

Supplemental Methods

Generation of the *cycA* complementing plasmid, pMO738, and the CycA complemented strain.

A 763-bp fragment containing the *cycA* gene and 343 bp of upstream DNA assumed to contain the *cycA* promoter, was amplified by Herc II polymerase (Stratagene, La Jolla, CA) with primers Dde_3182F and Dde_3182R (Table S1) and cloned into pCR4Blunt-TOPO (Invitrogen, Carlsbad, CA). The recombinant plasmid was transformed into One Shot® TOP10 Competent Cells (Invitrogen) and plated on LC with 50 µg kanamycin/ml. Individual transformants were screened by colony PCR (2) to identify plasmids containing the proper sized amplicon and then the cloned gene verified by sequencing both strands. All sequencing was performed at the DNA Core Facility at the University of Missouri. One plasmid isolate was kept as the source of *cycA* gene and named pMO736.

The *cycA* gene, expressed from its native promoter, was removed from pMO736 by restriction enzyme digests and ligated into the EcoRV site of pMO719, a plasmid containing a G20 replicon allowing maintenance in SRB strains. After selection of the recombinant plasmid and verification of the insert sequence, one isolate was named pMO738.

To obtain a complemented *cycA* mutant, I2 cells were transformed with pMO738 by electroporation as previously described (2), with the following exceptions. Rich lactate/sulfate (60/30) medium was used (instead of rich lactate/sulfite (60/40) medium) throughout growth, electroporation, recovery and selective plating of I2 and the electroporation parameters were set at 1550 V, 250 Ω, and 25 µF. Electroporated I2 cells recovered overnight in the presence of 800 µg kanamycin/ml and the final selection for maintenance of pMO738 was accomplished by plating with 800 µg kanamycin/ml and 800 µg spectinomycin/ml. The plasmids were isolated from Km^r/Sp^r transformants, transformed back into Alpha-Select™ Bronze Efficiency *E. coli* cells (Bioline), re-isolated and sequenced. Sequencing

revealed all the plasmid sequences matched the original pMO738 and one isolate was chosen as the complemented strain, I2 (pMO738), for comparison of phenotypes.

Generation of pMO9075, the complementation shuttle vector

In order to remove sequence repeats that resulted from the generation of pMO9072, sequential restriction digests and re-ligations were performed on pMO9072 to generate the complementation shuttle vector pMO9075 (Fig. S1). Initially, pMO9072 was digested with both EcoRV and BspEI (Fig. S1A), the linear fragment was purified, the ends from BspEI were made blunt, and then the remaining plasmid was re-ligated together. The newly constructed plasmid was captured in Alpha-Select™ Bronze Efficiency cells on LC plates containing 100 µg spectinomycin/ml. Plasmid was prepared from the cells, and primers pMO9075_F and pMO9075_R (Table S1) were used to verify the appropriate removal of the 125bp fragment by sequencing. The resulting intermediate plasmid was named pMO9072.5. A digestion with HpaI and XmnI was performed on pMO9072.5 (Figure S1B), the linear fragment was purified, then re-ligated and the newly constructed plasmid was captured in Alpha-Select™ Bronze Efficiency cells on LC plates containing 100 µg spectinomycin/ml. Plasmid was prepared from the cells, and primers pMO9073_F and pMO9073_R (Table S1) were used to verify the appropriate removal of the 307bp fragment by sequencing, and the resulting plasmid named pMO9073 (Fig. S1C). The last repeat sequence was removed by performing an EcoRI digest on pMO9073, the linear fragment was purified, then re-ligated and the newly constructed plasmid was captured in Alpha-Select™ Bronze Efficiency cells on LC plates containing 100 µg spectinomycin/ml. Plasmid was prepared from the cells, and primers pMO9075_F and pMO9075_R (Table S1) were used to verify the appropriate removal of the 289bp fragment by sequencing, and the resulting plasmid named pMO9075 (Fig. S1D).

Generation of the *qrcA* complementing plasmid, pMO756

The *qrcA* gene (Dde_2932) was amplified by Herc II polymerase with the primers 2932_SLICF and 2932_SLICR (Table S1). To generate the complementation plasmid, the pMO9075 plasmid was amplified in two fragments by Herc II polymerase. Fragment 1 (2781bp) was amplified with the primers pMO9075_SLIC_1F and pMO9075_SLIC_2R; while fragment 2 (2080 bp) was amplified with the primers pMO9075_SLIC_3F and pMO9075_SLIC_4R, and both fragments were gel purified. The SLIC method (3) was used to join the three DNA fragments together by transforming equal molar ratios of the three. Briefly, the PCR products were cleaned using Wizard SV Gel and PCR clean-up system (Promega) and quantified. All three fragments were added in equal ratios (~400ng) and water added to make the final volume 15 μ l. A T4 DNA polymerase prep mix was prepared freshly (6 μ l BSA (1mg/ml), 6 μ l NEB Buffer 2 (10x), 2.5 μ l sterile deionized water, and 0.5 μ l T4 DNA polymerase (3U/ μ l)) and then 5 μ l of this “T4 prep mix” was added to the tube with the fragments. The mixture was allowed to incubate at room temperature for 30 minutes and the reaction stopped by the addition of 2 μ l of dCTP (10 mM stock). To 50 μ l of Alpha-Select™ Silver Efficiency *E. coli* cells, 5 μ l of the reaction mixture was added and the cells transformed as described in the Bioline protocol. Following recovery, the cells were plated on LC plates containing 100 μ g spectinomycin/ml. Individual Spectinomycin resistant transformants were screened by colony PCR (primers pMO9075_F and pMO9075_R) to identify those containing the appropriately sized insertion fragment and the cloned gene verified by sequencing both strands. The resulting plasmid was named pMO756.

To obtain a complemented *qrcA* mutant, cells were transformed with pMO756 by electroporation as previously described above for the I2 complementation with the following exceptions. The electroporation parameters were set at 1500 V, 250 Ω , and 25 μ F. Electroporated QrcA mutant cells were recovered overnight in rich lactate/sulfate (60/30) medium and the final selection for maintenance

of pMO756 was accomplished by plating with 800 µg kanamycin/ml and 800 µg spectinomycin/ml. The plasmids were isolated from Km^r/Sp^r transformants, transformed back into Alpha-Select™ Bronze Efficiency *E. coli* cells, re-isolated and sequenced. Sequencing revealed all the plasmid sequences matched the original pMO756 and one isolate was chosen as the complemented strain, QrcA (pMO756), for comparison of phenotypes.

Supplemental References

1. **Hauser, L. J., M. L. Land, S. D. Brown, F. Larimer, K. L. Keller, B. J. Rapp-Giles, M. N. Price, M. Lin, D. C. Bruce, J. C. Detter, R. Tapia, C. S. Han, L. A. Goodwin, J-F. Cheng, S. Pitluck, A. Copeland, S. Lucas, M. Nolan, A. L. Lapidus, A. V. Palumbo and J. D. Wall.** 2011. Complete genome sequence and updated annotation of *Desulfovibrio alaskensis* G20. *J. Bacteriol.* **193**:4268-4269.
2. **Keller, K. L., and J. D. Wall.** 2011. Genetics and molecular biology of the electron flow for sulfate respiration in *Desulfovibrio*. *Front. Microbiol.* **2**:135, 10.3389/fmicb.2011.00135.
3. **Li, M. Z., and S. J. Elledge.** 2007. Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. *Nat. Methods* **4**: 251–256.
4. **Yang, S., T. J. Tschaplinski, N. L. Engle, S. L. Carroll, S. L. Martin, B. H. Davison, A. V. Palumbo, M. J. Rodriguez, and S. D. Brown.** 2009. Transcriptomic and metabolomic profiling of *Zymomonas mobilis* during aerobic and anaerobic fermentation. *BMC Genomics* **10**:34.

Supplemental Table 1. Primers used in this study

Target	Nucleotide Sequence (5' – 3')	Target/Use
Dde_3182F	AGGTGGACATGCTCTCCATTGTGA	Amplifying <i>cycA</i> gene
Dde_3182R	CTCGAACAAGCAAATCCGGACCAGT	Amplifying <i>cycA</i> gene
Dde_2932F	ATGGAGGACAGGCATTTAGACAGTTCA	Amplifying <i>qrcA</i> gene
Dde_2932R	CCTGCGACGAACTTCATGAATCCTCT	Amplifying <i>qrcA</i> gene
2932_SLICF	GCCCTGCAAT <u>TGCAGTCCCAGGAGGTACCAT</u> ATGGAGGACAGGCATTTAGACAGTTCA	Amplifying <i>qrcA</i> gene with overlaps required for SLIC
2932_SLICR	GATCGTGATCCCCTGCGCCATCAGATCCTTG CCTGCGACGAACTTCATGAATCCTCT	Amplifying <i>qrcA</i> gene with overlaps required for SLIC
pMO9075_SLIC_1F	CAAGGATCTGATGGCGCAGGG	Amplifying the pBG1 backbone fragment for SLIC from pMO9075
pMO9075_SLIC_2R	GGCCTTTTGCTGGCCTTTTGCTCACAT	Amplifying the pBG1 backbone fragment for SLIC from pMO9075
pMO9075_SLIC_3F	ATGTGAGCAAAAGGCCAGCAAAAGGC	Amplifying the <i>spec</i> gene and pUC ori backbone fragment for SLIC from pMO9075
pMO9075_SLIC_4R	<u>ATGGTACCTCCTGGGACTGCA</u> TTGCAGGGC TTCCCAACCT	Amplifying the <i>spec</i> gene and pUC ori backbone fragment for SLIC from pMO9075
pCR4_F	GCTATGACCATGATTACGCCAAGCTCAG	Screening inserts in pCR4
pCR4_R	ACGGCCAGTGAATTGTAATACGACTCAC	Screening inserts in pCR4
pMO719_F	GCTTGAATTCGACCCAGCTTTCTTGT	Screening inserts into EcoRV site of pMO719
pMO719_R	ATTTCTGTCCTGGCTGGTCTAGAGG	Screening inserts into EcoRV site of pMO719
pMO9073_F	GCAGCTGGCACGACAGGTTT	Screening for deletions from pMO9702.5 in the generation of pMO9073
pMO9073_R	CACCCAAAGGCTACACCACGTAC	Screening for deletions from pMO9702.5 in the generation of pMO9073

pMO9075_F	GCTGAAAGCGAGAAGAGCGCAC	Screening for deletions from pMO9702, pMO9073 and inserts into pMO9075
pMO9075_R	TGGGTTCGTGCCTTCATCCG	
Dde_0717F	TGCACAAGGAGCAGTTCAAG	Screening for deletions from pMO9702, pMO9073 and inserts into pMO9075
Dde_0717R	GACCAGCCAGTCCAGCTTAC	
Dde_1074F	GCTGTCCGTCTTCATCATTCTC	Amplify Dde_0717 by qRT-PCR ^a
Dde_1074R	ATACAAACGTGGCGCCTACTAC	Amplify Dde_0717 by qRT-PCR
Dde_1110F	AGCCCACCATCAAAGAACAC	Amplify Dde_1074 by qRT-PCR
Dde_1110R	AGCATATTTGTCAGCCCAGC	Amplify Dde_1074 by qRT-PCR
Dde_1253F	GTCATTCCCGTAAAACCGTG	Amplify Dde_1110 by qRT-PCR
Dde_1253R	TGGAAGGGTCTTCCTTGATG	Amplify Dde_1110 by qRT-PCR
Dde_2234F	TCCGCCGTGAAATATCTAGC	Amplify Dde_1253 by qRT-PCR
Dde_2234R	CTGACCTCTAGCAGGCAACC	Amplify Dde_1253 by qRT-PCR
Dde_2265F	CAACCTTCCGCCAGAACTAC	Amplify Dde_2234 by qRT-PCR
Dde_2265R	GGTCTGGGCTTCGAACATAC	Amplify Dde_2234 by qRT-PCR
Dde_2272F	AGCCGGTGTCCAAGTGAAC	Amplify Dde_2265 by qRT-PCR
Dde_2272R	CTTCGATGTCGTCACACAGG	Amplify Dde_2265 by qRT-PCR
Dde_3103F	GATTCCGGGCTTTGAATACC	Amplify Dde_2272 by qRT-PCR
Dde_3103R	CAGTGTACATGAGCGGCTTG	Amplify Dde_2272 by qRT-PCR
Dde_3156F	TATGCCGACTACAGAGCACG	Amplify Dde_3103 by qRT-PCR
Dde_3156R	TTATTGGTGGAGGTGGATGC	Amplify Dde_3103 by qRT-PCR
		Amplify Dde_3156 by qRT-PCR

^aPrimer pairs for qRT-PCR were designed as described (4).

Bold print indicates the ribosomal binding site (TGCAGTCCCAGGAGGTACCAT) with the primer

Supplemental Table 2. Change in gene annotations as per M. Price^a

Locus		Original Annotation		Reannotation
Dde_0473	FhcA	Formate dehydrogenase	FhcA	Putative cytoplasmic formate:hydrogen lyase, formate dehydrogenase subunit
Dde_0474	FhcD	Formate dehydrogenase, chain A	FhcB	Putative cytoplasmic formate:hydrogen lyase, FeS subunit 1
Dde_0475	FhcB	Hydrogenase, FeS protein	FhcC	Putative cytoplasmic formate:hydrogen lyase, hydrogenase subunit
Dde_0476	FhcC	[Fe] hydrogenase, large and small subunits	FhcD	Putative cytoplasmic formate:hydrogen lyase, FeS subunit 2
Dde_1250	GltA	Glutamate synthase (NADPH), homotetrameric	NfnA-2	Electron-bifurcating transhydrogenase 2, subunit
Dde_1251	COG-UbiB	Oxidoreductase, FAD-binding	NfnB-2	Electron-bifurcating transhydrogenase 2, subunit
Dde_1252	COG-CitT	Cation transporter	COG-CitT	Malate/fumarate uptake transporter, or antiporter with succinate
Dde_1253	COG-SfcA	Malic enzyme	SfcA	Malate:NADPH oxidoreductase, decarboxylating
Dde_2136	COG-HyaD	Hydrogenase expression/formation protein	COG-HyaD	Maturation protease for HysBA hydrogenase
Dde_2932	QrcA	hexaheme cytochrome <i>c</i>	QrcA	Type I cytochrome <i>c</i> ₃ :menaquinone oxidoreductase hexaheme cytochrome <i>c</i>
Dde_2933	QrcB	molybdopterin-containing family protein	QrcB	Type I cytochrome <i>c</i> ₃ :menaquinone oxidoreductase molybdopterin-containing subunit
Dde_2934	QrcC	periplasmic FeS protein	QrcC	Type I cytochrome <i>c</i> ₃ :menaquinone oxidoreductase molybdopterin-containing subunit
Dde_2935	QrcD	integral membrane protein	QrcD	Type I cytochrome <i>c</i> ₃ :menaquinone oxidoreductase integral membrane protein subunit

^aThe genome of *D. alaskensis* G20 has been reannotated since the original transcriptomic and proteomic data were collected. Designations from recent reannotations are used in the manuscript text while the 2011 annotation of *D. alaskensis* G20 (1) appears in all supplemental material.

Supplemental Table 3. Genes in *D. desulfuricans* *cycA*⁻ mutant I2 identified as differentially expressed by comparison of transcripts with those from wild-type *D. desulfuricans* G20 by microarray analysis

Locus	Protein	Annotation^a	Log₂ (I2/G20)^b
Lactate/sulfate (60/30) medium^c			
Up-regulated			
Dde_0899		Hypothetical protein	1.80
Dde_0900		Helix-turn-helix, Fis-type	1.70
Dde_0901		Hypothetical protein	1.62
Dde_0922		Adenine-specific DNA methylase-like	1.52
Dde_0923		Hypothetical protein	1.37
Dde_0924		Hypothetical protein	1.37
Dde_0929		Hypothetical protein	1.34
Dde_0930		Hypothetical protein	1.30
Dde_1154		Hypothetical protein	1.24
Dde_1155		Hypothetical protein	1.11
Dde_1156		Hypothetical protein	1.04
Dde_1157		Hypothetical protein	1.04
Dde_1981		Hypothetical protein	1.03
Down-regulated			
Dde_0645		Hypothetical protein	-1.19
Dde_1250	GltA	Glutamate synthase (NADPH), homotetrameric	-1.61
Dde_1251	COG-UbiB	Oxidoreductase, NAD-binding, putative	-1.79

Dde_1253	COG-SfcA	Malic enzyme	-2.74
Dde_1254	COG-FumA	Fumarate hydratase β subunit	-2.39
Dde_1255	COG-TtdA	Fumarate hydratase α subunit	-2.41
Dde_1256	FrdB	Fumarate reductase FeS protein	-0.90
Dde_1257	FrdA	Fumarate reductase flavoprotein subunit	-1.98
Dde_1258	FrdC	Fumarate reductase cytochrome b subunit	-2.47

Pyruvate (60) medium^d

Up-regulated

Dde_0001	DnaA	Chromosomal replication initiator protein	1.22
Dde-0280		Hypothetical protein	1.06
Dde_0282		TPR repeat	1.23
Dde_0283		Conserved hypothetical protein	1.29
Dde_0484		Conserved hypothetical protein	1.20
Dde_0597		Conserved hypothetical protein	1.93
Dde_0598		Hypothetical protein	1.34
Dde_0599		Hypothetical protein	1.71
Dde_0656	COG-FdnI	Fe-S-cluster-binding protein	1.14
Dde_0657	COG-HybA	Twin-arginine translocation pathway signal	1.61
Dde_0672		Hypothetical protein	1.26
Dde_0678	COG-PspE	Rhodanese-related sulfurtransferase	0.99
Dde_0681	COG-HybA	FeS-cluster-containing hydrogenase components	1.01
Dde_0682	COG-BisC	Twin-arginine translocation pathway signal	1.03

Dde_0707	FdhD	Formate dehydrogenase accessory protein	1.12
Dde_0715		Hypothetical protein	2.96
Dde_0717	FdhA-1	Formate dehydrogenase, α subunit	3.76
Dde_0718	FdhB-1	Formate dehydrogenase β subunit	3.33
Dde_0719	COG-MobB	Molybdopterin-guanine dinucleotide biosynthesis protein-like	1.61
Dde_0835		Polysaccharide deacetylase family protein	1.30
Dde_1076	COG-BisC	Thiosulfate reductase, putative	1.09
Dde_1194	COG-HisJ	ABC-type amino acid transport/signal transduction systems periplasmic component	1.14
Dde_1239		Hypothetical protein	1.10
Dde_1409	DnaB	Replicative DNA helicase	1.13
Dde_1440	ProC	Pyrroline-5-carboxylate reductase	1.04
Dde_1533	TypA	Small GTP-binding protein	1.33
Dde_1546	IclR	Regulatory protein	1.14
Dde_1581	DprA	DNA processing protein	1.00
Dde_1597		Hypothetical protein	1.35
Dde_1939		Hypothetical protein	1.44
Dde_1980		Transcriptional regulator	1.05
Dde_2006		Hypothetical protein	1.42
Dde_2129	COG-CarD	Transcriptional regulator	1.27
Dde_2153	TolQ	Transport protein	1.19
Dde_2154	COG-ExbB	Biopolymer transport protein	1.57

Dde_2155	COG-ExbD2	Biopolymer transport protein	1.43
Dde_2156	COG- TonB	Periplasmic protein, links inner and outer membranes	1.52
Dde_2484		Conserved hypothetical protein	1.09
Dde_2746		Hypothetical protein	1.13
Dde_2883		Sugar transferase	1.08
Dde_2926		Hypothetical protein	1.19
Dde_3016	NrdD	Ribonucleoside-triphosphate reductase	1.16
Dde_3028	CooS	Carbon-monoxide dehydrogenase, catalytic subunit	2.24
Dde_3029	CooC	Carbon monoxide dehydrogenase accessory protein	2.03
Dde_3103	CPG-CbiK	Cobalamin biosynthesis protein	1.27
Dde_3160		Conserved hypothetical protein	1.11
Dde_3161	NusA	Transcription termination factor NusA	1.04
Dde_3513	FdhA-3	Formate dehydrogenase, α subunit	1.46
Dde_3648		Hypothetical protein	1.28

Down-regulated

Dde_0132	COG-701	Permease	-0.99
Dde_0197		Conserved hypothetical protein	-1.35
Dde_0198		Hypothetical protein	-1.23
Dde_0199		Hypothetical protein	-1.45
Dde_0221	COG-CheY	Response regulator receiver	-1.52
Dde_0222		Conserved hypothetical protein	-2.53
Dde_0223	COG-HcaD	Flavoprotein oxidoreductase	-2.27

Dde_0324	COG-CheY	Response regulator receiver	-1.09
Dde_0348		Conserved hypothetical protein	-1.33
Dde_0398	COG-IlvB	Acetolactate synthase, large catalytic subunit	-1.19
Dde_0399	COG-IlvH	Acetolactate synthase III, small subunit	-1.13
Dde_0552		Conserved hypothetical protein	-1.46
Dde_0645		Hypothetical protein	-1.84
Dde_0646		Hypothetical protein	-1.08
Dde_0692	COG-CheY	Response regulator receiver	-1.63
Dde_0693	COG-CitT	putative transport protein	-1.81
Dde_0694	CheYI	Chemotaxis protein	-1.94
Dde_0696	CckA	Histidine kinase	-2.50
Dde_0724		Hypothetical protein	-2.18
Dde_0725		Fe-hydrogenase-like protein	-3.14
Dde_0821		Hypothetical protein	-1.34
Dde_0976		Hypothetical protein	-1.16
Dde_1074	COG-LdIP	L-lactate transport	-1.66
Dde_1140		Uncharacterized membrane protein	-1.06
Dde_1250	GltA	Glutamate synthase (NADPH), homotetrameric	-3.16
Dde_1251	COG-UbiB	Oxidoreductase, NAD-binding, putative	-3.12
Dde_1252	COG-CitT	Cation transporter	-3.43
Dde_1254	COG-FumA	Fumarate hydratase β subunit	-3.30
Dde_1255	COG-TtdA	Fumarate hydratase α subunit	-3.25

Dde_0324	COG-CheY	Response regulator receiver	-1.09
Dde_0348		Conserved hypothetical protein	-1.33
Dde_0398	COG-IlvB	Acetolactate synthase, large catalytic subunit	-1.19
Dde_0399	COG-IlvH	Acetolactate synthase III, small subunit	-1.13
Dde_0552		Conserved hypothetical protein	-1.46
Dde_0645		Hypothetical protein	-1.84
Dde_0646		Hypothetical protein	-1.08
Dde_0692	COG-CheY	Response regulator receiver	-1.63
Dde_0693	COG-CitT	putative transport protein	-1.81
Dde_0694	CheYI	Chemotaxis protein	-1.94
Dde_0696	CckA	Histidine kinase	-2.50
Dde_0724		Hypothetical protein	-2.18
Dde_0725		Fe-hydrogenase-like protein	-3.14
Dde_0821		Hypothetical protein	-1.34
Dde_0976		Hypothetical protein	-1.16
Dde_1074	COG-LdIP	L-lactate transport	-1.66
Dde_1140		Uncharacterized membrane protein	-1.06
Dde_1250	GltA	Glutamate synthase (NADPH), homotetrameric	-3.16
Dde_1251	COG-UbiB	Oxidoreductase, NAD-binding, putative	-3.12
Dde_1252	COG-CitT	Cation transporter	-3.43
Dde_1254	COG-FumA	Fumarate hydratase β subunit	-3.30
Dde_1255	COG-TtdA	Fumarate hydratase α subunit	-3.25

Dde_1256	FrdB	Fumarate reductase FeS protein	-2.46
Dde_1257	FrdA	Fumarate reductase flavoprotein subunit	-2.84
Dde_1258	FrdC	Fumarate reductase cytochrome b subunit	-3.01
Dde_1275	DctP	TRAP dicarboxylate transporter	-1.20
Dde_1343	HspC	Heat shock protein, HSP20 family	-1.12
Dde_1344	COG-IbpS	Heat shock protein, HSP20 family	-1.08
Dde_1347		Conserved hypothetical protein	-1.40
Dde_1605	COG-GrsT	Oleoyl-[acyl-carrier protein] hydrolase	-1.05
Dde_1614	COG-GntR	Regulatory protein GntR, HTH	-1.17
Dde_1615		Membrane protein, putative	-1.40
Dde_1616		Metallo-beta-lactamase family protein	-1.20
Dde_1709	FlaB1	Flagellin	-1.18
Dde_1710		Hypothetical protein	-1.03
Dde_2169		Conserved hypothetical protein	-1.24
Dde_2170		Conserved hypothetical protein	-1.21
Dde_2365		Conserved hypothetical protein	-2.02
Dde_2382		Hypothetical protein	-1.06
Dde_2451		Hypothetical protein	-1.28
Dde_2509		Conserved hypothetical protein	-1.26
Dde_2551		Conserved hypothetical protein	-1.00
Dde_2643	COG-UspA	Universal stress protein	-1.00
Dde_2824		Hypothetical protein	-3.12

Dde_3018		Flagellin, putative	-1.38
Dde_3211		Twin-arginine translocation pathway signal	-1.39
Dde_3539	COG-CoxL	Aldehyde oxidoreductase, putative	-1.02
Dde_3600		Conserved hypothetical protein	-1.21
Dde_3601		Hypothetical protein	-1.23
Dde_3733	GlpD	Glycerol-3-phosphate dehydrogenase	-1.06

^aGene annotation was obtained from <http://www.microbesonline.org>

^bLog₂ R, R = I2/G20 ratio of transcripts measured by microarray analysis. Only ratios with at least an absolute two-fold differential expression in I2 compared with G20 are reported.

^cLactate/sulfate (60/30) is MO basal salts with 60 mM sodium lactate and 30 mM sodium sulfate providing a medium for sulfate respiration.

^dMOPyr (60) is MO basal salts with 60 mM sodium pyruvate for growth by fermentation.

Supplemental Table 4. List of genes encoding proteins altered in protein abundance from *D. desulfuricans cycA*⁻ mutant I2 as compared to proteins from wild-type G20 as revealed by proteomic analysis

Locus	Protein	Annotation^a	Log₂ (I2/G20)^b
Lactate/sulfate (60/30) medium^c			
Up-regulated			
Dde_0115	FtsX	Cell division membrane protein	1.17
Dde_0139		ABC-type uncharacterized transport system, ATPase components.	1.22
Dde_0235		Predicted transcriptional regulator	1.37
Dde_0513		Aminotransferase, putative	1.77
Dde_0520	COG-LeuA	2-phosphinomethylmalic acid synthase, putative	1.00
Dde_0580	DchA	Decaheme <i>c</i> -type cytochrome	1.63
Dde_0583	RnfG	Electron transport subunit	1.38
Dde_0643		Signal transduction protein, putative	1.36
Dde_0651	HmcC	Integral membrane protein	1.01
Dde_0706	FdhE	Formate dehydrogenase formation protein	1.58
Dde_0717	FdhA-1	Formate dehydrogenase, α subunit, anaerobic	4.53
Dde_0718	FdhB-1	Formate dehydrogenase, β subunit	4.51
Dde_0719	COG-MobB	Molybdopterin-guanine dinucleotide biosynthesis protein	2.78
Dde_1328	COG-DppB	Dipeptide ABC transporter, nickel transport system permease protein	1.10
Dde_1334	COG-FatA	Acyl-ACP thioesterase	1.39

Dde_1378	ThiM	Hydroxyethylthiazole kinase	1.12
Dde_1390	COG-MoaB	Molybdopterin binding domain	1.48
Dde_1556	ThiH	Thiamin biosynthesis, thiazole moiety	1.68
Dde_1723	COG-RsbU	Regulatory protein, putative	1.22
Dde_1748	COG-HepA	ERCC4-like helicases	3.54
Dde_1814	GltB-1	Glutamate synthase,	4.32
Dde_2118		Conserved hypothetical protein	1.23
Dde_2212		Hypothetical protein	1.01
Dde_2218	Ion	Peptidase S16, ATP-dependent protease	1.37
Dde_2328	MetE	5-methyltetrahydropteroyltriglutamate--homocysteine	2.42
Dde_2334	SucCD	Succinyl-CoA ligase, alpha/beta subunit	2.26
Dde_2351		Conserved hypothetical protein	1.20
Dde_2507		Conserved hypothetical protein	1.14
Dde_2596	RecN	DNA repair protein	1.20
Dde_2597		Conserved hypothetical protein	2.57
Dde_2623		Conserved hypothetical protein	1.23
Dde_2703		Conserved hypothetical protein	1.16
Dde_2831	COG-LivK	Substrate-binding protein, putative	1.20
Dde_2960		TPR domain protein	1.18
Dde_3028	CooS	Carbon-monoxide dehydrogenase, catalytic subunit	2.02
Dde_3029	CooC	Carbon monoxide dehydrogenase accessory protein	2.32
Dde_3060	COG-WcaG	NAD-dependent epimerase/dehydratase family protein	1.18

Dde_3393		Tail fiber protein, putative	1.11
Dde_3523	COG-EutG	Alcohol dehydrogenase, iron containing	1.45
Dde_3556		Predicted acyltransferase	1.91
Dde_3620	COG-HisJ	ABC-type amino acid transport, periplasmic protein	6.24

Down-regulated

Dde_0054	PutA	Proline dehydrogenase	-1.14
Dde_0194		Conserved hypothetical protein	-1.45
Dde_0360		Predicted nucleoside-diphosphate sugar epimerase	-1.81
Dde_0417	GabT	4-aminobutyrate aminotransferase	-1.44
Dde_0479	WbaZ-2	Mannosyl transferase	-1.12
Dde_0550		Sensory box/GGDEF family protein	-1.28
Dde_0564		Conserved hypothetical protein	-1.03
Dde_0801	CobM	Precorrin-4 C11-methyltransferase	-1.30
Dde_0822		Conserved hypothetical protein	-1.46
Dde_1051	HtpX	Heat shock protein, integral membrane protein	-1.07
Dde_1065		Hypothetical protein	-1.03
Dde_1071		Multi-sensor signal transduction histidine kinase	-1.54
Dde_1120	FliD	Flagellar biosynthesis	-1.66
Dde_1210		Hydrogenase, iron-sulfur cluster-binding subunit, putative	-1.29
Dde_1213	COG-FrhD	Hydrogenase, Fe-S cluster binding subunit, putative	-1.47
Dde_1251	COG-UbiB	Oxidoreductase, NAD-binding, putative	-1.35
Dde_1257	FrdA	Fumarate reductase, flavoprotein subunit	-2.09

Dde_1323	COG-UbiD	3-octaprenyl-4-hydroxybenzoate carboxy-lyase family protein	-1.00
Dde_1370	COG-AmiC	N-acetylmuramoyl-L-alanine amidase	-1.81
Dde_1380		Conserved hypothetical protein	-1.22
Dde_1405	FlrC	Flagellar regulatory protein	-2.02
Dde_1411	RpsR	Ribosomal protein S18	-1.04
Dde_1415	TolC	Type I secretion outer membrane protein,	-4.33
Dde_1429	COG-HisJ	Putative periplasmic binding protein	-1.02
Dde_1534		Conserved hypothetical protein	-2.39
Dde_1570	FlaD	Flagellin	-2.59
Dde_1601	COG-MdlB	ABD-type multidrug transport system, ATPase and permease components	-1.21
Dde_1709	FlaB1	Flagellin and hook related proteins	-2.63
Dde_1713		Hypothetical protein	-1.34
Dde_1726		4-diphosphocytidyl-2C-methyl-D-erythritol synthase:	-2.52
Dde_1972	COG-PcnB	TRNA nucleotidyl transferase-related protein	-1.35
Dde_1987	COG-WcaA	Glycosyltransferase involved in cell wall biosynthesis	-1.60
Dde_2283	PdxA	Pyridoxal phosphate biosynthetic protein	-1.46
Dde_2396	RnpA	Bacterial ribonuclease P protein	-2.14
Dde_2470		Hypothetical protein	-2.29
Dde_2525	MetF	5,10-methylenetetrahydrofolate reductase	-1.01
Dde_2581	COG-Acd	1-aminocyclopropane-1-carboxylate deaminase	-2.33
Dde_2627		Conserved hypothetical protein	-1.01
Dde_2834	COG-LysR	Transcriptional regulator, putative	-1.38

Dde_2943	Aor-2	Aldehyde:ferredoxin oxidoreductase	-1.04
Dde_3094		Phosphoesterase, putative	-1.62
Dde_3126		Alcohol dehydrogenase, iron-containing	-2.12
Dde_3182	CycA	Cytochrome <i>c</i> ₃ , Tpl- <i>c</i> ₃	-3.62
Dde_3248	COG-PspE	rhodanese-like domain protein	-1.03
Dde_3424	HsdR	Type I site-specific deoxyribonuclease	-1.27
Dde_3530	COG-UbiB	NiFe hydrogenase gamma subunit, putative	-1.25
Dde_3637	Tme	Malic enzyme	-1.04
Dde_3647	KdsB	3-deoxy-D-manno-octulosonate cytidyltransferase	-1.02
Dde_3657	COG-UpgB	ABC-type sugar transporter, periplasmic component	-1.57
Dde_3698	COG-WcaA	Glycosyl transferase	-1.21

Pyruvate (60) medium^d

Up-regulated

Dde_0002	DnaN	DNA polymerase III, β subunit	1.06
Dde_0003	GyrB	DNA gyrase, β subunit	1.00
Dde_0004	GyrA	DNA gyrase, subunit A	1.65
Dde_0168	GlnH	Periplasmic glutamine-binding protein	1.91
Dde_0217	COG-PepP	Peptidase, putative	1.06
Dde_0410	GlpK	Glycerol kinase	2.60
Dde_0428	COG-RfaG	Glycosyltransferase	1.13
Dde_0518	COG-GatA	6-aminohexanoate-cyclic-dimer hydrolase	4.48
Dde_0546	MurA	UDP-N-acetylglucosamine 1- carboxyvinyltransferase	1.09

Dde_0557	COG-CaiC	Ligase/synthetase, putative	3.14
Dde_0598		Hypothetical protein	4.49
Dde_0652	HmcB	Transmembrane electron transfer protein	1.14
Dde_0679	COG-PspE	Sulfurtransferase, rhodanese-like	1.39
Dde_0682	COG-BisC	Oxidoreductase, putative	2.77
Dde_0706	FdhE	Formate dehydrogenase formation protein	2.08
Dde_0717	FdhA-1	Formate dehydrogenase, α subunit	8.57
Dde_0719	COG-MobB	Molybdopterin-guanine dinucleotide biosynthesis protein	4.42
Dde_0839	COG-RfaG	Glycosyltransferase	1.10
Dde_0988	AtpH	ATPase F1, δ subunit	1.56
Dde_0989	AtpF	ATP synthase F0, β subunit	1.04
Dde_1018		Chemotaxis protein CheY1, putative	1.27
Dde_1047	FtsZ	Cell division protein	1.13
Dde_1070	TalC	Transaldolase C	1.50
Dde_1098		Conserved hypothetical protein	1.35
Dde_1106	COG-AroA	5-enolpyruvylshikimate-3-phosphate synthase, putative	1.30
Dde_1129	PyrH	uridylate kinase	1.01
Dde_1185	DppA	Dipeptide transport protein	1.67
Dde_1198	COG-CheR	MCP methyltransferase	1.52
Dde_1207	COG-HrdC	Heterodisulfide reductase, C subunit	1.09
Dde_1214		Conserved hypothetical protein	1.05
Dde_1323	COG-UbiD	3-octaprenyl-4-hydroxybenzoate carboxy-lyase family protein	1.14
Dde_1328	COG-DppB	ABC transporter dipeptide/oligopeptide/nickel transport system permease protein	1.45
Dde_1439	Ndk	Nucleoside diphosphate kinase	2.46
Dde_1522	COG-BaeS	Histidine kinase	2.09

Dde_1586	FabK	Trans-2-enoyl-ACP reductase II	1.11
Dde_1594	MetG	Methionine tRNA synthetase	1.89
Dde_1646	COG-ThiJ	Peptidase	1.47
Dde_1672	PurM	Phosphoribosylaminoimidazole synthetase	1.78
Dde_1757	GpmA	phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	1.56
Dde_1759		Conserved hypothetical protein	1.23
Dde_1765	PyrG	CTP synthase	1.06
Dde_1769		Conserved hypothetical protein	1.48
Dde_1810	COG-ArgE	Peptidase	1.02
Dde_1814	GltB-1	Glutamate synthase, iron-sulfur cluster-binding subunit	3.55
Dde_2002	COG-AcrR	transcriptional regulator, TetR family	1.78
Dde_2052	RpoD	RNA polymerase sigma-70 factor	1.12
Dde_2056	RpsU	Ribosomal protein S21	1.12
Dde_2063	FusA	Translation elongation factor G	2.04
Dde_2091	COG-NadE	NAD ⁺ synthase, putative	1.94
Dde_2117		TPR domain protein, putative	2.20
Dde_2130	Rho	Transcription termination factor Rho	1.48
Dde_2134	HysB	[NiFeSe] hydrogenase, small subunit	2.62
Dde_2135	HysA	[NiFeSe] hydrogenase, large subunit,	1.11
Dde_2138	HynA-1	[NiFe] hydrogenase, large subunit	1.77
Dde_2140	HypC	Hydrogenase assembly chaperone	3.78
Dde_2236	Map	Methionine aminopeptidase	2.82
Dde_2238	RspsK	Ribosomal subunit protein	1.14
Dde_2325		Hypothetical protein	1.44
Dde_2394	YidC	Inner membrane protein, 60 kDa	1.47
Dde_2429	FabC	3-oxoacyl-(acyl-carrier-protein) reductase	1.58
Dde_2479		Conserved hypothetical protein	1.56
Dde_2498	HsdM	Type I restriction-modification system	1.46

Dde_2578	COG-Lrp	Heme biosynthesis protein, putative	1.68
Dde_2621	HemC	Porphobilinogen deaminase	1.73
Dde_2688	ThiD	Phosphomethylpyrimidine kinase	2.24
Dde_2727	COG-UspA	Universal stress protein family	1.15
Dde_2764	COG-HisJ	Amino acid binding protein, putative	1.19
Dde_2858		Cytochrome c-554 precursor	2.14
Dde_2933	MopB	Molybdopterin-binding protein	1.65
Dde_2934	MopC	Cytoplasmic FeS protein	1.32
Dde_2949		Conserved hypothetical protein	1.72
Dde_2979	COG-CynT	Carbonic anhydrase	1.88
Dde_2984	PrfA	Peptide chain release factor 1	1.33
Dde_3012	PyrE	Orotate phosphoribosyl transferase	1.96
Dde_3028	CooS	Carbon-monoxide dehydrogenase, catalytic subunit	4.18
Dde_3029	CooC	Carbon monoxide dehydrogenase accessory protein	3.62
Dde_3125		Conserved hypothetical protein	1.06
Dde_3243		Conserved hypothetical protein	1.43
Dde_3459	PurE	Phosphoribosylaminoimidazole carboxylase	1.79
Dde_3484	TrpE	Anthranilate synthase, component I	1.06
Dde_3486	AroA	3-phosphoshikimate 1-carboxyvinyltransferase	1.86
Dde_3489	COG-FbaB	Phospho-2-dehydro-3-deoxyheptonate aldolase, putative	1.36
Dde_3490	COG-FbaB	Phospho-2-dehydro-3-deoxyheptonate aldolase, putative	1.52
Dde_3641		Sulphate permease/anion transporter	1.03
Dde_3646	CarA	Carbamoyl-phosphate synthase, glutamine subunit	2.22
Dde_3673	PotD	Periplasmic polyamine transport protein	1.52
Dde_3712	COG-UspA	Universal stress protein family	1.44
Down-regulated			
Dde_0011	HisS	Histidyl-tRNA synthetase	-2.56

Dde_0060		Conserved hypothetical protein	-1.70
Dde_0076	MtnS	Methylthioribose salvage protein	-2.00
Dde_0098	COG-UspA	Universal stress protein family	-1.20
Dde_0111		Zinc resistance-associated protein, putative	-2.40
Dde_0184	COG-GlnQ	ATP-binding protein	-1.30
Dde_0204		Uncharacterized ACR protein	-1.27
Dde_0303		Transcriptional regulator, putative	-1.32
Dde_0312	COG-GlcD	Oxidoreductase, FAD/iron-sulfur cluster-binding domain protein, putative	-1.45
Dde_0325		Hypothetical protein	-2.26
Dde_0327		Response regulator receiver, CheY-like	-1.38
Dde_0377	PurH	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.72
Dde_0408	COG-GlpA	Glycerol-3-phosphate dehydrogenase, putative	-2.60
Dde_0432	Gmd	GDP-mannose 4,6-dehydratase	-2.18
Dde_0532		Sensory box/GGDEF family protein	-1.89
Dde_0692		Response regulator containing CheY-like receiver	-6.25
Dde_0693	COG-CitT	Transport protein, putative	-4.54
Dde_0695		Response regulator containing CheY-like receiver	-5.33
Dde_0765		CBS domain protein	-1.39
Dde_0863		Conserved hypothetical protein	-2.32
Dde_0970		Conserved hypothetical protein	-1.82
Dde_1081	ArgJ	Arginine biosynthesis protein	-1.75
Dde_1145		Conserved hypothetical protein	-1.21
Dde_1283	UidR	Repressor for uid operon	-1.18
Dde_1304	b0965	Coenzyme A binding protein, putative	-1.51
Dde_1310		Conserved hypothetical protein	-3.13
Dde_1425	COG-Dut	DeoxyUTP pyrophosphatase subfamily 1	-1.20
Dde_1444		Conserved hypothetical protein	-1.13

Dde_1600	FepA	Receptor precursor-mostly Fe transport	-1.34
Dde_1608		Polyketide synthase modules and related proteins	-1.41
Dde_1634		Conserved hypothetical protein	-1.60
Dde_1834		Unknown function domain protein	-1.30
Dde_1843		Iron-sulfur cluster binding protein	-1.38
Dde_1974		Conserved hypothetical protein	-1.30
Dde_1997		Conserved hypothetical protein	-2.06
Dde_2013	DegQ	Serine endoprotease	-1.15
Dde_2043	PdxJ	Pyridoxal phosphate biosynthetic protein	-1.01
Dde_2104	CheB-1	Response regulator receiver	-2.24
Dde_2116	RpoH	Sigma 70 family protein KO	-1.05
Dde_2121	CysS	CysteinyI-tRNA synthetase	-1.12
Dde_2142	IleS	Isoleucyl-tRNA synthetase	-1.86
Dde_2169		Conserved hypothetical protein	-1.08
Dde_2220	ClpP	Peptidase S14	-1.82
Dde_2265	Sat	ATP-sulfurylase	-1.44
Dde_2317	COG-Acd	Acetyl-CoA synthetase, putative	-1.70
Dde_2334	SucCD	Succinyl-CoA ligase, alpha/beta subunit	-1.35
Dde_2462		Conserved hypothetical protein	-1.91
Dde_2592		Conserved hypothetical protein	-1.34
Dde_2819		Hypothetical protein	-2.85
Dde_3103	COG- CbiK	Cobalamin biosynthesis protein	-1.21
Dde_3200		Fe-S cluster-binding/ATPase domain protein	-1.13
Dde_3476	IcdA	Isocitrate dehydrogenase	-1.26
Dde_3635	GltA	Glutamate synthase	-2.51
Dde_3636	COG-UbiB	Electron transfere protein, putative	-1.40
Dde_3707		Conserved hypothetical protein	-1.10

^a Gene annotation was obtained from <http://www.microbesonline.org>

^b $\text{Log}_2 R$, $R = I2/G20$ ratio of abundance values obtained from proteomic analysis of proteins with at least a two-fold up or down change in abundance in protein content in I2 versus G20.

^c Lactate/sulfate (60/30) is MO basal salts with 60 mM sodium lactate and 30 mM sodium sulfate providing a medium for sulfate respiration.

^d MOPyr (60) is MO basal salts with 60 mM sodium pyruvate for growth by fermentation.

Supplemental Table 5. Validation of microarray data by quantitative reverse transcription PCR analysis^a.

Locus	Annotation	qRT-PCR		Microarray	
		Lactate/sulfate (60/30)	Pyruvate (60)	Lactate/sulfate (60/30)	Pyruvate (60)
Dde_0717	Formate dehydrogenase α subunit	-1.15	8.28	0.72	3.76
Dde_1074	L-lactate transport	-0.49	-2.88	0.12	-1.66
Dde_1110	Adenylylsulfate reductase α subunit	0.46	-1.55	0.50	NR ^b
Dde_1253	Malic enzyme	-4.54	-5.90	-2.74	-3.71
Dde_2234	30S ribosomal protein S13	-1.01	1.39	0.03	0.51
Dde_2265	ATP-sulfurylase	-0.16	-2.10	0.08	NR
Dde_2272	Iron-sulfur binding protein, putative	0.01	0.01	0.30	-0.29
Dde_3103	Cobalmin biosynthesis protein	-0.05	4.76	0.20	1.27
Dde_3156	Flagellar biosynthesis	-0.16	-0.26	0.37	-0.64

^aData presented are expressed as $\text{Log}_2 R$, $R=(I2/G20)$

^bNR: data do not meet criteria for reproducibility

Supplemental Table 6. Protein Abundance Averages of G20 and I2 for Lactate/Sulfate (60mM/30mM) Medium (See Excel file)

Supplemental Table 7. Protein Abundance Averages of G20 and I2 for Pyruvate (60mM) Medium (See Excel file)

Supplemental Table 8. Protein Abundance Values for Each Technical Replicate for the Biological Replicates of G20 and I2 in Lactate/Sulfate (60mM/30mM) Medium (See Excel file)

Supplemental Table 9. Protein Abundance Values for Each Technical Replicate for the Biological Replicates of G20 and I2 in Pyruvate (60mM) Medium (See Excel file)

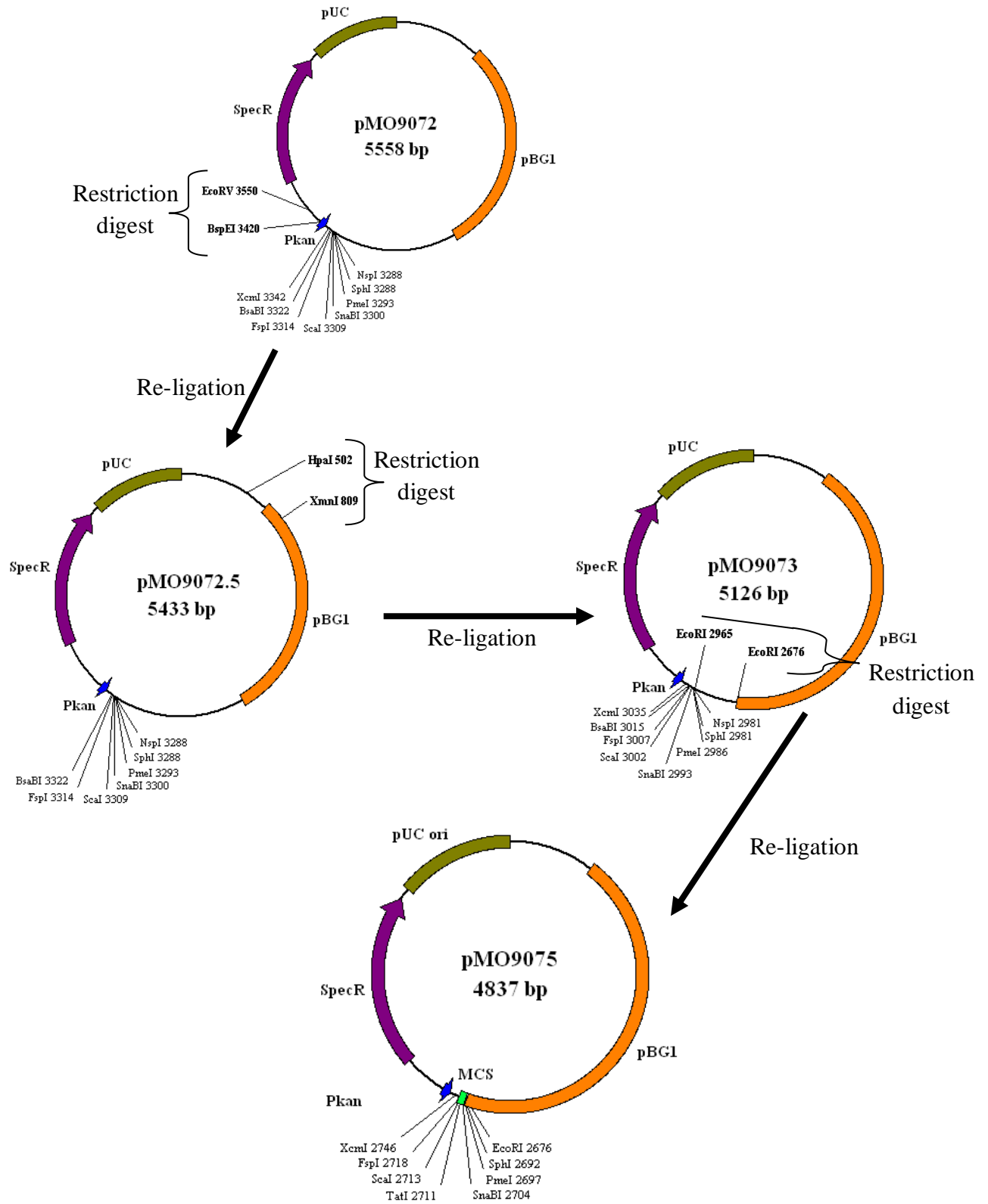
Supplemental Table 10. Proteomics analysis summary for *D. alaskensis* G20 and CycA mutant, I2^a

	G20	I2
Lactate/sulfate medium^b		
Total proteins identified	1965	1926
Proteins meeting confidence criteria ^c	1666	1613
Found only in wild type or mutant	162	109
Differentially expressed Log ₂ R ≥ 1 ^d	50 (R = I2/G20)	42 (R = I2/G20)
HP/CHP ^e among differentially expressed	9	8
Pyruvate medium^f		
Total proteins identified	1579	1425
Proteins meeting confidence criteria	838	761
Found only in wild type or mutant	106	183
Differentially expressed Log ₂ R ≥ 1	55 (R = I2/G20)	87 (R = I2/G20)
HP/CHP among differentially expressed	15	10

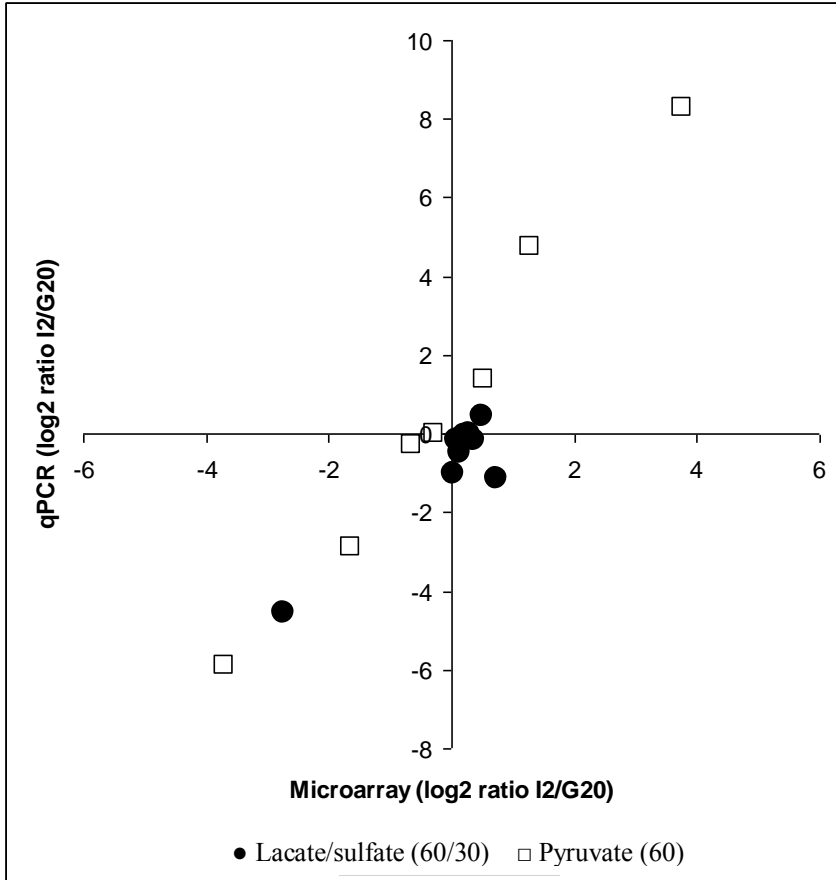
^a Accurate mass tag and time proteomics analysis performed by Environmental Molecular Sciences Laboratory of the Pacific Northwest National Laboratory.

^b Lactate/sulfate medium is MO basal salts plus 60 mM sodium lactate and 30 mM sodium sulfate.

^c Criteria given in Methods and Materials.



Supplemental Figure 1. Generation of pMO9075, the complementation shuttle vector



Supplemental Figure 2. Comparison of selected microarray data with qRT-PCR data.

Microarray data were validated using for real-time quantitative reverse transcription-PCR (qRT-PCR) as described previously (4), except that the Bio-Rad MyiQ2 Two-Color Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and Roche FastStart SYBR Green Master reagent (Roche Applied Science, Indianapolis, IN) were used for this experiment.