

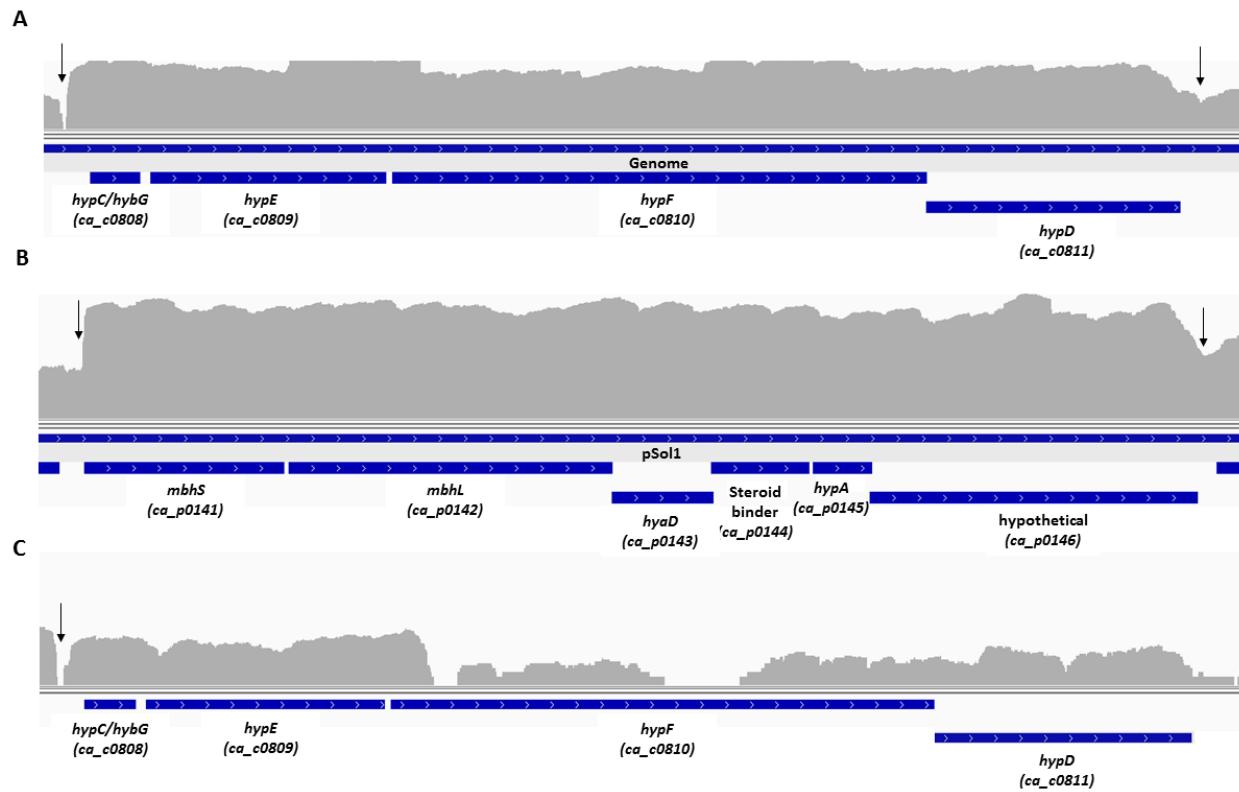
## Supplemental Information

**Supplemental table S1.** Primers used in this study

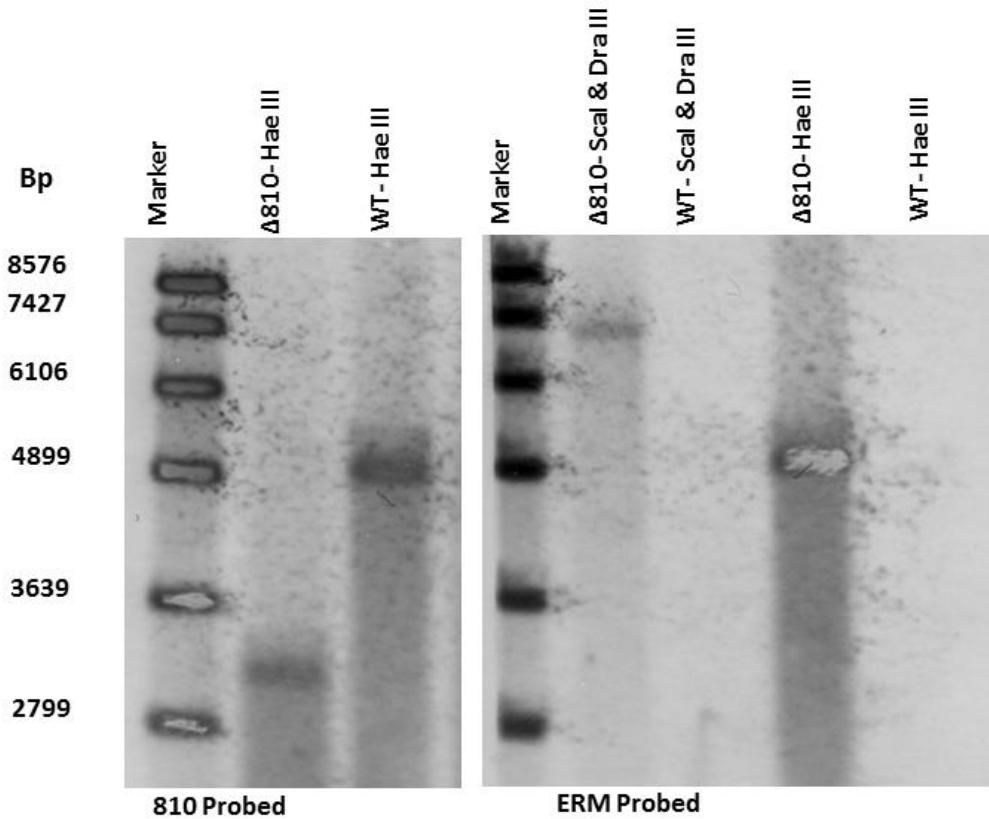
Primer Name	Sequence	Use
ca_c0810 sense	AGGAAAAAAACAGCTGTGAATTACAGG	Southern blot/PCR check
ca_c0810 antisense	CACATTTCTAACATGCCATTATATTGAC	Southern blot/PCR check
Intron probe sense	GACAATACTGCTCATAGTAACGGTAC	Southern blot
Intron probe antisense	<u>CTTTTAACGAGTGAAAAGTACTCAACC</u>	Southern blot

**Supplemental table S2.** Primers generated from TargeTron Gene Knockout System site for interruption of *ca\_c0810* at the sense sequence site 153|154S and the Intron sequence generated using the primers. The designed Intron sequence was cloned into pMTL007C-E2.

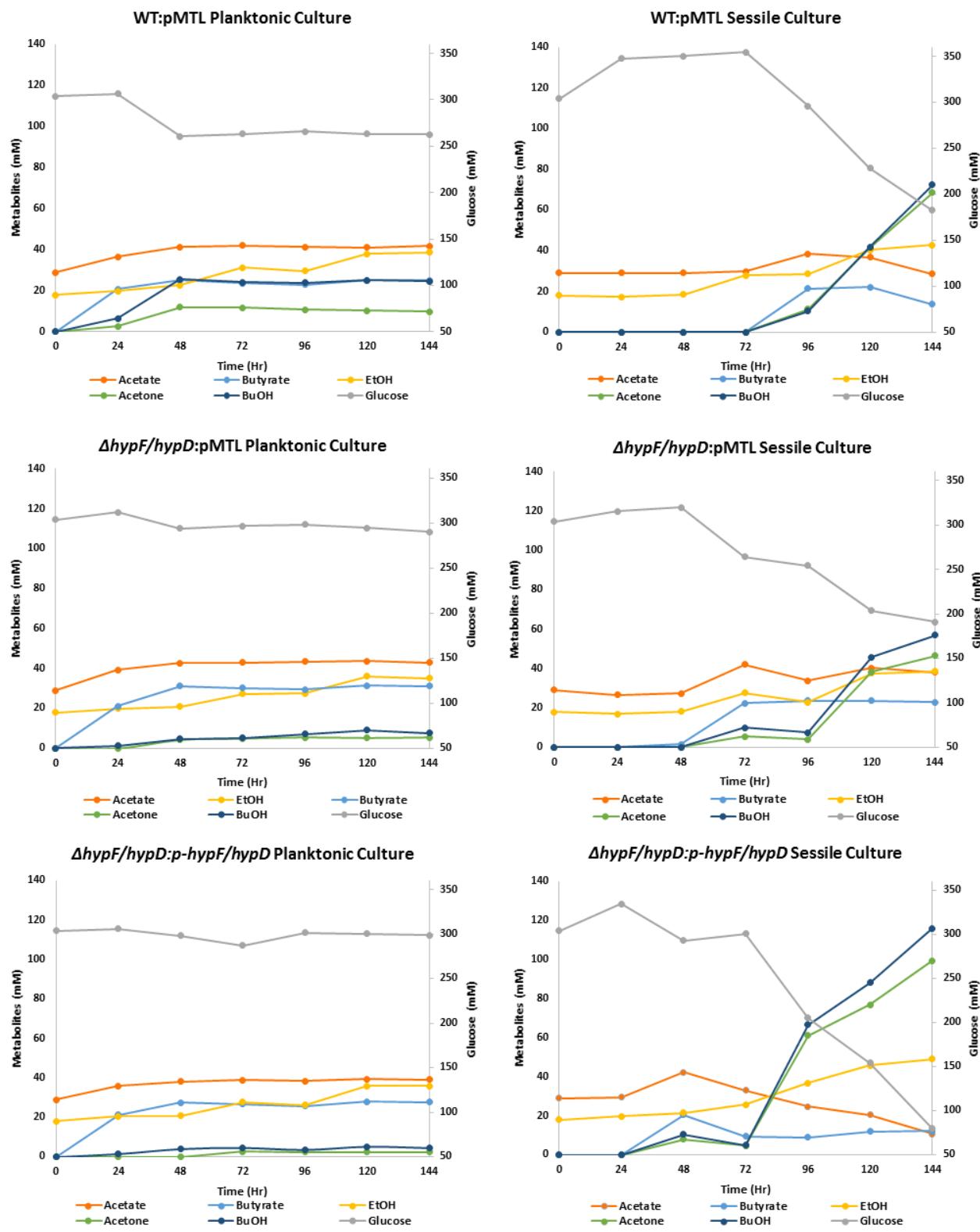
Ca_c0810 153 154s Intron Design Primers and Sequence	
<b>153 154s-IBS</b>	AAAAAAAGCTTATAATTATCCTTAGGTGACAG <u>TTCA</u> GTGCGCCCAGATAGGGTG
<b>153 154s-EBS1d</b>	CAGATTGTACAAATGTGGTGATAACAGATAAGTCAG <u>TTCAAATAACTAC</u> CTT
<b>153 154s-EBS2</b>	TGAACGCAAG <u>TTCTAATT</u> CGATT <u>CACCTCGA</u> TAGAGGAAAGTGTCT
<b>Designed Intron Sequence</b>	AAGCTTATAATTATCCTTAG <u>GTGACAG</u> <u>TTCA</u> GTGCGCCCAGATAGGGTGTAA GTCAAGTAG <u>TTAAGGTACTACT</u> CTGTAA <u>GATAACACAGAAAACAGCCAAC</u> CT AACCGAAAAG <u>CGAAAG</u> CTGAT <u>ACGGG</u> ACAGAGCACGGTGGAA <u>AGCGAT</u> G AGT <u>TACCTAAAGACAATCGGGTACGACTGAGTCGCAATG</u> TAAT <u>CGAT</u> ATAA GGT <u>TAAAGTTGT</u> TTACT <u>GAACGCAAG</u> <u>TTCTAATT</u> CGAT <u>TCACCTCGA</u> T GAGGAAAG <u>GTCTGAAAC</u> CT <u>AGTACAAAGAAAGG</u> TAAG <u>TTATTGAACT</u> G ACTT <u>ATCTGTTATCACCAC</u> ATT <u>GTACA</u>



**Supplemental Figure S1.** [NiFe]-hydrogenase genes occur in two operons identified by RNA-Seq data analysed in Rockhopper and DOOR<sup>2</sup> predictions. Arrows indicate the beginning and end of operons determined by RNA-Seq read coverage in Rockhopper. The operons are expressed from left to right indicated by the white arrows on the blue bar. The operon on the genome includes *ca\_c0808* through *ca\_c0811* in panel A. The operon on the pSol megaplasmid includes *ca\_p0141* through *ca\_p0146* in panel B. Panel C shows a decrease in read coverage of operon *ca\_c0808-ca\_c0811* from the  $\Delta$ *hypF/hypD* mutant indicating gene interruption.

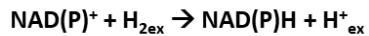


**Supplemental Figure S2.** Southern blot indicates *ca\_c0810* gene is interrupted by ClosTron group II intron. Wild type and  $\Delta hypF/hypD$  genomic DNA was digested with endonucleases and probed for intron insertion using probes for *ca\_c0810* gene or insertion of the erythromycin (erm) gene into the *ca\_c0810* gene. The expected sizes/number of base pairs of bands for mutant and wild type bands for the gene probe after digestion with HaeIII are 3160 bp and 5009 bp. There should not be bands in the wild type lanes when probed for the erm gene. Band sizes in the  $\Delta hypF/hypD$  lane after digestion with Sca/DraIII is 7004 bp or HaeIII is 5155 bp.



**Supplemental Figure S3.** Representative metabolite profiles of planktonic and sessile cultures grown in minimal medium containing 6.0% glucose from an N=3 experiment. Metabolite concentrations vs. time for WT:pMTL (empty vector),  $\Delta$ hypF/hypD:pMTL (empty vector), and  $\Delta$ hypF/hypD:p-hypF/hypD.

$\Delta hypF/hypD:p-hypF/hypD$  (complement) cultures. The left Y axes correspond to metabolite concentrations in mM for acetate, ethanol (EtOH), butyrate, acetone, and butanol (BuOH). The right Y axes correspond to glucose concentration in mM vs time.



$$\text{Rxn potential} = E_{H_{\text{ex}}} + E_{\text{NAD(P)H}} + 2 \times \text{membrane potential}$$

$$E_{H_{\text{ex}}} = -0.0592 \times pH_{\text{ex}}$$

$$E^{\circ'}_{\text{NAD(P)H}} = -0.320 \text{ V}$$

$$E_{\text{NAD(P)H}} = E^{\circ}_{\text{NAD(P)H}} - 0.0296 \times \log (\text{NAD(P)}^+/\text{NAD(P)H}) + 0.0296(7-pH_{\text{int}})$$

**Supplemental Figure S4.** Reaction equations, calculations and energy values used for determining contributions of pH and membrane potentials to electron transport and electron acceptors.