

Supplementary Material

Supplementary Table 1. Bacterial strains and plasmids used in this study

| Bacterial strain or plasmid | Relevant characteristic(s) ^{<i>a</i>} for these studies | Source or reference |
|-----------------------------|--|--|
| Escherichia. coli | | |
| strains | | |
| a-select (silver | deoR endA1 recA1 relA1 gyrA96 hsdR17($r_k^- m_k^+$) | Bioline |
| efficiency) | phoA supE44 thi-1 Δ (lacZYA-argF)U169 φ 80lacZ Δ M15 λ^{-} F ⁻ | |
| Desulfovibrio vulgaris | | |
| Hildenborough | Wild-type strain, ATCC29579, 5FU ^s G418 ^s Sp ^s | ATCC 29579, received ca. 2003 |
| JW710 | <i>D. vulgaris</i> Hildenborough Δupp ; Km ^s ; 5FU ^r ; | (Keller et |
| J W / 10 | parental strain | al., 2009) |
| JW9251 | JW710, Δ DVU2305-6::(<i>P_{npt}-npt-upp</i>); Km ^r , 5FU ^s ; marker exchange deletion | This study |
| JW9253 | JW9251, Δ DVU2305-6 Δ (<i>P_{npt}-npt-upp</i>); Km ^s ; 5FU ^r ; markerless deletion | This study |
| JW9271 | JW710, Δsat ; markerless deletion | (Hillesland et al., 2014) |
| JW9477 | JW710, Δ DVU2210::(<i>P_{npt}-npt-upp</i>); Km ^r , 5FU ^s ; marker exchange deletion | This study |
| JW9479 | JW9477, Δ DVU2210 Δ (<i>P_{npt}-npt-upp</i>); Km ^s ; 5FU ^r ; markerless deletion | This study |
| JW9480 | JW9271, $\Delta sat \Delta DVU2210::(P_{npt}-npt-upp)$; Km ^r , 5FU ^s ; marker exchange deletion | This study |
| JW9481 | JW9480, $\Delta sat \Delta DVU2210 \Delta (P_{npt}-npt-upp)$; Km ^s ; 5FU ^r ; markerless deletion | This study |
| JW9484 | JW9477, (<i>P_{npt}-npt-upp</i>)::DVU2210 _{A583C} ; Km ^s ; 5FU ^r ; creation of site-directed mutation in DVU2210 | This study |
| JW9485 | JW9480, $\Delta sat (P_{npt}-npt-upp)$::DVU2210 _{A583C} ; Km ^s ; 5FU ^r ; creation of site-directed mutation in DVU2210 | |
| JW9498 | JW9477, (<i>P_{npt}-npt-upp</i>)::DVU2210; complement, replacement of marker with wild-type DVU2210 gene Km ^s ; 5FU ^r | |
| JW9499 | JW9480, Δsat , (P_{npt} -npt-upp)::DVU2210; complement, replacement of marker with wild-type DVU2210 gene; Km ^s ; 5FU ^r | This study |

| JW9503 | JW710, Δ DVU1975::(<i>P_{npt}-npt-upp</i>); Km ^r , 5FU ^s ; | This study |
|----------------------|--|-------------|
| JW9505 | marker exchange deletion $W_{0503} = ADV(11075 A(B - ant ann)) Km^{St} 5EU^{T}$ | This study |
| J W 9303 | JW9503, Δ DVU1975 Δ (<i>P_{npt}-npt-upp</i>); Km ^s ; 5FU ^r ; markerless deletion | This study |
| JW9506 | JW9271, $\Delta sat \Delta DVU1975::(P_{npt}-npt-upp); Km^r$, | This study |
| 3 11 3 3 10 3 | 5FU ^s ; marker exchange deletion | This study |
| JW9507 | JW9506, $\Delta sat \Delta DVU1975 \Delta (P_{npt}-npt-upp)$; Km ^s ; | This study |
| | 5FU ^r ; markerless deletion | j |
| JW9510 | JW9481, Δ <i>sat</i> ΔDVU2210 ΔDVU1975::(<i>P_{npt}-npt-</i> | This study |
| | <i>upp</i>); Km ^r , 5FU ^s ; marker exchange deletion | J |
| JW9511 | JW9510, Δsat ΔDVU2210 ΔDVU1975 $\Delta(P_{npt}-npt-$ | This study |
| | <i>upp</i>); Km ^s ; 5FU ^r ; markerless deletion | 5 |
| JW9512 | JW9271, $\Delta sat \Delta DVU2305-6::(P_{npt}-npt-upp); Km^r$, | This study |
| | 5FU ^s ; marker exchange deletion | 2 |
| JW9513 | JW9512, $\Delta sat \Delta DVU2305-6 \Delta (P_{npt}-npt-upp)$; Km ^s ; | This study |
| | 5FU ^r ; markerless deletion | - |
| JW9514 | JW9481, Δsat ΔDVU2210 ΔDVU2305-6::(<i>P</i> _{npt} -npt- | This study |
| | <i>upp</i>); Km ^r , 5FU ^s ; marker exchange deletion | - |
| JW9515 | JW9514, Δsat ΔDVU2210 ΔDVU2305-6 Δ(Pnpt- | This study |
| | <i>npt-upp</i>); Km ^s ; 5FU ^r ; markerless deletion | |
| JW9516 | JW9479, ΔDVU2210 ΔDVU1975::(<i>P_{npt}-npt-upp</i>); | This study |
| | Km ^r , 5FU ^s ; marker exchange deletion | |
| JW9517 | JW9516, Δ DVU2210 Δ DVU1975 Δ (<i>P_{npt}-npt-upp</i>); | This study |
| | Km ^s ; 5FU ^r ; markerless deletion | |
| JW9518 | JW9479, ΔDVU2210 ΔDVU2305-6::(<i>P_{npt}-npt-upp</i>); | This study |
| | Km ^r , 5FU ^s ; marker exchange deletion | |
| JW9519 | JW9518, Δ DVU2210 Δ DVU2305-6 Δ (<i>P_{npt}-npt-</i> | This study |
| _ | <i>upp</i>); Km ^s ; 5FU ^r ; markerless deletion | |
| JW9520 | JW9253, ΔDVU2305-6 ΔDVU1975::(<i>P_{npt}-npt-upp</i>); | This study |
| | Km ^r , 5FU ^s ; marker exchange deletion | |
| JW9521 | JW9520, ΔDVU2305-6 ΔDVU1975 $\Delta(P_{npt}-npt-$ | This study |
| | <i>upp</i>); Km ^s ; 5FU ^r ; markerless deletion | |
| JW9522 | JW9507, Δ <i>sat</i> ΔDVU1975 ΔDVU2305-6::(<i>P</i> _{npt} -npt- | This study |
| | <i>upp</i>); Km ^r , 5FU ^s ; marker exchange deletion | |
| JW9523 | JW9522, Δ sat Δ DVU1975 Δ DVU2305-6 Δ (<i>P_{npt}-npt-</i> | This study |
| | <i>upp</i>); Km ^s ; 5FU ^r ; markerless deletion | T |
| JW9524 | JW9517, ΔDVU2210 ΔDVU1975 ΔDVU2305- | This study |
| | 6::(P_{npt} - npt - upp); Km ^r , 5FU ^s ; marker exchange | |
| 1110525 | deletion | T1 1 |
| JW9525 | JW9524, Δ DVU2210 Δ DVU1975 Δ DVU2305-6 | This study |
| 1110526 | $\Delta(P_{npt}-npt-upp)$; Km ^s ; 5FU ^r ; markerless deletion | |
| JW9526 | JW9511, $\Delta sat \Delta DVU2210 \Delta DVU1975 \Delta DVU2305-$ | This study |
| | 6::(<i>P_{npt}-npt-upp</i>); Km ^r , 5FU ^s ; marker exchange | |
| | deletion JW9526, Δ <i>sat</i> ΔDVU2210 ΔDVU1975 ΔDVU2305- | This study |
| JW9527 | | I DIG GTUDI |

| Plasmids | | |
|--------------|--|----------------------|
| pCR8/GW/TOPO | Cloning vector containing <i>aadAI</i> and pUC <i>ori</i> cassette; Sp ^r , non-replicating in <i>D. vulgaris</i> Hildenborough | Invitrogen |
| pCR4-TOPO | Cloning vector containing <i>npt</i> ; Km ^r | Invitrogen |
| pMO746 | pCR4-TOPO containing <i>upp</i> in an artificial operon with <i>npt</i> under the <i>npt</i> promotor; Km ^r | (Parks et al., 2013) |
| pMO9250 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 660bp region upstream of DVU2305 followed by the synthetic operon P_{npt} -npt-upp from pMO746 and a 633bp fragment downstream of DVU2306; Km ^r ; for marker-exchange deletion of DVU2305-6, Δ (DVU2305-6)::(P_{npt} -npt-upp) | This study |
| pMO9252 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 660bp region upstream of DVU2305 followed by a 633bp fragment downstream of DVU2306; Sp ^r ; for markerless deletion of DVU2305-6 | This study |
| pMO9476 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 493bp region upstream and 4 bp of DVU2210 followed by the synthetic operon P_{npt} - <i>npt-upp</i> from pMO746 and 501bp region downstream of DVU2210; Km ^r ; for marker-exchange deletion of DVU2210 Δ DVU2210::(P_{npt} - <i>npt-upp</i>) | This study |
| pMO9478 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 493bp region upstream and 4bp of DVU2210 followed by a 501bp fragment downstream of DVU2210; Sp ^r ; for markerless deletion of DVU2210 | This study |
| pMO9482 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 493bp region upstream of DVU2210 followed by DVU2210 and 501bp downstream; Sp ^r ; for complementation of the deleted DVU2210 | This study |
| pMO9483 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 493bp region upstream of DVU2210 followed by DVU2210(A583C) and 501bp downstream; Sp ^r ; for site-directed mutation construct of DVU2210. | This study |
| рМО9502 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 500bp region 67bp upstream of DVU1975 followed by the synthetic operon P_{npt} -npt-upp from pMO746 and 453bp region 48bp downstream of DVU1975; Km ^r ; for marker-exchange deletion of DVU1975 Δ DVU1975::(P_{npt} -npt-upp) | This study |

| pMO9504 | pCR8/GW/TOPO <i>aadAI</i> and pUC <i>ori</i> cassette plus 500bp region 67bp upstream of DVU1975 followed | This study |
|---------|---|------------|
| | by a 453bp region 48bp downstream of DVU1975; Sp ^r ; for markerless deletion of DVU1975 | |

^{*a*}Definitions: WT: wild-type; *upp*: uracil phosphoribosyltransferase (gene locus ID DVU1025); *sat*: sulfate adenylyl transferase (gene locus ID DVU1295); *npt*: neomycin phosphotransferase II, confers resistance to kanamycin, contains a mutation of C→A at -34bp; *aad*A1: aminoglycoside adenyltransferase with its native promotor, confers resistance to spectinomycin. 5FU^r: 5-fluorouracil resistance due to absence of *upp* gene; Km^r: kanamycin resistance, Sp^r: spectinomycin resistance, DVU2210_{A583C}: variant of DVU2210 in which the adenine at nucleotide 583 is changed to cytosine causing an amino acid change of S195R in the encoded protein.

Supplementary Table 2. Primer Information

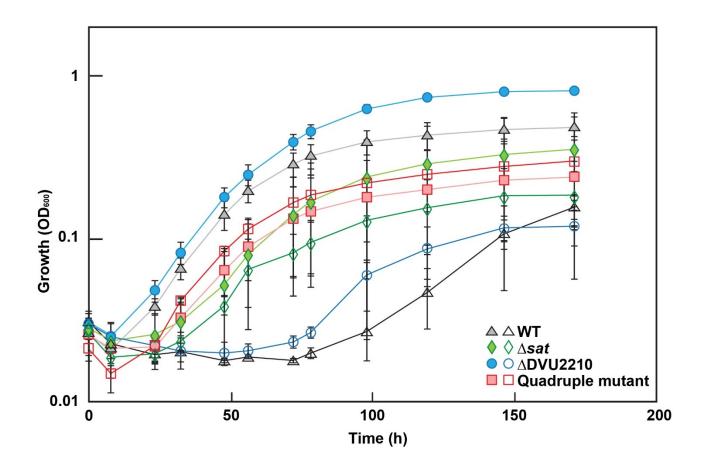
| Primer name | Primer sequence (5'-3') ^a | Purpose |
|----------------|--------------------------------------|--|
| SpecRpUC-F | CCAGCCAGGACAGAAATGCCTCG | Amplification of plasmid |
| | | backbone (<i>aadAI</i> -pUC) |
| | | from pCR8/GW/TOPO |
| SpecRpUC-R | ATGTGAGCAAAAGGCCAGCAAAAGGC | Amplification of plasmid |
| | | backbone (<i>aadAI</i> -pUC) |
| | | from pCR8/GW/TOPO |
| Kan gene Prom | CCGGAATTGCCAGCTGGGGCGC | Amplification of (P _{npt} -npt- |
| Nterm | | <i>upp</i>) from pMO746 |
| upp gene Cterm | CTTACTTGGTGCCGAATATCTTGTCGC | Amplification of (P _{npt} -npt- |
| | | <i>upp</i>) from pMO746 |
| SpacDpUC up | | Sequencing of cloned |
| SpecRpUC-up | CGCCTGGTATCTTTATAGTCCT | regions |
| pMO719-XbaI- | TGGGTTCGTGCCTTCATCCG | Sequencing of cloned |
| dn | IGOUTICOTOCCTTCATCCO | regions |
| DVU1975-upF | <u>GCCTTTTGCTGGCCTTTTGCTCACAT</u> | Amplification of upstream |
| | GTTCTCGACGACATCAAGCCCG | region of DVU1975 |
| DVU1975-upR | GCGACAAGATATTCGGCACCAAGTAAG | Amplification of upstream |
| | TAGTTTTGTTCCTCGACTCGAACTTGCATC | region of DVU1975, |
| | | specific for marker- |
| | | exchange deletion |
| DVU1975-dnF | GCGCCCCAGCTGGCAATTCCGG | Amplification of |
| | GGCACTTTCGTGTCTTGTAACGCC | downstream region of |
| | | DVU1975 |
| DVU1975-dnR | <u>CGAGGCATTTCTGTCCTGGCTGG</u> | Amplification of |
| | AACGGCGAAAGGTACGAGGC | downstream region of |
| | | DVU1975, specific for |
| | | marker-exchange deletion |
| DVU1975- | GGCGTTACAAGACACGAAAGTGCC | Amplification of upstream |
| MLD-upR | TAGTTTTGTTCCTCGACTCGAACTTGCATC | region of DVU1975, |

| | | specific for marker-less |
|---------------|--|---|
| | | deletion |
| DVU1975- | GATGCAAGTTCGAGTCGAGGAACAAAACTA | Amplification of |
| MLD-dnF | GGCACTTTCGTGTCTTGTAACGCC | downstream region of DVU1975, specific for marker-less deletion |
| DVU2210-upF | GCCTTTTGCTGGCCTTTTGCTCACAT | Amplification of upstream |
| D · CLEIC opi | GCTTCCTGCGCAAGGCGT | region of DVU2210 for |
| | | deletion plasmids and upstream-DVU2210- |
| | | downstream for |
| | | complementation and SNP strains |
| DVU2210-upR | GCGACAAGATATTCGGCACCAAGTAAG | Amplification of upstream |
| | TCATGGGTGTTTGTGGTGCAGAC | region of DVU2210, |
| | | specific for marker- |
| | | exchange deletion |
| DVU2210-dnF | <u>GCGCCCCAGCTGGCAATTCCGG</u> | Amplification of |
| | CCCTGTACCTCGGCGTGTAG | downstream region of |
| | | DVU2210, specific for |
| | | marker-exchange deletion |
| DVU2210-dnR | <u>CGAGGCATTTCTGTCCTGGCTGG</u> | Amplification of |
| | CGAGACGGAGATGGACCGC | downstream region of |
| | | DVU2210 for deletion |
| | | plasmids and upstream- |
| | | DVU2210-downstream |
| | | for complementation and SNP strains |
| DVU2210- | <u>CTACACGCCGAGGTACAGGG</u> | Amplification of upstream |
| MLD-upR | TCATGGGTGTTTGTGGTGCAGAC | region of DVU2210, |
| | | specific for marker-less |
| | | deletion |
| DVU2210- | <u>GTCTGCACCACAAACACCCATGA</u> | Amplification of |
| MLD-dnF | CCCTGTACCTCGGCGTGTAG | downstream region of |
| | | DVU2210, specific for |
| | | marker-less deletion |
| DVU2210- | CGAAGAGGAGTCCATCCTTCAAGGC <u>C</u> | Introduction of A583C in |
| S195R-F | GTTGCGAACTTGTGGAACGCCA | DVU2210 conferring |
| | | S195R; for introducing |
| | | the DVU2210 variant into |
| | | the marker-exchange |
| DVU2210- | | mutant Introduction of A583C in |
| S195R-R | TGGCGTTCCACAAGTTCGCAAC <u>G</u> GCCTTGAAGGATGGACTCCTCTTCG | |
| 517JK-K | | DVU2210 conferring S195R; for introducing |
| | | the DVU2210 variant into |
| | | the marker-exchange |
| | | mutant |
| <u> </u> | | matant |

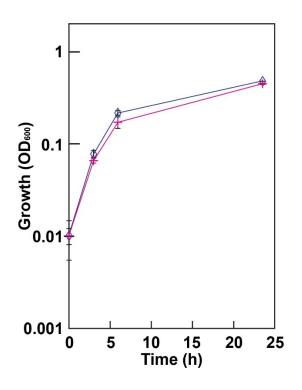
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| DVU2305-6- | <u>GCCTTTTGCTGGCCTTTTGCTCACAT</u> | Amplification of upstream |
|------------|------------------------------------|---------------------------|
| upF | CCGTCAGACCCGGCTGAAG | region of DVU2305-6 |
| DVU2305-6- | GCGACAAGATATTCGGCACCAAGTAAG | Amplification of upstream |
| upR | GCCTCTCTCCTGCTGTGGC | region of DVU2305-6, |
| | | specific for marker- |
| | | exchange deletion |
| DVU2305-6- | GCGCCCCAGCTGGCAATTCCGG | Amplification of |
| dnF | AGGCAGCAGACACGACAATATCAGAAG | downstream region of |
| | | DVU2305-6 |
| DVU2305-6- | CGAGGCATTTCTGTCCTGGCTGG | Amplification of |
| dnR | CACGAGTGCTACATCGATATCCAGTACGT | downstream region of |
| | | DVU2305-6, specific for |
| | | marker-exchange deletion |
| DVU2305-6- | CTTCTGATATTGTCGTGTCTGCTGCCT | Amplification of upstream |
| MLD-upR | GCCTCTCTCCTGCTGTGGC | region of DVU2305-6, |
| | | specific for marker-less |
| | | deletion |
| DVU2305-6- | GCCACAGCAGGAGAGAGGC | Amplification of |
| MLD-dnF | AGGCAGCAGACACGACAATATCAGAAG | downstream region of |
| | | DVU2305-6, specific for |
| | | marker-less deletion |

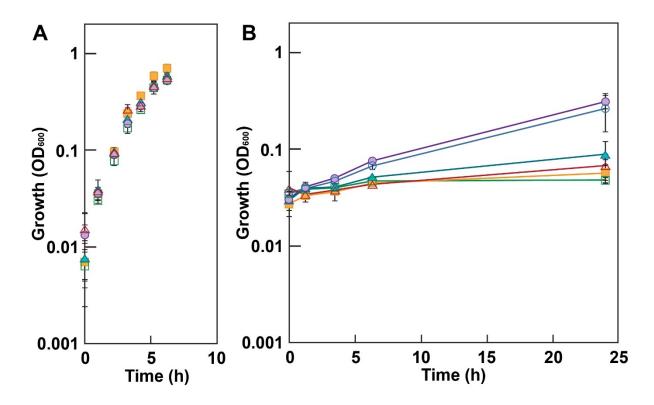
^{*a*}<u>Underlined</u> region of primer is overhang used for SLIC with plasmid backbone or P_{npt} -npt-upp; Underlined, bolded, and italicized <u>letter</u> indicates nucleotide changed in DVU2210 variant.



Supplementary Figure 1. Growth of *Desulfovibrio vulgaris* strains with sodium tungstate. Wild-type (WT) and mutant *D. vulgaris* strains were grown fermentatively at 34°C on MOYPc with (open symbols) and without (closed symbols) an added 5 mM tungstate. The quadruple mutant was lacking *sat*, DVU2210, DVU1975, and DVU2305-6. Growth was measured by optical density at 600nm (OD₆₀₀). Error bars denote the standard deviation from the average of triplicate cultures.



Supplementary Figure 2. Growth of *Clostridium sporogenes* ATCC 7955 with and without molybdate. *C. sporogenes* was grown anoxically in a modified Tanner's medium with no additional molybdate (diamonds) or an additional 7.5 mM sodium molybdate (crosses). Medium (10 ml) was inoculated with 0.2 ml of an overnight culture and incubated statically at 30°C. The modified Tanner's medium (Tanner, 2007) contained the following per liter: 0.8 g sodium chloride, 1 g ammonium chloride, 0.1 g potassium chloride, 0.1 g potassium phosphate monobasic, 0.2 g magnesium sulfate heptahydrate, 0.04 g calcium chloride dihydrate, 5 g glucose, 2 g yeast extract, 10 g TES, 1 ml vitamins, 5 ml trace elements. The vitamin solution was made at a 10x concentration of that described in Brandis and Thauer (1981). The trace elements solution was the same as that described for the MO medium in this study. All solutions were made in anoxic milliQ water generated by boiling the water under nitrogen gas. Growth was measured by optical density at 600nm (OD₆₀₀). Error bars denote the standard deviation from the average of triplicate cultures.



Supplementary Figure 3. *E. coli* growth in the presence of sodium molybdate. *E. coli* cultures were grown on LC (A) or M9 medium (Atlas, 2010) with 1.25 μ M FeCl₂ (B) with 0, 3, or 10 mM sodium molybdate (designated by circles, triangles, and squares, respectively). Two *E. coli* strains were analyzed, one containing the *D. vulgaris* wild-type DVU2210 on plasmid pMO9582 (open symbols) and the other containing the modified DVU2210 (A583C) on plasmid pMO9583 (closed symbols). Starter cultures were prepared by inoculating a scrape from a frozen stock into 50 ml flasks containing 5 ml of LC with spectinomycin. These were incubated at 37°C and shaken at 150 rpm. Following overnight incubation, 1 ml of each culture was washed to remove medium components by centrifuging the cells at 17,000 x g, removing the supernatant, resuspending the pellet in 1 ml of phosphate buffered saline pH 7.4, and repeating centrifugation and resuspension. Test tubes containing 3 ml of medium were inoculated with 0.03 ml of the washed starter cultures and incubated at 37°C while shaking at 150 rpm. Growth was measured by optical density at 600nm (OD₆₀₀). Error bars denote the standard deviation from the average of triplicate cultures.

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