

## Supplementary Information for Agapakis et. al. “Insulation of a synthetic hydrogen metabolism circuit in bacteria”

>Cr. HydA1; AAL23572

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**Figure S1. Sequence of codon optimized commercially synthesized genes.** Gene names listed as Organism.Gene Name; GenBank Accession Number (Cr=*Chlamydomonas reinhardtii*, So=*Spinacia olearcea*)

Gene	GenBank Accession	Primer (Italics indicate Biobrick sites)
Ca HydA 5'	CAC3230	<i>CCTTTCTAGA</i> ATGAAAACAATAATCTTAA
Ca HydA 3'		AAGGCTGCAGCGGCCGCTACTAGT TTCATGTTTTGAAACATTTTTA
Cs HydA 5'	CAU09760	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGATAAACATAGTAATTGATGAAA
Cs HydA 3'		AAGGCTGCAGCGGCCGCTACTAGT TTTATTGTATTTAAGTGAATAAT
So HydA 5'	SO_3920	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGACAACGACAACCTTATCAACCAG
So HydA 3'		AAGGCTGCAGCGGCCGCTACTAGT AATGTTACCCAGCCATGAAGA GCCT
So HydB 5'	SO_3921	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGAACAAGAAAAACACCTATTTG
So HydB 3'		AAGGCTGCAGCGGCCGCTACTAGT AGAGCTTAATTTGGTGCATCGACA
Tm HydA 5'	AF044577	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGAAAATTTACGTTGATGGAAGAGAAGTTAT
Tm HydA 3'		AAGGCTGCAGCGGCCGCTACTAGT TCAGCCATTTTTCGAAAGCTCCTCCAGCACCTTCTC
Ca PFOR 5'	CAC2229	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGAAAAAATGAAACTATGG
Ca PFOR 3'		AAGGCTGCAGCGGCCGCTACTAGT TTATTGATTAGCTAATCTTT
Da PFOR 5'	CAA70873	AAACATATGGGAAAGAAAATGATGACG
Da PFOR 3'		AAACCTAGGCTACTTCTTCGTCGGCTTGC
Ec ydbK 5'	CAQ31878	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATTACTATTGACGGTAATGGCG
Ec ydbK 3'		AAGGCTGCAGCGGCCGCTACTAGT TTAATCGGTGTTGCTTTTTCCGC
Ca Fd 5'	CAC0303	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGGCATATAAATAACAGA
Ca Fd 3'		AAGGCTGCAGCGGCCGCTACTAGT CTCTTGAAGTGGAGCTCCTA
Zm Fd 5'	EU956042	<i>CCTTGAATTCGCGGCCGCATCTAGA</i> ATGGCTGTATACAAGGTGAAGCTTG
Zm Fd 3'		AAGGCTGCAGCGGCCGCTACTAGT CAGGTCGCCTTCCTTGTGGGTGTGG
Ca mut. 5'		GTGGACAATGTTCCAGAAGAGAAAATTGTGAGTTCCTTAAACTTG
Cs mut. 5'		GCAGCAAACCTAGCTGAGTTCATGAATAGCGG
SoB mut.		CGCGGTAGAAAAGTACTACAAAGAGTTCGGTGGCGAGCCATTAGGACATATGTCCC
CrHydA P2K		CCGCTTCTAGAGCTGCAAAGGCCGCAGAAAGCTCCTTTGTC
CrHydA D126K		CGCTATGTTAGAAAAAGCTATCCTAAGCTAATTCCATACGTGAGC
CrHydA E5K		CCGCTTCTAGAGCTGCACCAGCCGCAAAGGCTCCTTTGTC
CrHydA M119K		CCAGGTTGGATCGCTAAGTTAGAAAAAGCTATCC

**Table S1: Primers used for cloning and mutagenesis of heterologous pathway components**



Ca ---MKTIIINGVQFNTDEDTILKFARDNNIDISALCFLNNCNNDINKCEICTVEVEG-T 56  
 Cs ---MINIVIDEKTIQVQENTTVIQAALANGIDIPSLCYLNECGN-VGKCGVCAVEIEGKN 56  
 Cr -----  
 So MNKKKHLFAEDSFFLSRRKFMAVGAAFVAALAIPIGWFT-----S 40  
 Tm ---MKIYVDGREVIINDNERNLLEALKNVGIEIPNLCYLSEASIYG---ACRMLLVEING 54

Ca GLVTACDTLIEDGMIINTNSDAVNEKIKSRISQLLDIHEFKCGPCNRRENCEFLKLVIKY 116  
 Cs NLALACITKVEEGMVVKTNSEKQERVKMRVATLLDKHEFKCGPCPRENCEFLKLVIKT 116  
 Cr -----  
 So KLERNEYIKARSQGLYKDDSLAKTRVSHANPAVEKYYKEFGGEPLGHMSHELLHTHFVD 100  
 Tm QITTSCTLKPYEGMKVKTNTPFIYEMRRILELILATHNRDCTTCDRNGSCKLQKYAEDF 114

Ca KARASKPFLPKDKTEYVDEKSKSLTVDRTKCLLCGRVCNACGKNTETAYMKFLNKNKGTI 176  
 Cs KAKANKPFVVEDKSYIDIRSKSIVIDRDKCVLCGRCEAACTKTGTGTAISICKSESGRI 176  
 Cr -----  
 So RTKLSSMTTTYQPGEIQG---LIKINASKCKGCDACKQFCPTHAINGASGAVHS----- 152  
 Tm GIRKIR--FEALKKEHVRDESAPVVRDTSKCILCGDCVRVCEEIQGVGVIEFAKRGFESV 172

Ca IGAEDEKCFDDTNCLLCGQCIACPVAALSE-KSHMDRVKNALNAPEKHVIVAMAPSVRA 235  
 Cs VQATGGKCFDDTNCLLCGQVAAACPVGALTE-KTHVDRVKEALEDPNKHVIVAMAPSI 235  
 Cr -----  
 So -----IDEDKCLSCGQCLINCPFSAIEETHSALETVIKKLADKNTTVVGIAPAVRV 204  
 Tm VTTAFDTPLIETECVLCGQCVAYCPTGALSIRNDIDKLI EALES-DKIVIGMIAPAVRA 230  
 . \* . : : . . \* : \*\* : \*

Ca SIGELFNMGFGVDVTGKIYALRQLGFDKIFDINFGADMTIMEEATELVQRIEN----- 289  
 Cs SMGELFKLGYGVDVTGKLYASMRALGFDKVFDFINFGADMTIMEEATEFIERVKN----- 289  
 Cr AIAETLGLAPGATTPKQLAEGLRRLGFDEVFDTLFGADLTIMEEGSELHLRLEHLEAHP 100  
 So AIGEEFGLGTGELVTGKLYGAMNQAGF-KIFDCNFAADLTIMEEGSEFIHRLHANVKGEA 263  
 Tm AIQEEFGIDEDVAMAELKVSFLKTIGFDKVFVDFGADLVAYEEAHEFYERLKK----- 284  
 : : \* : : . . : : . \*\* : \*\* \* . \* : . \* : \* . \* : \*

Ca --NGPF**PMFTSCCPGWVR**QAENYYPELLNLSAKSPQQIFGTASKTYYPISGLDPKNV 347  
 Cs --NGPF**PMFTSCCPAWVR**QVENYYPEFLENLSAKSPQQIFGAASKTYYPQISGISAKDV 347  
 Cr HSDEPL**PMFTSCCPGWIA**MLEKSYPDLIYVSSCKSPQMLAAMVKSYLEKKGIAPKDM 160  
 So NAG-PL**PQFTSCCPGWVR**YLETRYPALLPNLSTAKSPQQMAGTVAKTYGAKVYQMOPENI 322  
 Tm --GERL**PQFTSCCPAWVK**HAEHTYPQYLQNLSSVKSPQQALGTVIKKIYARKLVPEEKI 342  
 . : \* \* \* \* \* . \* : \* \* : : \* : \* \* : : \* . . : : :

Ca FTV**TVMPCTSKK**FEADRPQME-----KDGLRDIDAVITTRRELAKMIKDAKIPF 395  
 Cs FTV**TIMPCTAKK**FEADREEMY-----NEGIKNIDAVLTTRELAKMIKDAKINF 395  
 Cr VMV**SIMPCTRKQ**SEADRDWF-----CVDADPTLRQLDHVITTVLGNIFKGERGINL 211  
 So FTV**SVMPCTSKK**LEASRPEFNSAWQYHQEHGANSPSYQDIDAVLTTREMAQLLKLDDIDL 382  
 Tm FLV**SFMPCTAKK**FEAEREEHEG-----IVDIVLTTRELAQLIKMSRIDI 386  
 . \* : \* \* \* \* \* \* : \* \* . : \* \* \* \* \* : \* \* \* \* \* \* : :

Ca AKLEDSEADPAMGEYSGAGAFGATGGVMEAAALRSKDFAEAELEDEIEYKQVRGLNGIK 455  
 Cs ANLEDEQADPAMGEYTGAGVIFGATGGVMEAAALRTAKDFVEDKDLTDIEYQIRGLQGIK 455  
 Cr AELPEGEWDNPMGVGSGAGVLFGTGGVMEAAALRTAYELFTGTPLRSLSEVRGMDGIK 271  
 So ANTAEYQGDSLSEYTGAGTIFGTTGGVMEAAALRTAHKVLGTGEMAKLEFEPVRGLKGVK 442  
 Tm NRVEPQPFRPYGVSSQAGLGFKGAGGVFSCVLSVLNNEIG---IEKVDVKSPE--DGIR 441  
 . \* . : \* \* \* \* \* : \* \* \* \* \* : \* \* \* \* \* : \* \* \* \* \* \* : :

Ca EAEVEINNNKYN-----VAV 470  
 Cs EATVEIGGENYN-----VAV 470  
 Cr ETNITMVPAPGSKFEELLKHRAAARAEAAAHGTPGPLAWDGGAGFTSEDGRGGITLRVAV 331  
 So SASVSLFDTELN-----QDVTVNVAV 463  
 Tm VAEVTLKDGTSFKG-----AV 457  
 : : : \* \*

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Ca      INGAS-NLFKFMKSGMINEKQYHFIEVMACHGGCVNGGGQPHVNPKDLEKVDIKKVRASV 529
Cs      INGAA-NLAEFMNSGKILEKQYHFIEVMACPGGCVNGGGQPHVSAKEREKVDVRTVRASV 529
Cr      ANGLG-NAKKLITKMAGEAKYDFVEIMACPAGCVNGGGQPRSTDKA-----ITQKRQAA 385
So      VHDMGNNEPVLRDVMAGTSPYHFIEVMACAGGCVNGGGQP-----IEGKSSW 512
Tm      IYGLG-----KVKKFLEERKDVEIIEVMACNYGCVNGGGQPYPNDSR-----IREHRAKV 507
      . .      : .      . : : * : * * * * . * * * * *      :

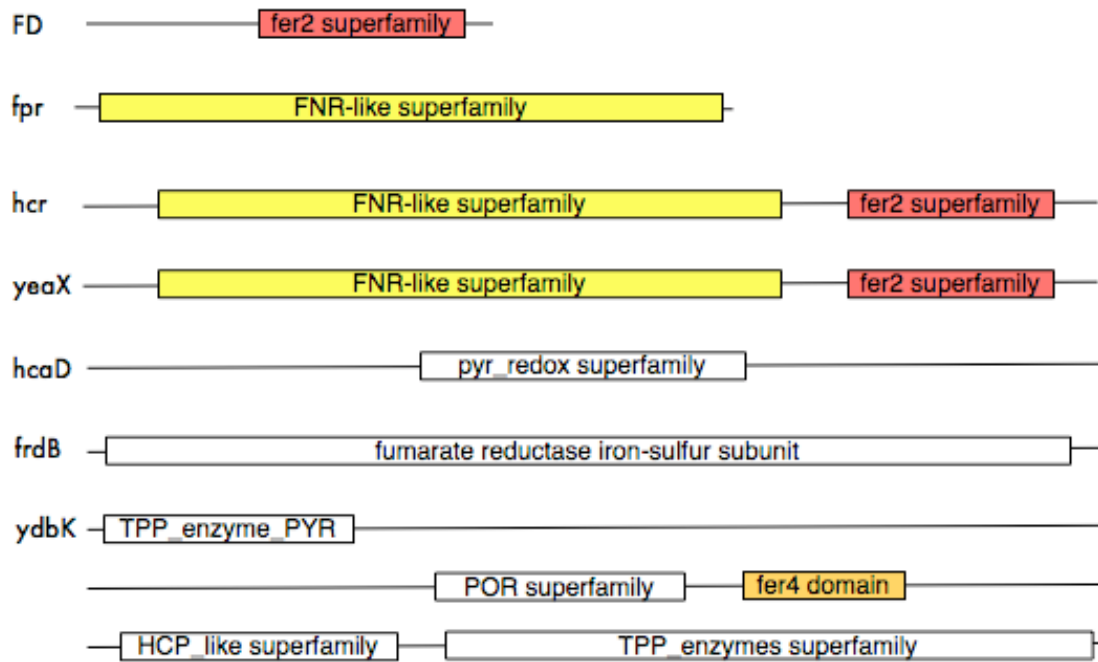
Ca      LYNQDEHLSKRKSHENTALVKMYQNYFGKPGEGRAHEILHFYK----- 574
Cs      LYNQDNLEKRKSHKNTALLNMYDYMGAPGQGAHELLHLKYNK----- 574
Cr      LYNLDEKSTLRRSHENPSIRELYDTYLGEPLGHKAHELLHHTHYVAGGVEEKDEKKTSSGR 445
So      LGNI----- 516
Tm      LRDTMGIKSLTPVENLFLMKLYEEDLKD--EHRHEILHTTYRPRRRYPEKDVEILPVP 565
      * :

Ca      -----
Cs      -----
Cr      C----- 446
So      -----
Tm      NGEKRTVKVCLGTSCYTKGSYEILKKLVYVKENDMEGKIEVLGTFCVENCGASPNVIVD 625

Ca      -----
Cs      -----
Cr      -----
So      -----
Tm      DKIIIGGATFEKVLLEELSKNG 645

```

**Figure S3. Sequence alignment of five hydrogenases** Protein sequences of *Clostridium acetobutylicum* (Ca), *Clostridium saccharobutylicum* (Cs), *Chlamydomonas reinhardtii* (Cr), and *Thermotoga maritima* (Tm) HydA and *Shewanella oneidensis* HydB + HydA aligned using ClustalW web server (<http://www.ebi.ac.uk/Tools/clustalw/>). Catalytic site binding area highlighted in bold with critical cysteine residues in red.



**Figure S4. Deletion of competing reactions leads to hydrogen circuit insulation.** A.) Domain structure of deleted ferredoxin-homology genes. FD-ferredoxin; fpr-flavodoxin:NADP<sup>+</sup> reductase; hcr-NADH oxidoreductase; yeaX-predicted oxidoreductase; hcaD-ferredoxin:NAD<sup>+</sup> reductase; frdB-fumarate reductase; ydbK-putative pyruvate-ferredoxin oxidoreductase. Genes were identified by BLAST homology search of the *Escherichia coli* genome against *Spinacia olearcea* ferredoxin I. Domain structure schematized from NCBI conserved domain search.

