	GCC	GCA	GCC
168	A	GCC	GCC	GCC	GCA	GCC	GCC	GCA	GCC
169	N	AAC	AAC	AAC	AAT	AAT	AAC	AAT	AAC
170	A	GCT	GCG	GCG	GCG	GCG	GCG	GCG	GCG
171	A	GCC	GCC	GCC	GCC	GCC	GCC	GCC	GCC
172	A	–	GCA	GCA	GCA	GCA	GCA	GCA	GCA
173	R	–	CGC	CGC	CGC	CGC	CGC	CGC	CGC
174	V	–	GTG	GTG	GTG	GTG	GTG	GTG	GTG
175	D	–	GAC	GAC	GAC	GAC	GAC	GAC	GAC
176	Y	–	TAC	TAC	TAC	TAC	TAC	TAC	TAC
177	K	–	AAG	AAG	AAG	AAG	AAG	AAG	AAG
178	D	–	GAC	GAC	GAC	GAC	GAC	GAC	GAC
179	D	–	GAT	GAT	GAT	GAT	GAT	GAT	GAT
180	D	–	GAC	GAC	GAC	GAC	GAC	GAC	GAC
181	D	–	GAT	GAT	GAT	GAT	GAT	GAT	GAT
182	K	–	AAG	AAG	AAG	AAG	AAG	AAG	AAG
183	A	–	GCG	GCG	GCG	GCG	GCG	GCG	GCG
184	A	–	GCC	GCC	GCC	GCC	GCC	GCC	GCC
185	A	–	GCG	GCG	GCG	GCG	GCG	GCG	GCG
186	STOP	TAG	TAG	TAG	TAG	TAG	TAG	TAG	TAG

Table S4: Primers used in this study. Sequence added for cloning is shown in italics.

Primer	**Sequence (5′→ 3′)**	**Restriction sites/features**
CM_syn_fae0-f	*ACTGCAGCGCAT***ATG**GCAAAAATCACCAAGGTTCAGGTC	*Pst*I, *Nde*I, **Start codon**
CM_syn_fae0-r	*GACGCGTGCGGCCGC*GTTGGCGGCGAAGGGGTG	*Mlu*I, *Not*I
CM_syn_fae-uf	*AGCTAG*CACATCGCCCGCAAGCAC	*Nhe*I
CM_syn_fae-ur	TGG*GCCCTGCAGCCTTGTCATCGTCATCCTTGTAGTCCATATG*GGGTCTCTCCCTGGATTCCTG	*Apa*I, *Pst*I
CM_syn_fae-df	*AGGGCCCACGCGTGGACTACAAGGACGATGACGATAAGGCGGCCGCG***TAG**ATCGGAGGCAGTCCTGGGCAGAG	*Apa*I, *Not*I, *Mlu*I, **Stop codon**
CM_syn_fae-dr	*CACCGGT*CAGTCGGATGCGGATCTCGTG	*Age*I
DA_p51_f	*ATTAAAGATCT*AGGGAGAGACCCCAT**ATG**G	*Bgl*II, **Start codon**
DA_p43_r	*ATTAGAAT****TC*****A**CGCGGCCGCCTTATCGTC	*Eco*RI, **Stop codon**

Table S5: Plasmids used in this study. *"up"* and *"down"* refer to *fae* upstream and downstream regions

Plasmid	**Features**	**Reference**
pCM433	Allelic exchange vector; Amp^{R}, Chl^{R}, Tet^{R}	Marx 2008
pDAFu0d	pCM433::*fae-up::fae*^{WT}*::fae-down*	This study
pDAFu1d	pCM433::*fae-up::fae*^{AF}*::fae-down*	This study
pDAFu2d	pCM433::*fae-up::fae*^{AR}*::fae-down*	This study
pDAFu3d	pCM433::*fae-up::fae*^{RN}*::fae-down*	This study
pDAFu4d	pCM433::*fae-up::fae*^{VA}*::fae-down*	This study
pDAFu5d	pCM433::*fae-up::fae*^{CO}*::fae-down*	This study
pDAFu6d	pCM433::*fae-up::fae*^{AC}*::fae-down*	This study
pDAF0	pDAFu0d without N-terminal FLAG tag	This study
pDAF1	pDAFu1d without N-terminal FLAG tag	This study
pDAF2	pDAFu2d without N-terminal FLAG tag	This study
pDAF3	pDAFu3d without N-terminal FLAG tag	This study
pDAF4	pDAFu4d without N-terminal FLAG tag	This study
pDAF5	pDAFu5d without N-terminal FLAG tag	This study
pDAF6	pDAFu6d without N-terminal FLAG tag	This study
pHC115	Kan^{R}, cumate regulator, P_{mxaF} promoter	Hagemeier et al. 2000
pDA115-F0	pHC115::*fae*^{WT}	This study
pDA115-F1	pHC115::*fae*^{AF}	This study
pDA115-F2	pHC115::*fae*^{AR}	This study
pDA115-F3	pHC115::*fae*^{RN}	This study
pDA115-F4	pHC115::*fae*^{VA}	This study
pDA115-F5	pHC115::*fae*^{CO}	This study
pDA115-F6	pHC115::*fae*^{AC}	This study

Table S6: Strains used in this study.

Strain	Label	Features	Reference
CM501	–	*Methylobacterium extorquens* wild-type strain	Marx 2008
CM2720	WT*	CM501 variant	This study
CM2721	–	CM1175 variant (with P_{tacA}-mCherry; Lee, Chou & Marx 2009)	This study
CM2563	Del	CM2006 variant (isolate of CM198.1, Marx & Lidstrom 2002), Δ*fae*	This study
CM2556	WT	CM2563::*fae*^{WT}	This study
CM2565	AF	CM2563::*fae*^{AF}	This study
CM2558	AR	CM2563::*fae*^{AR}	This study
CM2559	RN	CM2563::*fae*^{RN}	This study
CM2560	VA	CM2563::*fae*^{VA}	This study
CM2561	CO	CM2563::*fae*^{CO}	This study
CM2562	AC	CM2563::*fae*^{AC}	This study
CM2574	WT	CM2563 transformed with pDA115-F0	This study
CM2575	AF	CM2563 transformed with pDA115-F1	This study
CM2576	AR	CM2563 transformed with pDA115-F2	This study
CM2577	RN	CM2563 transformed with pDA115-F3	This study
CM2578	VA	CM2563 transformed with pDA115-F4	This study
CM2579	CO	CM2563 transformed with pDA115-F5	This study
CM2580	AC	CM2563 transformed with pDA115-F6	This study

Table S7: Primers used for quantitative real time PCR

Gene (allele)	Primer	Sequence (5' → 3')	Product length
rpsB	DA_p54_f	TCGGCTCAGTACTACGTCAACT	176 bp
	DA_p54_r	CTTCTCGAGCTTGTCCTTCTCAC	
fae^{WT}	DA_p55_f	TGGCAACGAAGTCGCTCACA	156 bp
	DA_p55_r	ATCAGCGTGTTCGGCTTGCA	
fae^{AF}	DA_p56_f	CGGCAACGAGGTGGCCCAT	159 bp
	DA_p56_r	AACATCAGGGTGTTCGGCTTGC	
fae^{AR}	DA_p66f	ACACGGATTTACAAGTTTGTTGGCA	149 bp
	DA_p66r	TGTACTGCCATTGCTACTCCGTG	
fae^{RN}	DA_p58_f	GTTGGCAGTGATCGCACCGA	151 bp
	DA_p58_r	GCCCTCTGCCACTGCATCTT	
fae^{VA}	DA_p67f	ACCAAGGTGCAGGTGGGAGA	159 bp
	DA_p67r	GTGCGATTACTGCCAACAACGA	
fae^{CO}	DA_p60_f	CCTGCCGAGACAGCCTTCTG	152 bp
	DA_p60_r	ATCTGTACGGCCTGCCTTGC	
fae^{AC}	DA_p61_f	GACGGCAACGAGGTAGCCC	157 bp
	DA_p61_r	TCAGGGTGTTCGGCTTGCAC	