Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacterial System

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National Renewable Energy Laboratory

Project ID
PD039

J. Craig Venter
INSTITUTE

Overview

Timeline

- Project start date: 5-01-05
- Project end date: 1-30-2014*
- Percent complete: 80%

Budget

- Total project funding
  - DOE share: $2.019M for JCVI
  - DOE share: $1.51M for NREL
  - JCVI cost-share: $820K
- Funding received for FY11
  - $123K for JCVI
  - $250K for NREL
- Planned funding for FY12
  - $150K for JCVI
  - $350K for NREL

Barriers

- Barriers addressed
  - Production Barrier AI: Continuity of H_2 production

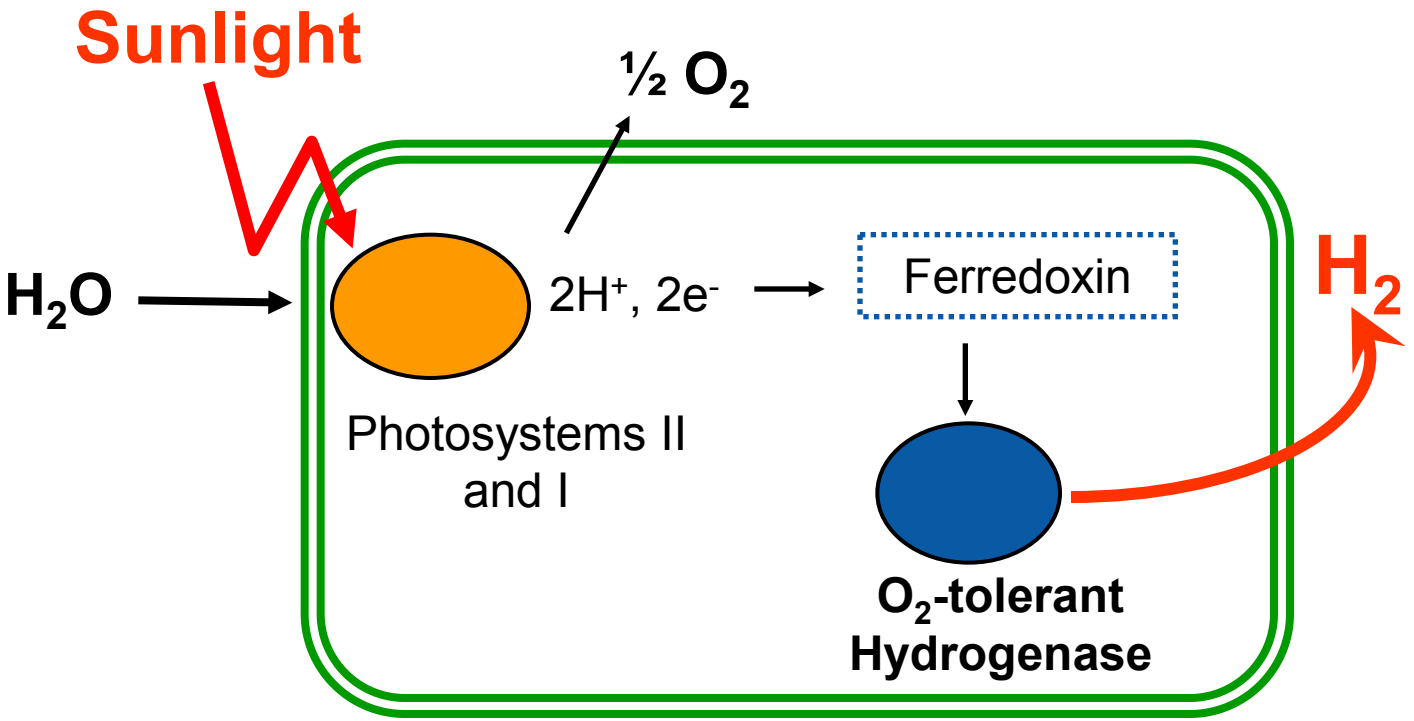
Partners

- J. Craig Venter Institute
- National Renewable Energy Laboratory

*NREL project continuation and direction determined annually by DOE.

Objective-Relevance:

Develop an O_2-tolerant cyanobacterial system for continuous light-driven H_2 production from water



Characteristics	2009 Status	2011 Target	2018 Target
Duration of continuous photoproduction	Zero to 30 seconds in air	Produce one cyanobacterial recombinant evolving H_2 through an O_2-tolerant NiFe- hydrogenase	Demonstrate H_2 production in air in a cyanobacterial recombinant

Milestones

Task 1. (JCVI and NREL)

Month/ Year	Milestone	% Comp
Dec-10	Determine electron mediator requirement	JCVI,100%
May-11	Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase	NREL, 100%
Sept-11	Confirm expression of the CBS hydrogenase maturation proteins in recombinant *Synechocystis*	NREL, 100%
May-12	Identify two additional hydrogenase maturation genes via quantitative PCR and the analysis of CBS genome	NREL, 100%
Sept-12	Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase	NREL, 80%

Task 2. (JCVI)

Month/ Year	Milestone	% Comp
Aug-11	Construct a cyanobacterial hybrid to express an active environmental hydrogenase	JCVI, 100%
Apr-13	Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity.	JCVI, 5%
Nov-13	Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase.	JCVI, 5%
Jan-14	Measure light-dependent H_2 production in modified strains.	JCVI, 5%

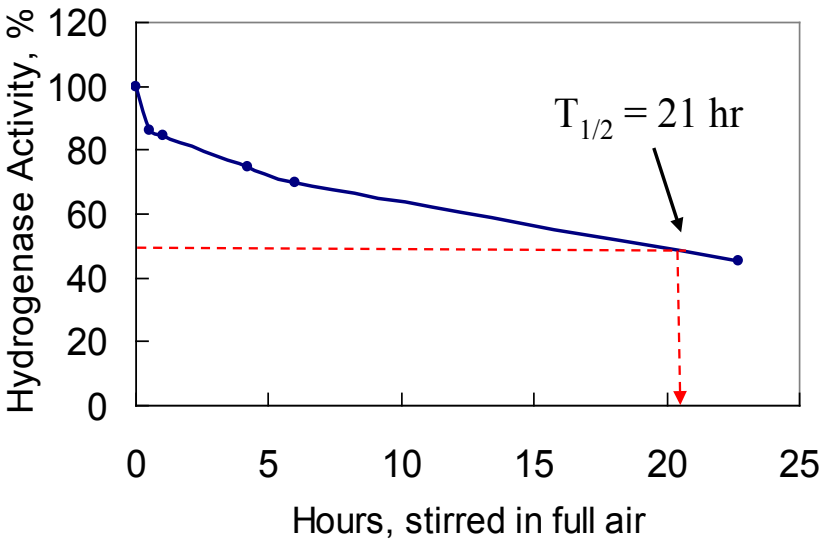
Go/No Go Decision: Jan-2013

Demonstrate 5x increase hydrogenase activity from environmental H2ase in cyanobacteria as measured by in vitro H2 evolution assay.

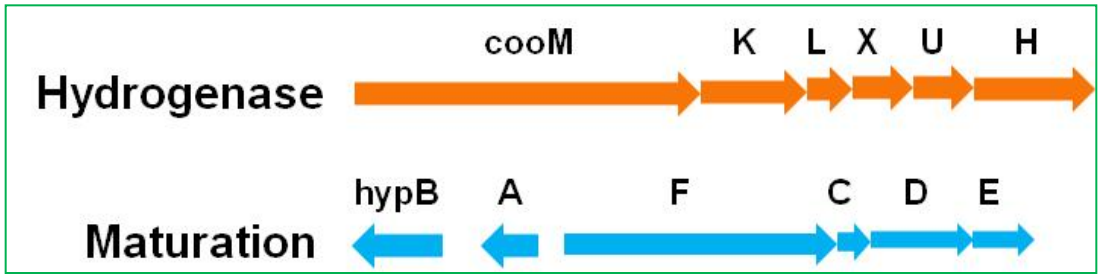
Task 1: NREL Approach

- Transfer an O_2-tolerant NiFe-hydrogenase from the bacterium *Rubrivivax gelatinosus* CBS (hence "CBS", an NREL isolate) into the model cyanobacterium *Synechocystis* sp. PCC 6803

CBS hydrogenase half-life in air: 21 hr



Cloned hydrogenase structural and putative maturation genes

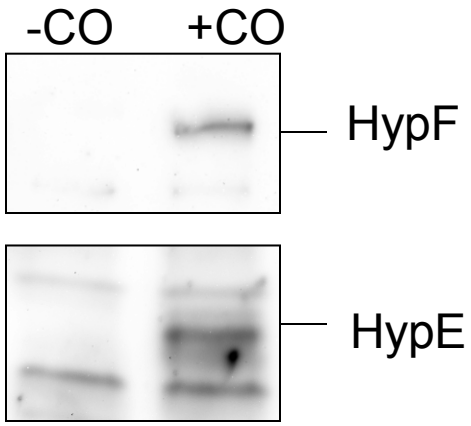


NREL approach is complementary to that of JCVI in harnessing two of Nature's O_2-tolerant hydrogenases and transferring into two model cyanobacteria.

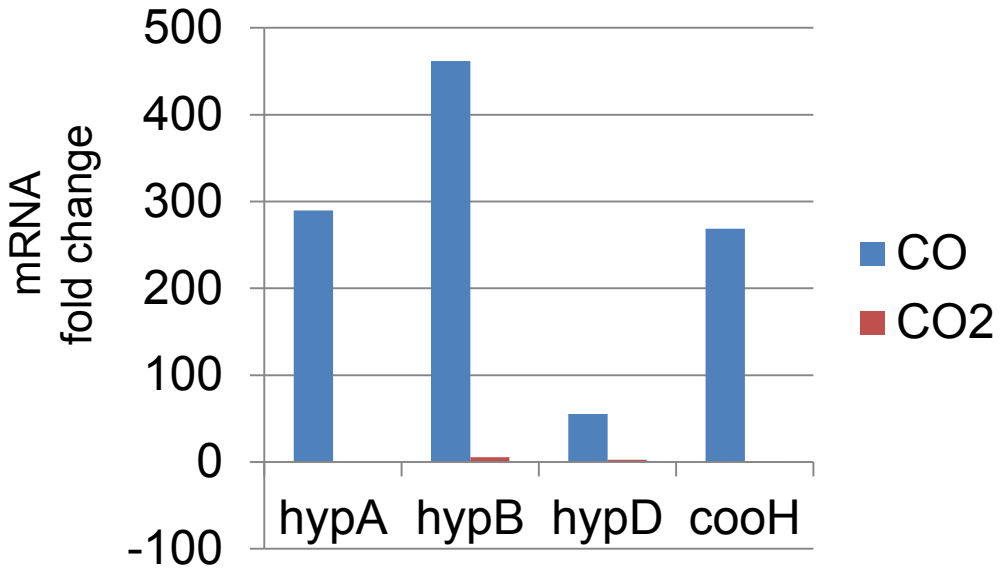
Task 1: NREL Technical Accomplishments

Determined Function of the *hypABDF* Maturation Genes

- Protein immunoblot verified *hypF* as a maturation gene
  - Protein expression is specific to CO.



- Quantitative RT-PCR verified *hypABD* as maturation genes
  - Gene expression is specific to CO

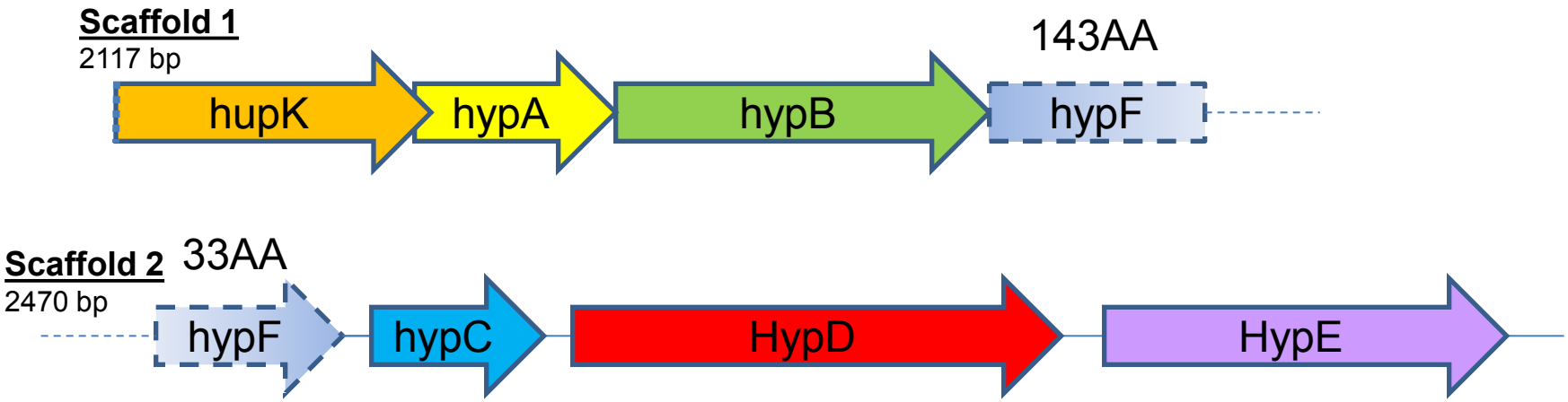


- Completed Milestone "Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase (5/11).
- Completed Milestone "Identify two additional hydrogenase maturation genes via quantitative PCR and analysis of CBS genome (5/12).

Task 1: NREL Technical Accomplishments

Identified Additional Hydrogenase Maturation Genes

- NREL collaborates with Michigan State U. (leveraging Office of Science funding) and Pacific Biosciences (free service) in sequencing and annotating CBS genome.
  - 5 MB genome size, 3,582 genes with predicted function.
- Analysis of CBS genome revealed a new set of *hyp* genes.



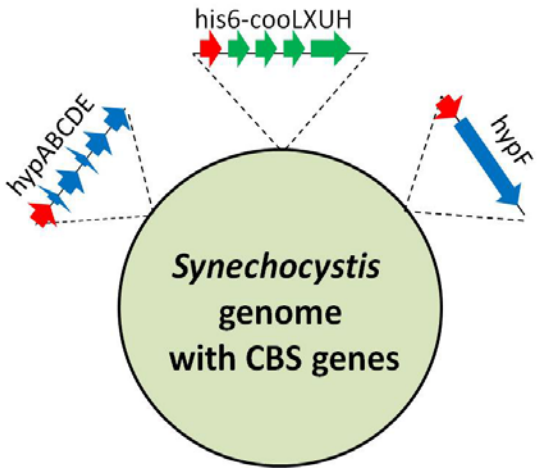
- Via PCR we filled the gap in the *hypF* gene and confirmed these maturation genes are in a single operon.

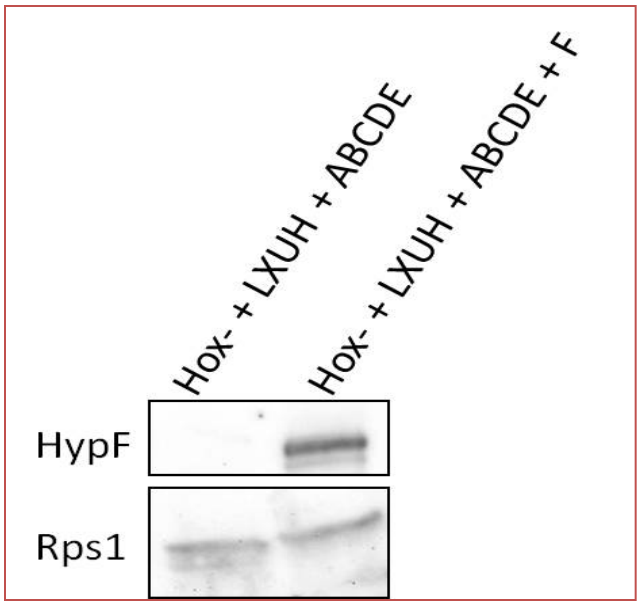
- **Completed Milestone "Identify two additional hydrogenase maturation genes via quantitative PCR and the analysis of CBS genome (5/12).**

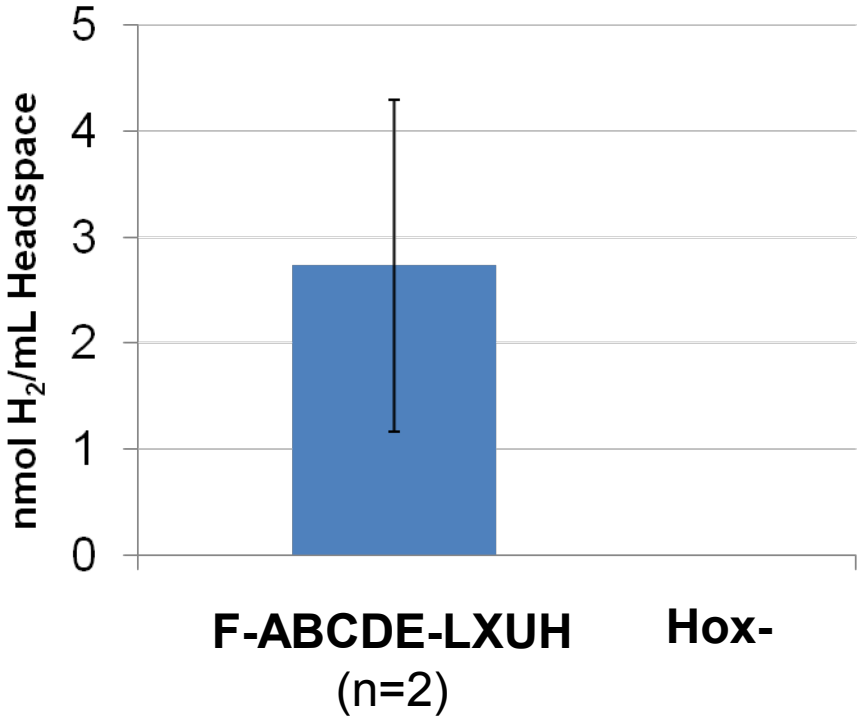
Task 1: NREL Technical Accomplishments

Synechocystis Recombinant #1 with CBS Hydrogenase Activity

- **Ten** CBS genes were transformed into a *Synechocystis* recombinant in which its native hydrogenase was inactivated (Hox-).
- The recombinant expressed HypF protein and displayed CBS hydrogenase activity.





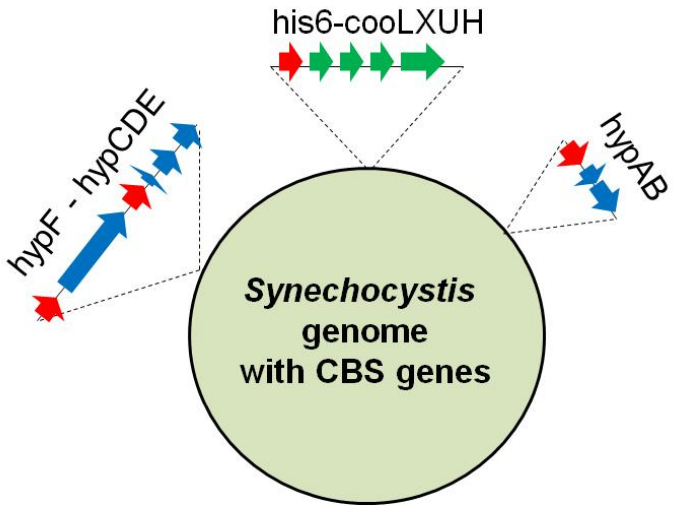


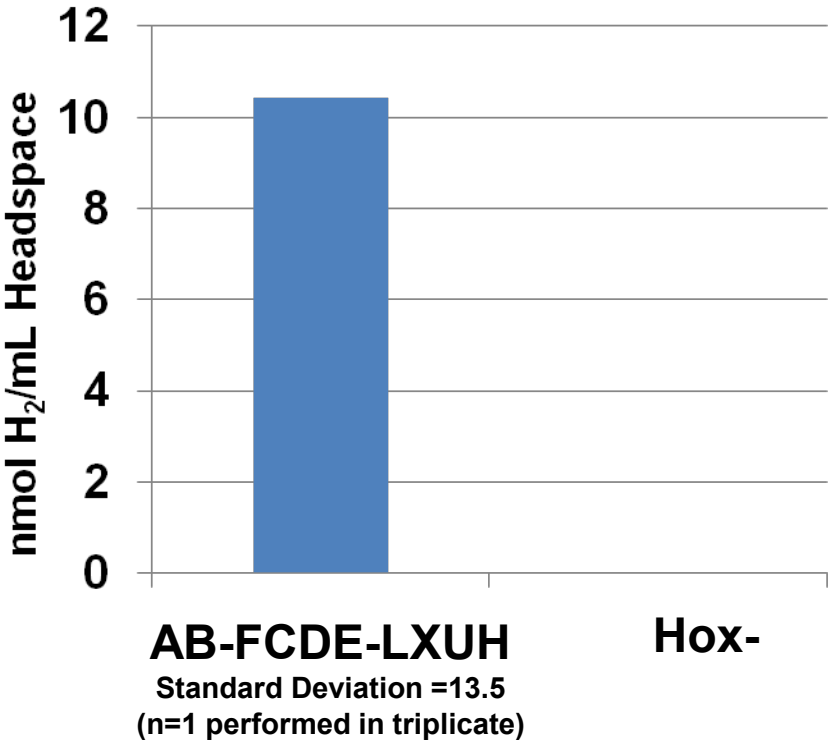
- **Completed** Milestone "*Confirm expression of the CBS hydrogenase maturation proteins in recombinant Synechocystis*" (9/11).
- **On track** to complete Milestone "Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase" (9/12).

Task 1: NREL Technical Accomplishments

Synechocystis Recombinant #2 with CBS Hydrogenase Activity

- Generated a second *Synechocystis* recombinant displaying CBS hydrogenase activity, with different promoters arrangement.
- Promoter engineering is ongoing to improve activity further.

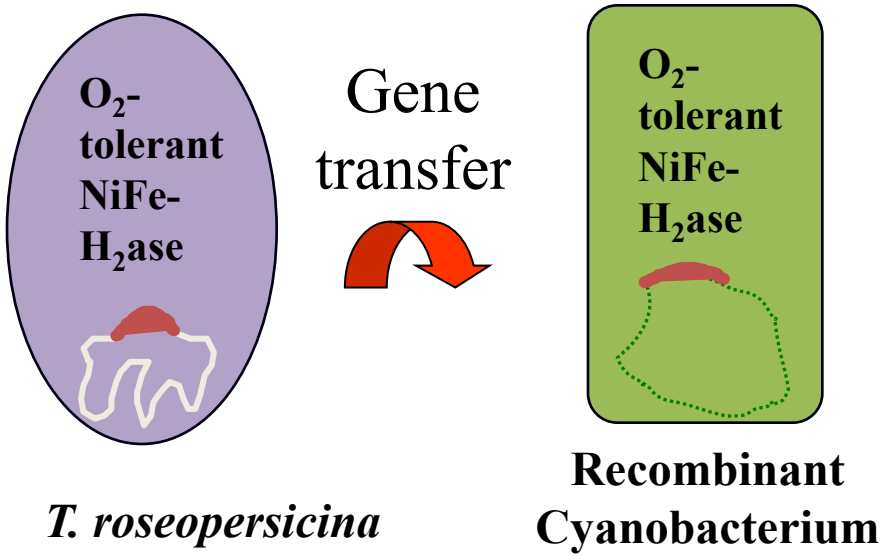


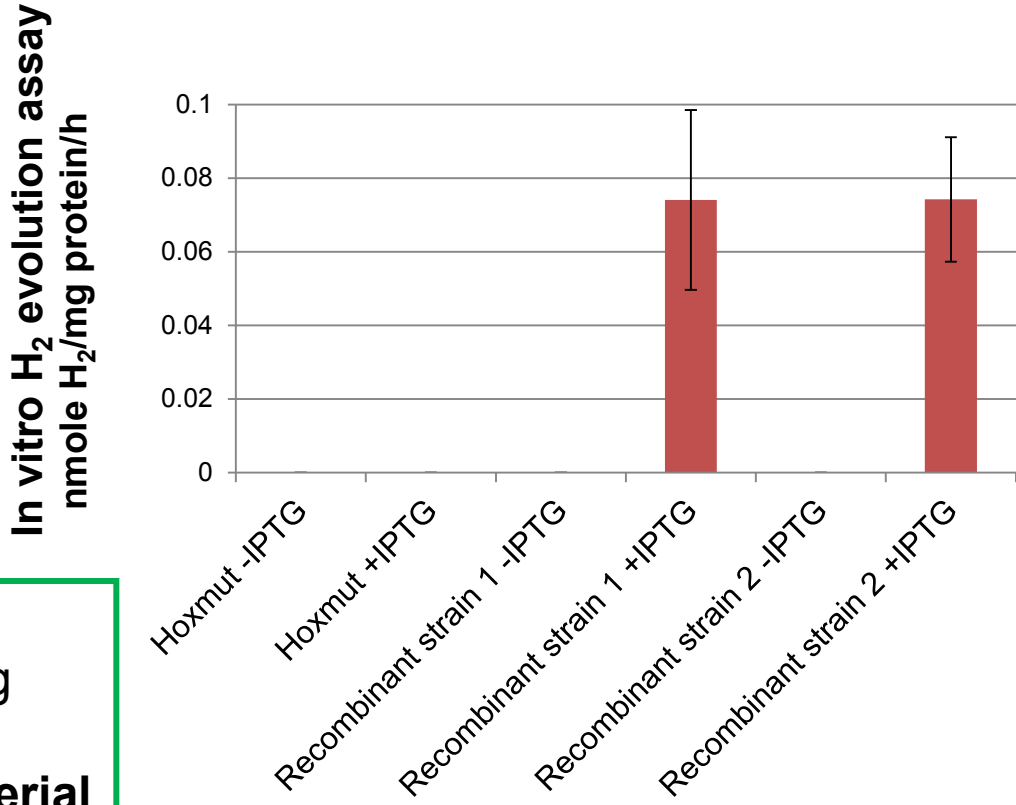


On track to complete Milestone "Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase" (9/12).

Task 1: JCVI Technical Accomplishments

Transferring a known O_2-tolerant NiFe-hydrogenase from *T. roseopersicina* into cyanobacterium *Synechococcus sp.* PCC 7942



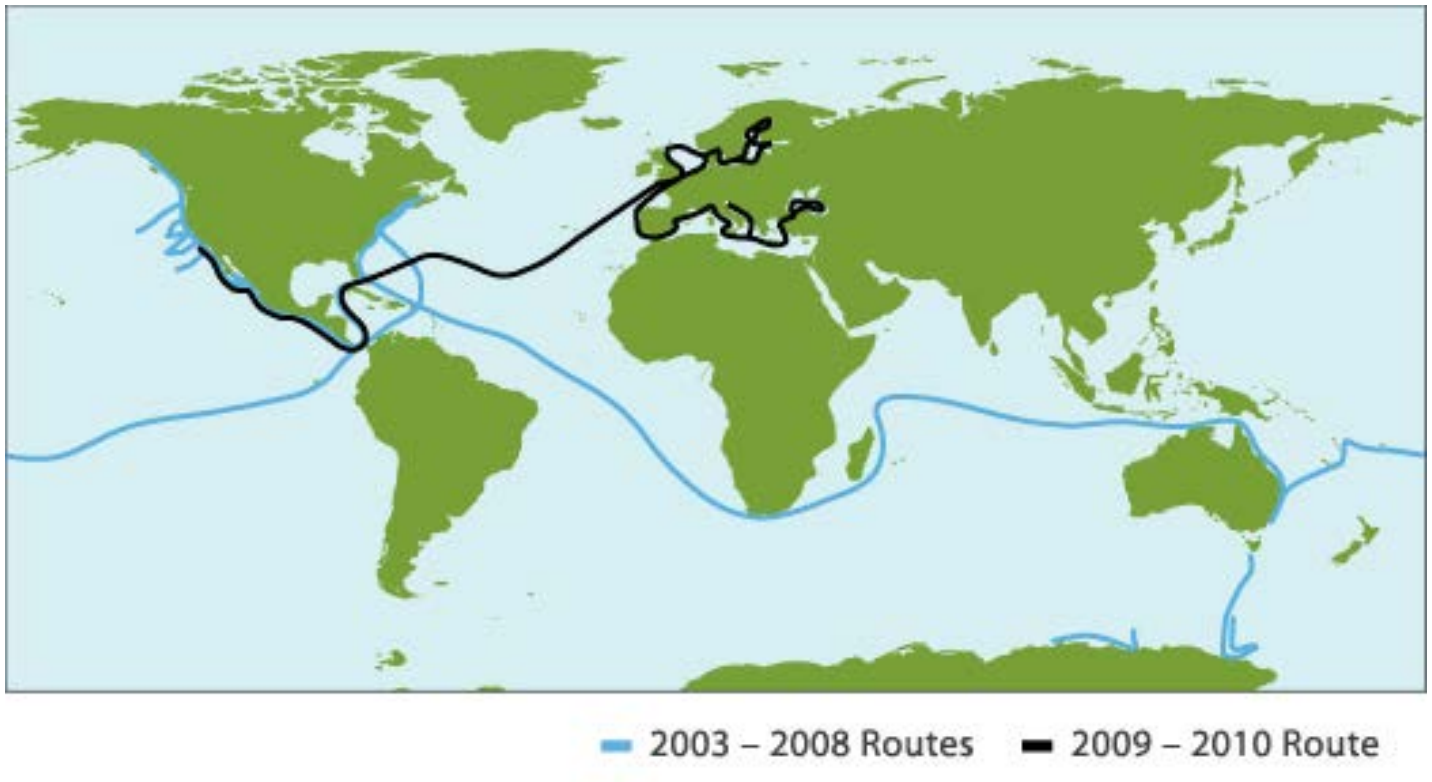


- Effort in FY2011-12 focused on increasing expression of heterologous hydrogenase – using Envi. Hydrogenase as model.
- **Completed milestone "Construct cyanobacterial hybrid to express active *Thiocapsa* hydrogenase" (8/11).**

- JCVI approach is complementary to that of NREL in harnessing Nature's O_2-tolerant hydrogenases and their transfer into two model cyanobacteria.

Task 2: JCVI Technical Approach

Task 2. Identifying novel O_2-tolerant hydrogenases through metagenomic analysis of marine microbes in the global ocean and transferring the hydrogenases into cyanobacteria



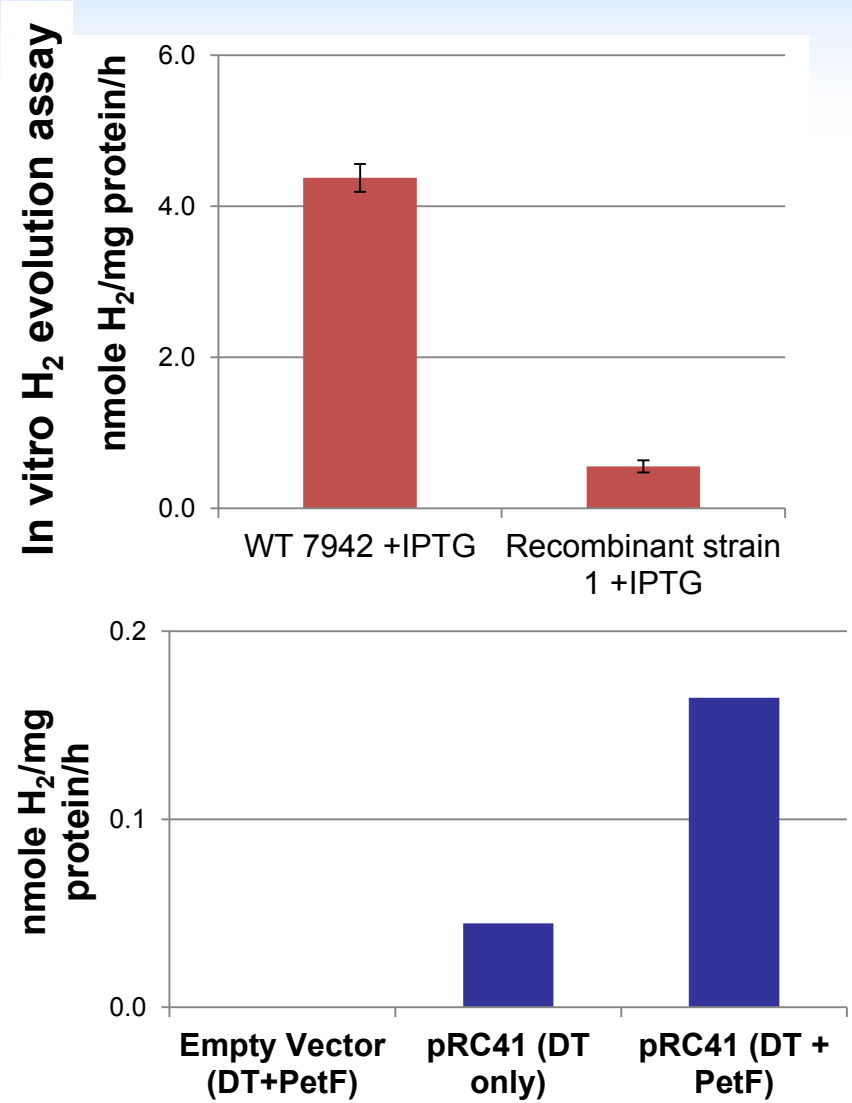
Sorcerer II Expedition: a Global Ocean Sampling Project accomplished by JCVI

- This approach is complementary to two approaches in the Task 1 about harnessing nature's O_2-tolerant hydrogenases and their transfer into cyanobacteria.

Task 2: JCVI Technical Accomplishments

- Previously, we reported transfer of hydrogenase from environmental DNA to *Synechococcus* PCC 7942 (Milestone reached 4/10)
- Environmental hydrogenase is more thermostable and O_2-tolerant than *Thiocapsa* HynSL.
- Cyanobacterial ferredoxin (PetF*) can act as an electron mediator to the environmental hydrogenase in *E. coli* crude extract.

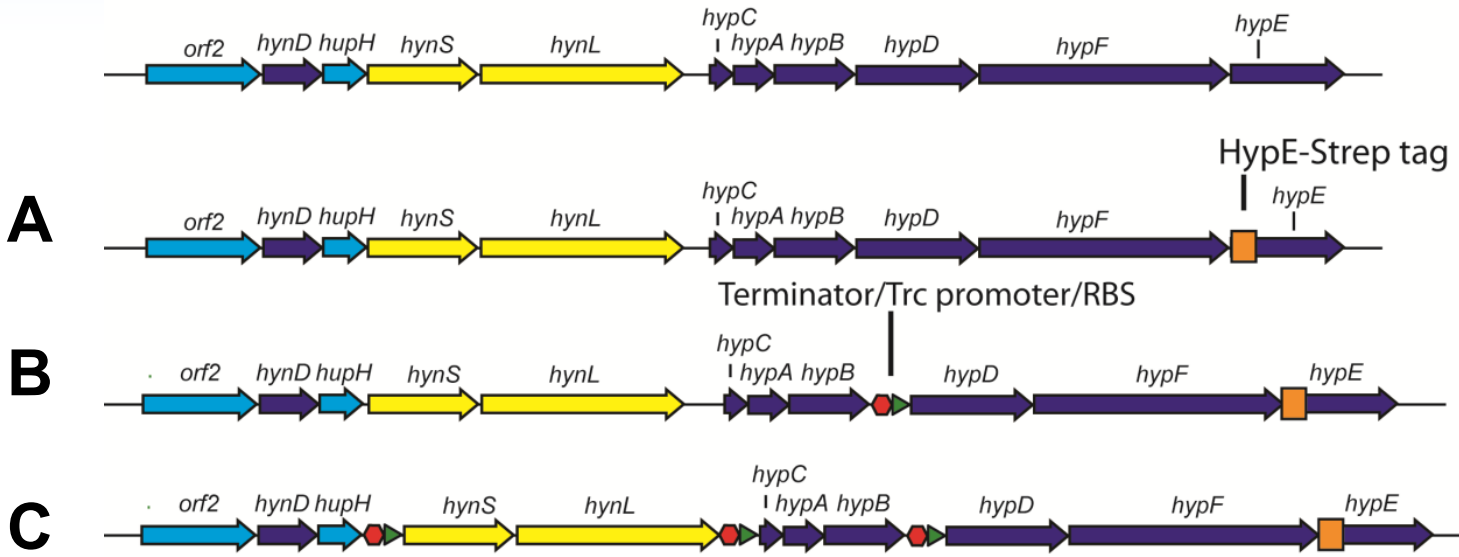
  * PetF cloned and purified by NREL and provided to JCVI through our collaboration

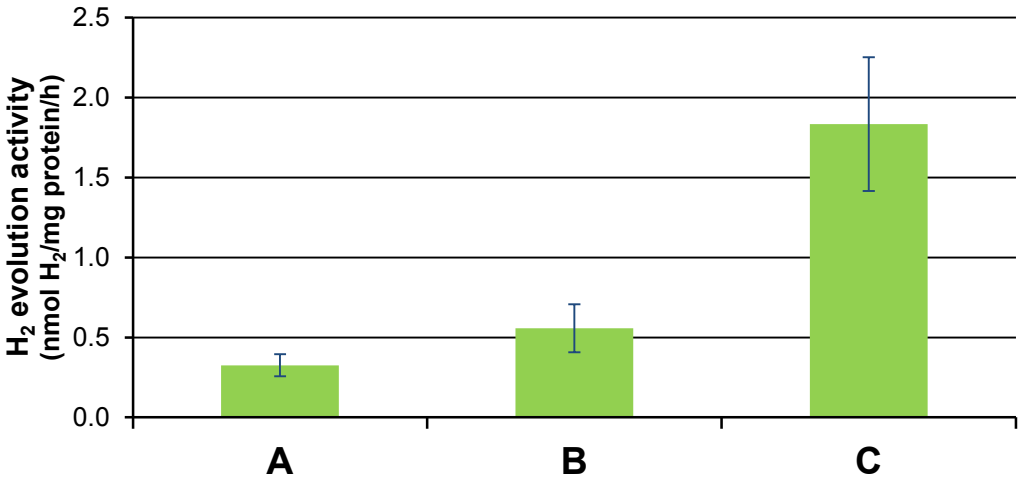


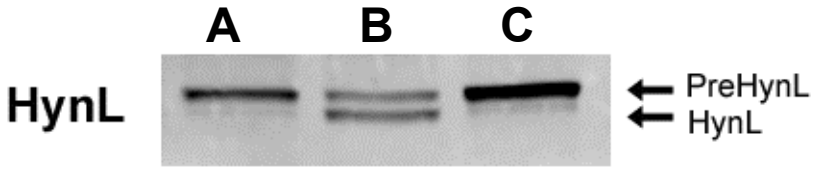
Completed Milestone "Determine Electron Mediator Requirement" (12/10)

Task 2: JCVI Technical Accomplishments

Engineer additional promoters into environmental hydrogenase gene cluster to increase hydrogenase activity in cyanobacteria.





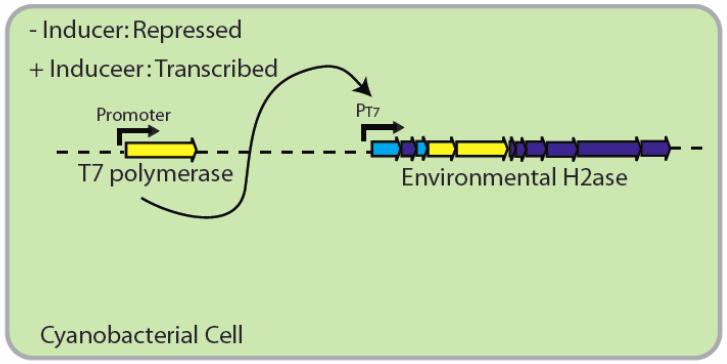


On track to complete Milestone "Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity." (4/13).

Task 2: JCVI Technical Accomplishments

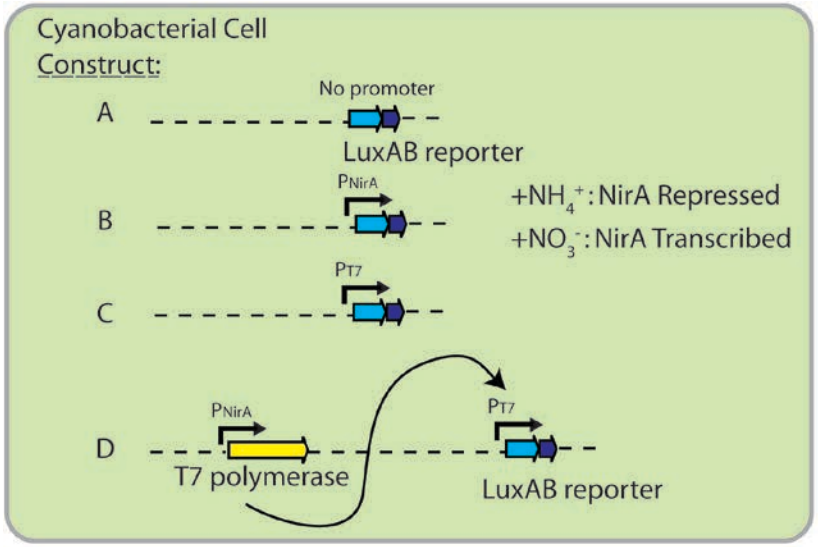
Increasing hydrogenase activity – Continued:

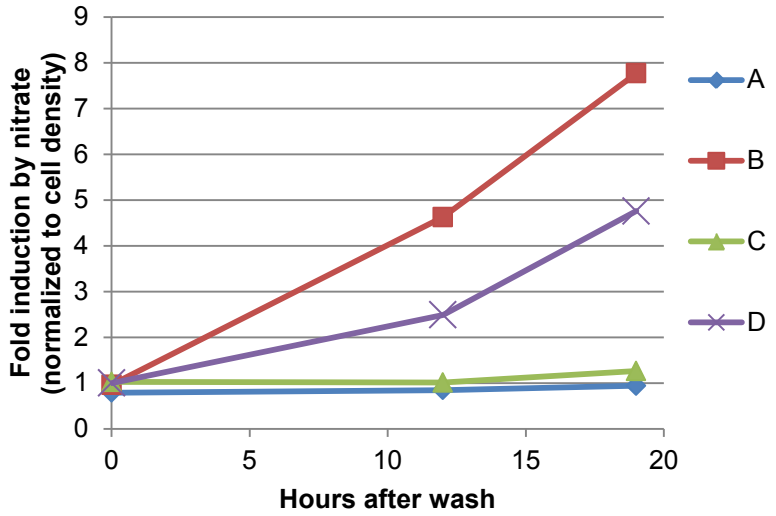
- Engineer T7 polymerase strategy for hydrogenase expression.



<u>Advantages of T7 polymerase:</u>

- Tighter control of transcription
- Better transcriptional processivity, may function better with long transcripts.

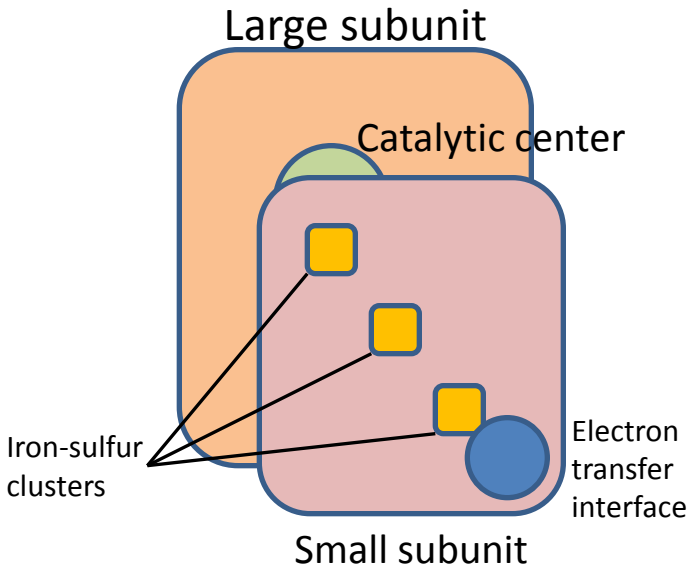


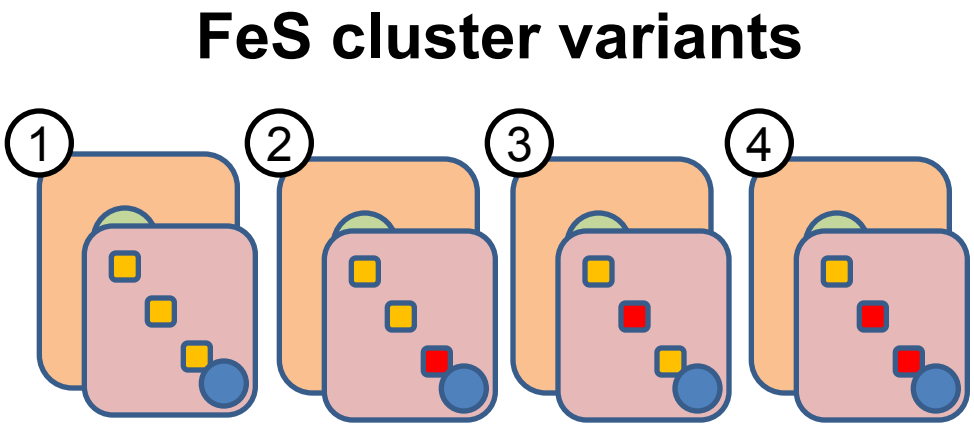


<u>On track</u> to complete Milestone "Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity." (4/13).

Task 2: JCVI Technical Accomplishments

Point mutants alter electrochemistry of the hydrogenase small subunit "molecular wire"

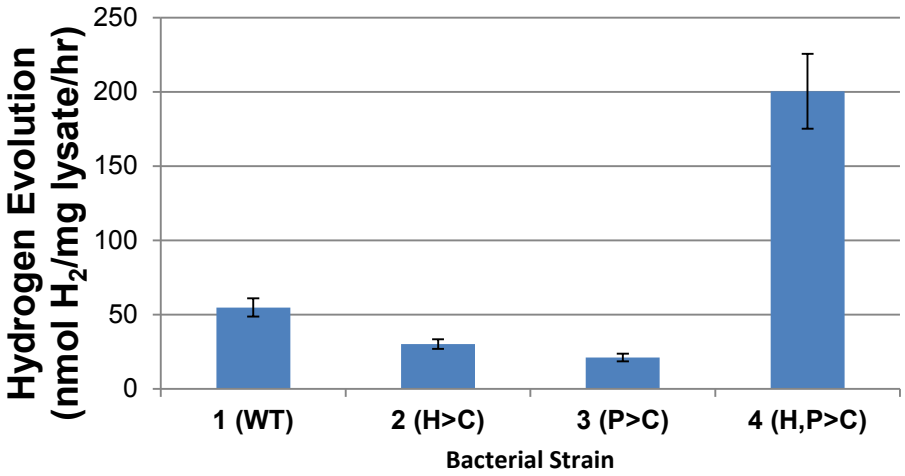


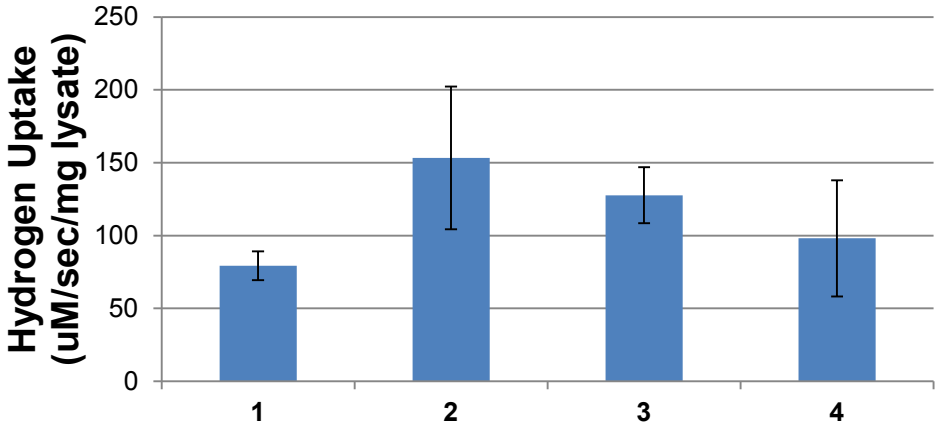


Tests the potential to further modify the environmental hydrogenase to favor H_2 production *in vivo* using PetF as an electron mediator.

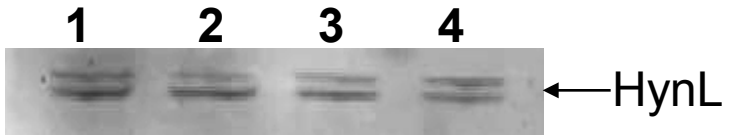
Task 2: JCVI Technical Accomplishments

Double mutant has increased evolution activity relative to uptake





Western blot of HynL shows equivalent expression in all samples



<u>On track</u> to complete Milestone "Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase." (11/13).

Collaborations

JCVI

- Vanderbilt University
  - Expressing O_2-tolerant hydrogenases in cyanobacteria

NREL

- Michigan State University
  - Annotate CBS genome
  - Leverage funding from DOE Office of Science
- Pacific Biosciences
  - Provide **free** CBS genome sequencing

Proposed Future Work

JCVI

- Continue work to increase O_2-tolerant hydrogenase expression 100-fold in cyanobacteria via optimization of transcriptional regulation of hydrogenase and accessory genes and by engineering FeS cluster mutations (FY12 and 13)
- Improve electron transfer between ferredoxin and hydrogenase small subunit by mutational analysis and constructing ferredoxin-hydrogenase fusion proteins (FY12 and 13)
- Link environmental-hydrogenase to S. elongatus photosynthetic pathways (FY13).

NREL

- Probe roles of a set of newly discovered hydrogenase maturation genes in CBS identified from the sequenced genome (FY12).
- Manipulate promoters to increase CBS hydrogenase activity in the *Synechocystis* recombinants (FY12 & FY13).
- Link CBS hydrogenase to the *Synechocystis* photosynthetic pathways to enhance photolytic H_2 production (FY13).

Summary

JCVI

- Increased expression of the environmentally-derived hydrogenase in cyanobacteria by modifying the transcriptional strategy.
- Developed a novel T7 polymerase strategy for expression of hydrogenase.
- Identified mutations leading to increased hydrogenase activity in the direction of hydrogen evolution.

NREL

- CBS genome sequencing and annotation is near complete, at no cost to DOE.
- A new set of hydrogenase maturation genes is identified through the above genome sequencing effort.
- Confirmed roles of four hydrogenase maturation genes involved in the assembly of the CBS O_2-tolerant hydrogenase.
- Generated two *Synechocystis* recombinants which displayed active CBS hydrogenase activity, albeit improvement is needed.