Photobiological Hydrogen Research

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Project ID: PDP4

May 15, 2007

Overview

Timeline

- Start: June 2006
- Finish: May 2008
- 40% Complete

Budget

DOE: $565,000 (2-years)
FIU Cost share: $141,252
Total: $706,252

Barriers

(Y) Rate of Hydrogen Production
(Z) Continuity of Photoproduction
(AA) Systems Engineering

Partners

NREL
- Dr. PinChing Maness
- Dr. Maria Ghirardi
- Dr. Jianping Yu

Objectives

Overall

Identify which **structural** and **active site** maturation genes of the O_2 tolerant NiFe-hydrogenase from the photosynthetic bacterium ***Rubrivivax gelatinosus CBS*** are critical to optimal expression of the enzyme in *E. coli*. Expression in *E. coli* will facilitate eventual expression of the hydrogenase in cyanobacteria at NREL.

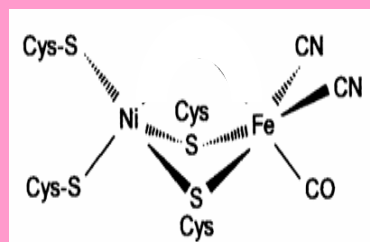
July 2006 - March 2007

Clone the structural genes (CooM, CooK, CooL, CooX, CooU and CooH) of the *R.gelatinosus* NiFe-hydrogenase into duet expression vectors under T7 promoter.

Clone the maturation genes (HypA, HypB, HypC, HypD, HypE, and HypF) of the *R. gelatinosus* NiFe-hydrogenase into duet expression vectors under T7 promoter.

Transform the completed clones, along with the hydrogenase large subunit, into a proper *E.coli* host, such as BL21(DE3*), to express an active NiFe-hydrogenase.

Typical Active Site of Energy -Conserving NiFe-hydrogenase



Approach Overview

Optimizing biological hydrogen production requires understanding the enzymatic pathways through which H_2 is formed at the molecular level. Work on this project is divided into (1) fundamental aspects designed to understand the protein expression system of O_2 tolerant NiFe-hydrogenase from the photosynthetic bacterium *R. gelatinosus CBS* in *E. coli* host and (2) applied aspects focusing on H_2 production by *E. coli*.

Task 1.0

July 2006-May 2007
Completed Task : 85 %

- **Clone structural genes with the large subunit of hydrogenase (CooH) into Duet Vectors: CooM, CooK, CooL, CooU, and CooX**

-One of the gene carries strep -II tag (CooH: large subunit of NiFe-hydrogenase)
-Confirm cloning by DNA sequencing of the plasmids

Task 2 .0

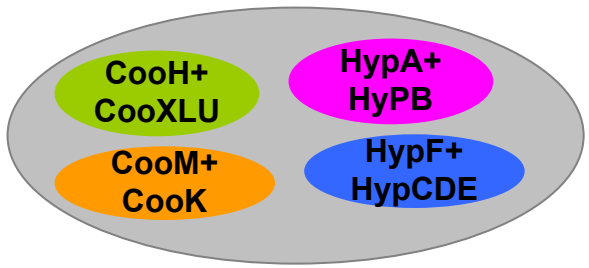
Sep. 2006-Aug. 2007
Completed Task : 85 %

•Clone active site assembly genes into Duet Vectors: HypA, HypB, HypC, HypD, HypE, HypF, and lytR
(putative transcriptional regulator gene; not needed in IPTG-inducible T7 polymerase system)
-Confirm cloning by DNA sequencing of the plasmids.
For fully active hydrogenase in *E. coli*, active site maturation is vital.

Task 3 .0

March 2007-Nov. 2007
Completed Task :5 %

Transform the cloned NiFe-hydrogenase genes into an *E. coli.*
-Confirm transformation efficiency by agarose gel.
-Test H_2 gas production by using Gas Chromatography.
-Test hydrogenase activity by Methyl Viologen assay.
-Confirm hydrogenase expression by western blotting and MS analysis. Co-transform plasmids carrying the essential genes for expression of active hydrogenase and H_2 production.

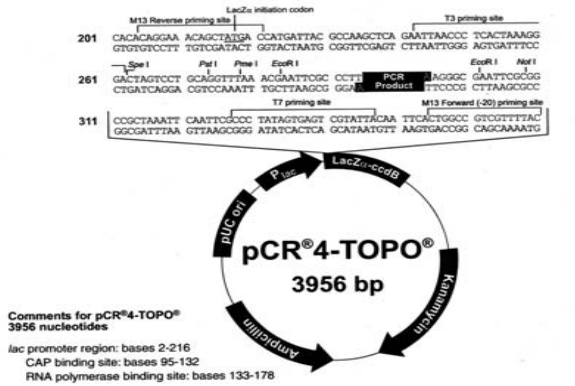


Technical Accomplishments/Progress/Results

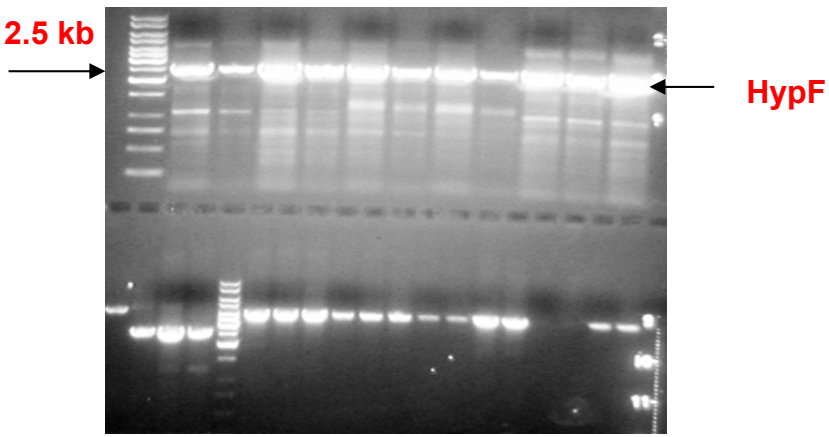
- **Assembly and the structural genes of Hydrogenase cloned into PCR-4-TOPO Vector**
  The T-A cloning vector is a useful tool for DNA sequencing of the PCR-amplified genes and helps increase the cloning efficiency of the genes into target expression vectors. The following genes were cloned into the vector:

  HypF
  HypCDE
  CooH (without st-tag)

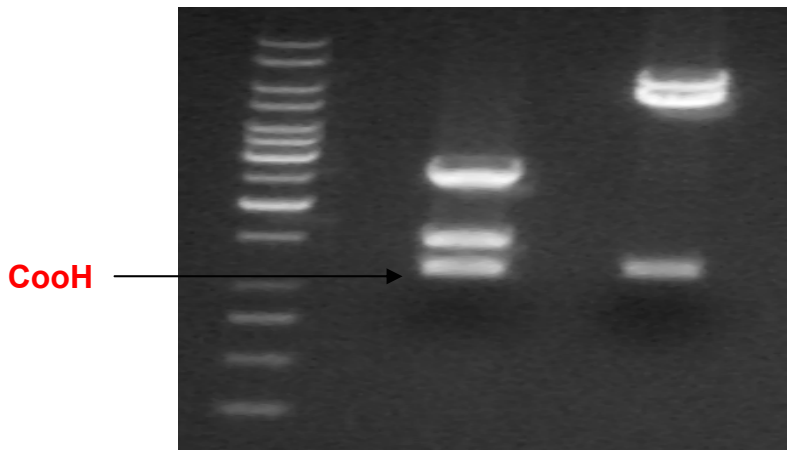
- **DNA sequencing confirmed cloning in PCR-4-TOPO Vector**



PCR amplified HypF from genomic DNA of CBS



Agarose gel picture of CooH in TOPO

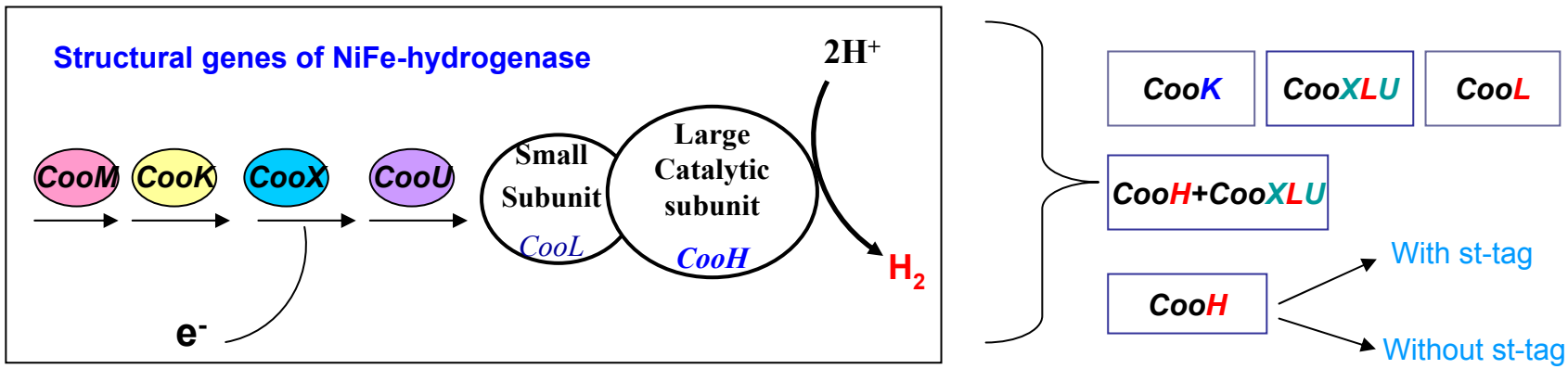


Technical Accomplishments/Progress/Results

Task 1.0

•Structural genes to be cloned into Duet Vectors under T7 Polymerase

We have cloned five structural genes of the O_2 tolerant NiFe-hydrogenase from R.gelatinosus CBS (identified by Vanzin et al., NREL, unpublished data). Three of these genes were first constructed in TOPO cloning vectors and transferred into Duet vectors under T7 polymerases. PCR amplification of ***CooM*** (encodes membrane-anchoring protein of hydrogenase) has been completed; however, cloning into a Duet vector is pending.



- **Gene modification to carry strep II- tag**

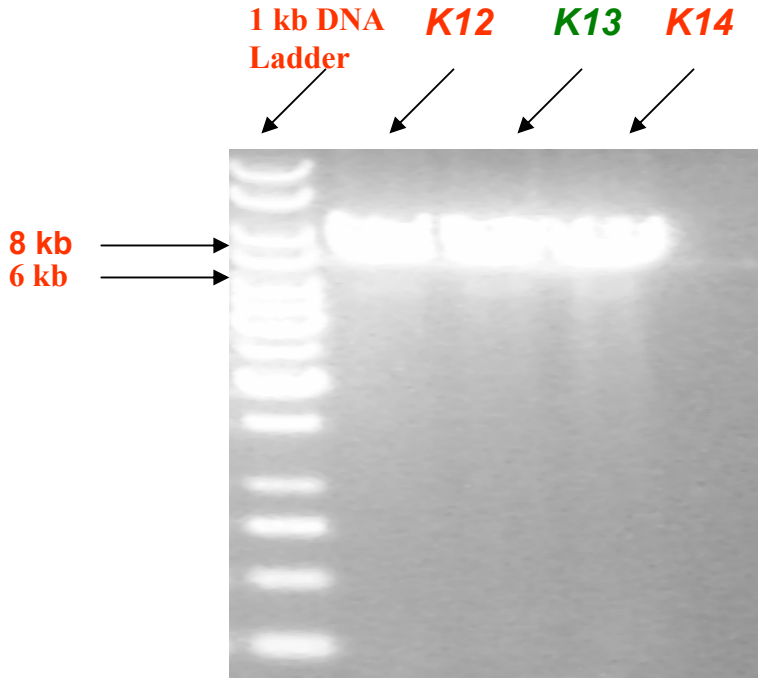
  CooH: hydrogenase large subunit, which carries the active metal center, has been cloned with strep II-tag in pCDF Duet-1 (from Novagen).

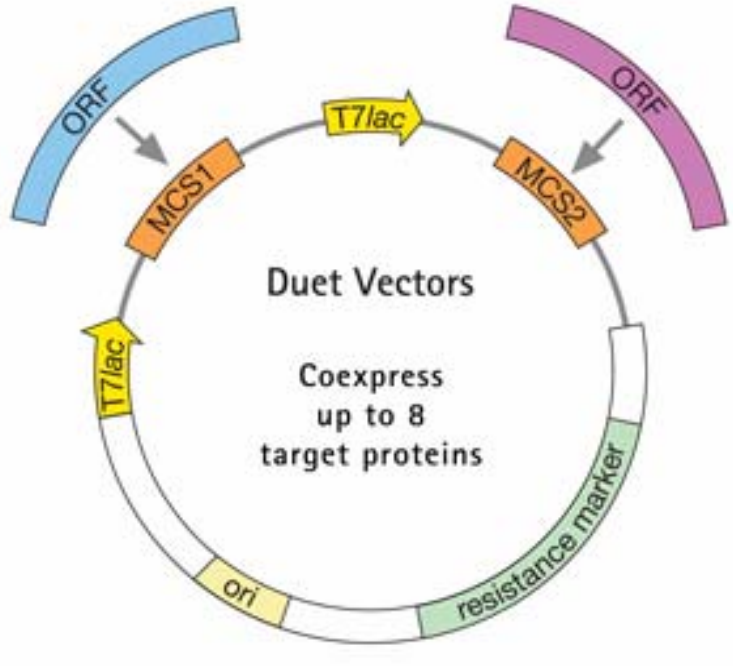
- **Cloned plasmids to be sequenced**

  The successful cloning in Duet vectors was confirmed by DNA sequencing.
  None of the plasmids has DNA mutations.

Technical Accomplishments/Progress/Results

Task 1 .0 (cont'd)



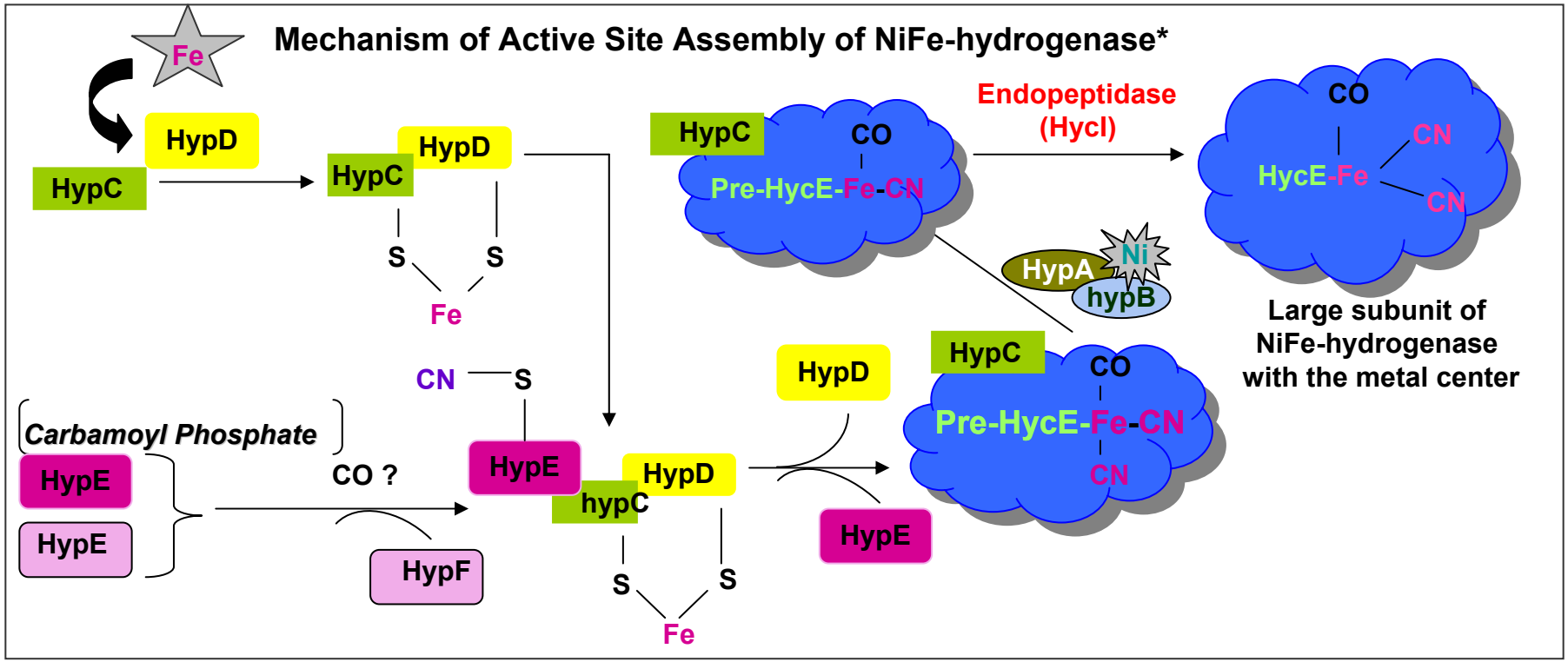


CooK, **one of the structural genes of the *CBS hydrogenase,* was cloned into pRSF Duet-1 vector. K13, colony #13 of CooK, was fully sequenced confirming the cloning.**

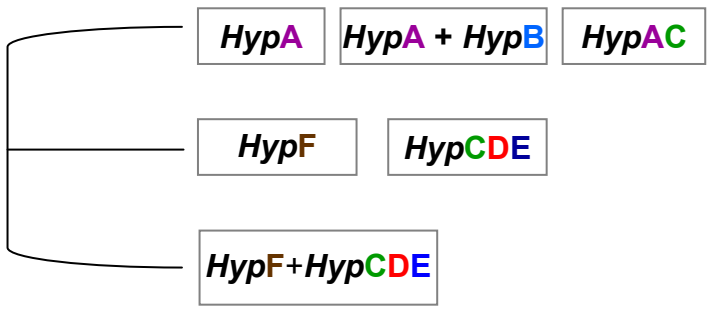
Duet vectors allow the transformation of the cell by 8 genes at a time.

Technical Accomplishments/Progress/Results

Task 2.0



- **Active site assembly genes to be cloned in Duet vectors**
  We have cloned hypA to HypF genes of the CBS hydrogenase into Duet vectors. *R. gelatinosus* hydrogenase lacks a C-terminal extension, so it doesn't need endopeptidase HycI to complete its maturation process. The scheme is prepared assuming the assembly mechanism of the active site of NiFe-hydrogenase from *R.gelatinosus* is similar to the assembly mechanism of hydrogenase 3 from *E. coli*.
- **Gene cloning was confirmed by DNA sequencing.**

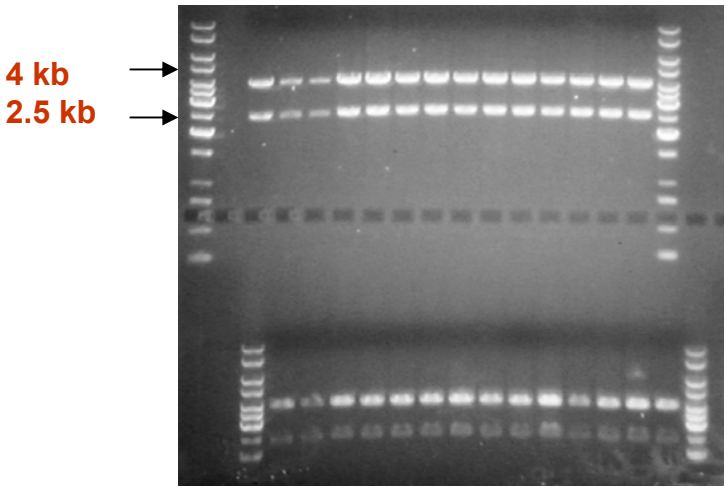


*Adapted from Blokesch et al., Biochem. Soc. Trans. 2002, 30, (674–680).

Technical Accomplishments/Progress/Results

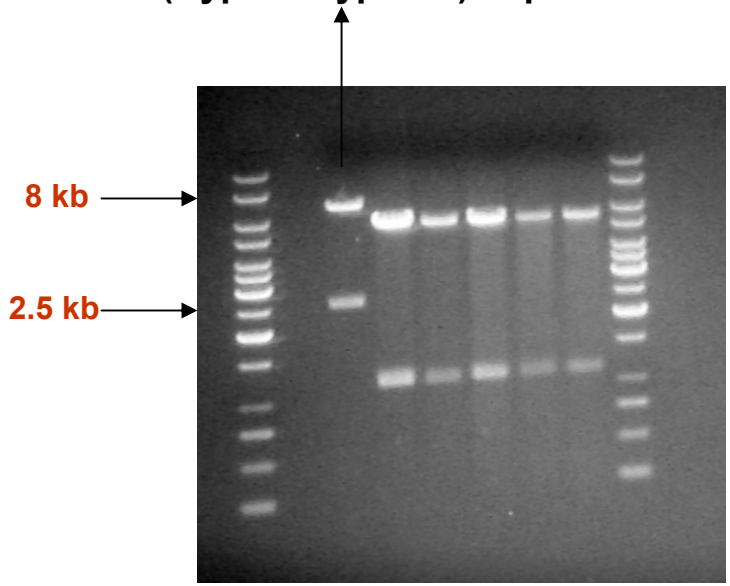
Task 2.0 (cont'd)

HypF in pACYC Duet-1



Lines 3-16 of upper gel and lines 4-17 of lower gel represent colonies carrying HypF. The HypF gene size is 2.5 kb. The pACYC Duet-1 size is 4 kb.

(HypF + HypCDE) in pACYC Duet-1

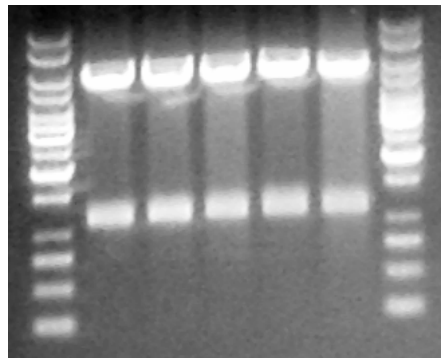


Line 3: HypF+HypCDE cloning into pACYC Duet-1. Plasmid size is ~ 7.5 kb.

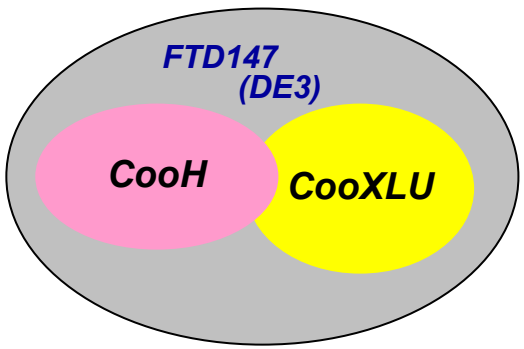
Technical Accomplishments/Progress/Results

Task 3.0

- We have co-transformed structural genes on Duet vectors into *E. coli*: CooH+ CooXLU were co-transformed into FTD147 (DE3)
- We have confirmed gene transformation by using DNA gel assay. Transformation efficiency: 100 %
- Headspace gas analysis by using GC showed no detectable H_2 gas.
- Hydrogenase activity assay detected no activity.
- Protein detection and the remainder of the work is underway.

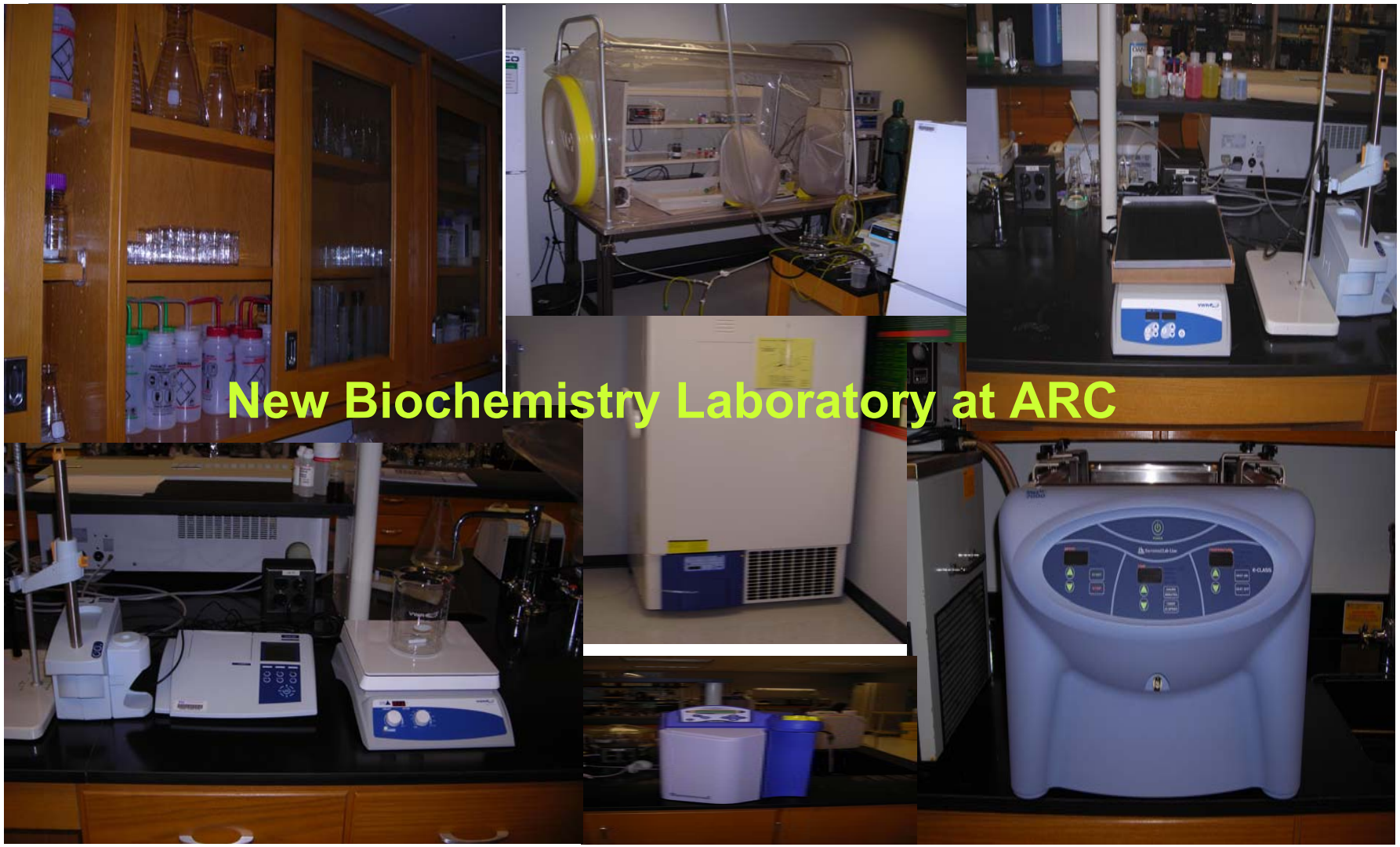


Agarose gel shows the transformation efficiency of CooH + CooXLU into FTD147(DE3)



•T7 Polymerase gene has been transferred into host strain FTD147 (DE3) via phage lysogenization by our collaborators at NREL. Zero-background strain will be used as an alternative to BL21(DE3*), which were originally selected as an *E. coli* host for the protein expression in this project. BL21 (DE3*), a commercial strain, has a low-capacity endogenous hydrogenase, which may cause poor expression of CBS hydrogenase.

Technical Accomplishments/Progress/Results



Remaining Work (FY2007-08)

•**Complete Cloning**
-CooM (may be needed for active hydrogenase expression) will be cloned into an appropriate vector other than the Duet vectors.

•**Complete Co-transformations**
To test how many genes of NiFe-hydrogenase are needed to obtain a fully functional enzyme, we will co-transform auxiliary and structural hydrogenase genes in *E. coli*.

-Coo and Hyp genes into BL21(DE3*)
-Coo and Hyp genes into MC4100FTD147(DE3**)** (The strain was developed from the original strain MC4100 of Frank Surgent (France) by Dr. Wecker at NREL; it has zero background)

•**Optimize the Conditions of Protein Expression for Hydrogenase**
-Express active NiFe-Hydrogenase in BL21(DE3*) and/or
-Express active NiFe-Hydrogenase in MC4100FTD147(DE3)

•**Purify and Characterize Active Hydrogenase**
-Following expression of active hydrogenase we will purify and characterize the NiFe-Hydrogenase of *R.gelatinosus* from the *E. coli* host.

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•**Optimize H_2 Production by Physiological Means**
-Study growth and H_2 generation under fermentation conditions to assess H_2 production capability.

Summary

Task 1.0

- We have cloned the structural genes of O_2-tolerant NiFe-hydrogenase of *R. gelatinosus* CBS (CooK, CooH with and without st-tag, CooL, CooX and CooU) in Duet protein expression vectors under T7 polymerase from the *R.gelatinosus* CBS strain isolated by NREL scientist Dr. PinChing Maness.
- We have amplified and sequenced CooM, which encodes the membrane anchoring protein of the NiFe-hydrogenase in *R. gelatinosus* CBS.

Task 2.0

- We have cloned the active site assembly genes (HypA to HypF) of NiFe- hydrogenase into TOPO cloning and Duet expression vectors for future transformations into foreign hosts.

Task 3.0

- Large subunit gene CooH without st-tag and structural genes CooXLU of the CBS NiFe-hydrogenase were co-transformed into FT147(DE3), which was developed by Jianping Yu (NREL). After IPTG induction, recombinant cells did not generate H_2 gas. Protein expression and other parameters have not been tested yet. The maturation genes and other structural genes may need to be co-transformed into *E. coli* along with the large subunit (CooH) of the NiFe-hydrogenase to express the fully functional hydrogenase. This work is underway.

Task 4.0

- Following **tasks 1-3** we will purify and characterize the membrane-bound anaerobic NiFe-hydrogenase of *R.gelatinosus* from the *E. coli* host.

Benefits from this Project

This project aims at determining the minimum number of auxiliary and structural genes required for the expression of a fully functional NiFe-hydrogenase from *R.gelatinosus* CBS in *E. coli*.

- **New genetic constructs developed in this project will be used in cyanobacterial expression work at NREL.**
- **The research will improve our understanding of how solar-driven, water-splitting cyanobacteria can become hydrogen-producing vehicles.**
- **Once the molecular manipulations are complete, H_2 production via fermentation using *E. coli* and cyanobacteria will serve as a technology platform for commercialization.**

Acknowledgments

- This research project is supported by the US DOE (Contract DE-FG36-06GO86047):
  - DOE Technology Development Manager: Roxanne Garland
  - DOE Project Officer: Jill Gruber

- We wish to thank NREL scientists Dr. Jianping Yu, Dr. Maria Ghirardi, and Dr. PinChing Maness for their collaboration.

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