

# Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacteria System

Qing Xu and Hamilton O. Smith  
J. Craig Venter Institute

May 23, 2005

**Project ID # PDP31**

This presentation does not contain any proprietary or confidential information

**J. Craig Venter**

I N S T I T U T E

# Overview

## Timeline

- Project start: 5-01-05
- Project end: 4-30-08
- Percent complete: N/A

## Budget

- Total project funding
  - \$720K (Contractor share)
  - \$2,880K (DOE share)
- Funding for FY04: \$0
- Funding for FY05: \$350K

## Barriers

- Barriers addressed
  - Barrier Z, continuity of H<sub>2</sub> photo-production

## Partners

- Pin-Ching Maness, National Renewable Energy Laboratory

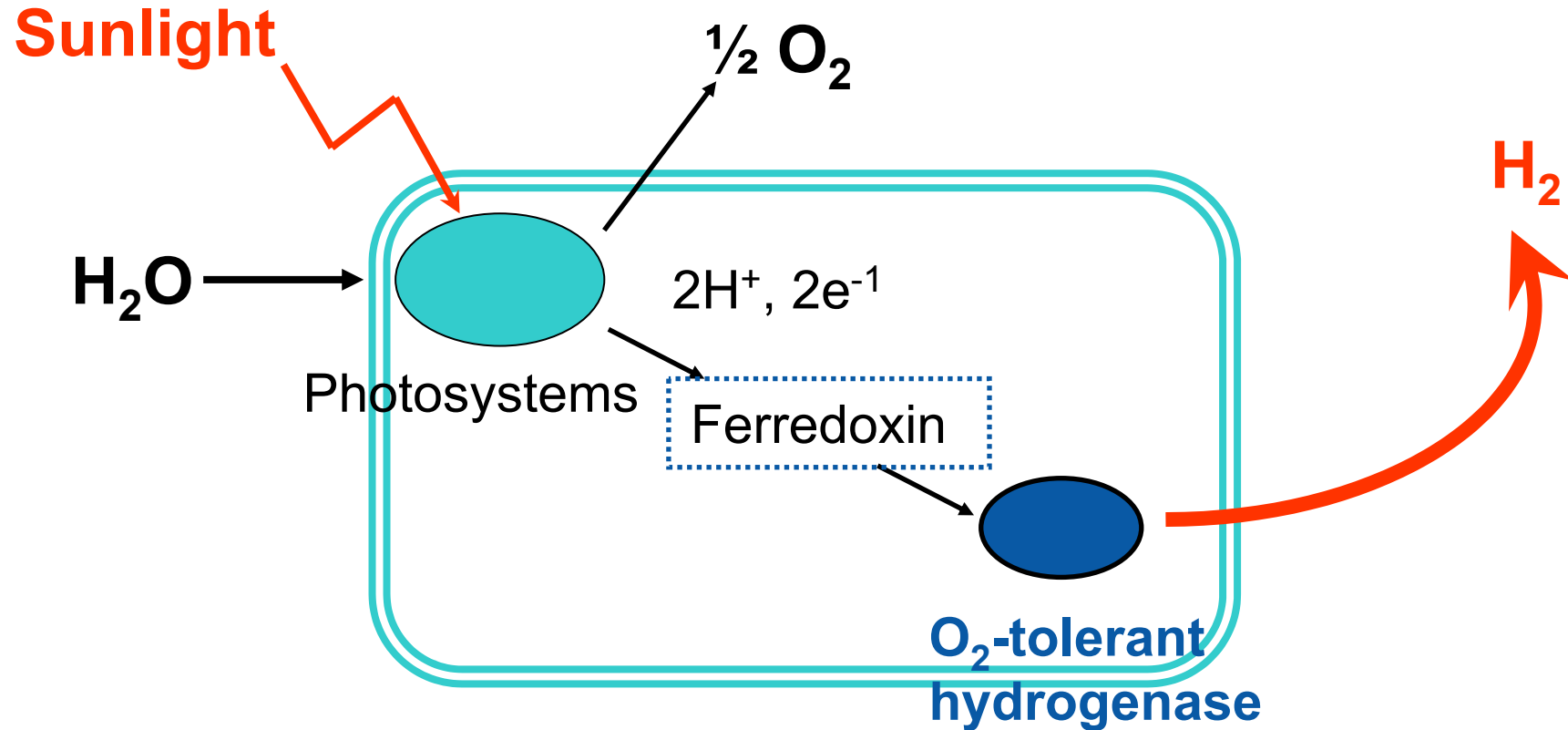
# Objective

- Develop an O<sub>2</sub>-tolerant cyanobacterial system for sustained and continuous light-driven H<sub>2</sub>-production from water
  - Transfer and express known O<sub>2</sub>-tolerant hydrogenases in cyanobacteria
  - Identify novel O<sub>2</sub>-tolerant hydrogenases from VI's ongoing sampling in international waters and transfer them into cyanobacteria

# Approach to address hydrogenase O<sub>2</sub>-sensitivity barrier

- *Problem:* Cyanobacteria have the ability to split water photolytically into O<sub>2</sub> and H<sub>2</sub>, but their hydrogenases are highly O<sub>2</sub>-sensitive. In contrast, a few anoxygenic photosynthetic bacteria have O<sub>2</sub>-tolerant H<sub>2</sub>-evolving hydrogenases, but they can not use water as the electron donor.
- *Approach:* Use genetic techniques to transfer O<sub>2</sub>-tolerant H<sub>2</sub>-evolving hydrogenases from anoxygenic bacteria into cyanobacteria

# Cyanobacterium Transformed with an O<sub>2</sub>-tolerant [NiFe]-Hydrogenase

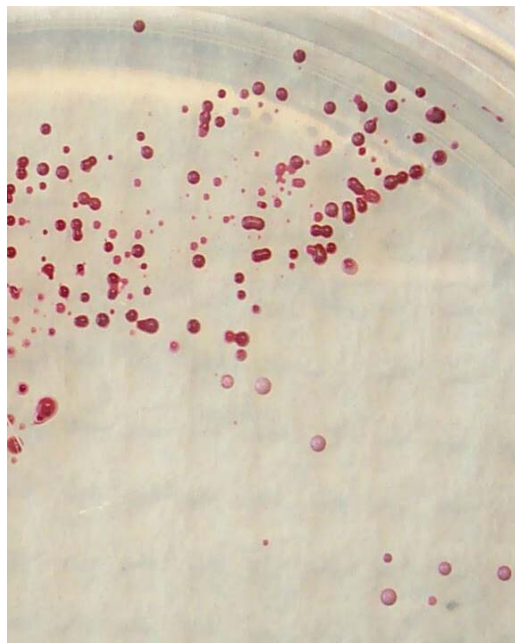


- The overall goal is to produce a cyanobacterial recombinant to produce H<sub>2</sub> continuously

# Technical Approach 1.1 (Venter Institute)

- Goal: Introducing a known O<sub>2</sub>-tolerant hydrogenase from *Thiocapsa roseopersicina* into cyanobacteria (*Synechococcus* and *Synechocystis*)

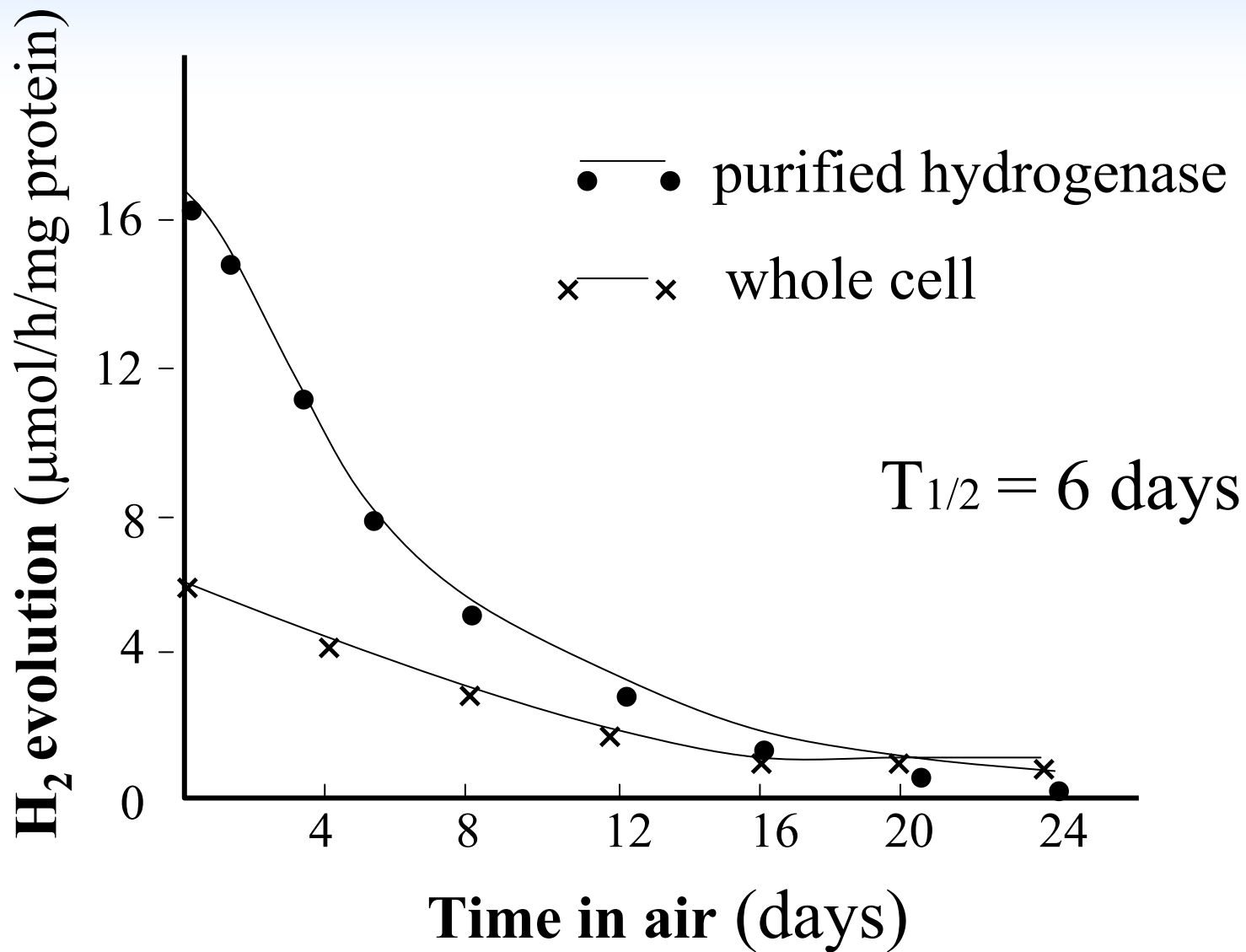
*Purple sulfur photosynthetic bacteria Thiocapsa roseopersicina*



J. Craig Venter

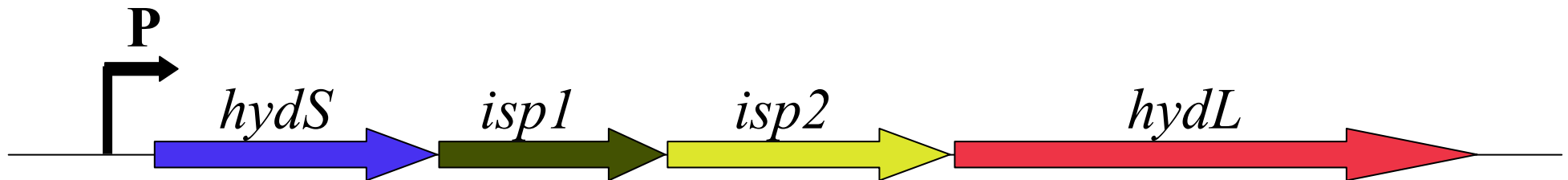
I N S T I T U T E

# O<sub>2</sub>-tolerant hydrogenase Hyd from *T. roseopersicina*



*Biochimica et Biophysica Acta* 523:335-343 (1978)

# The gene locus of *T. roseopersicina* O<sub>2</sub>-tolerant hydrogenase



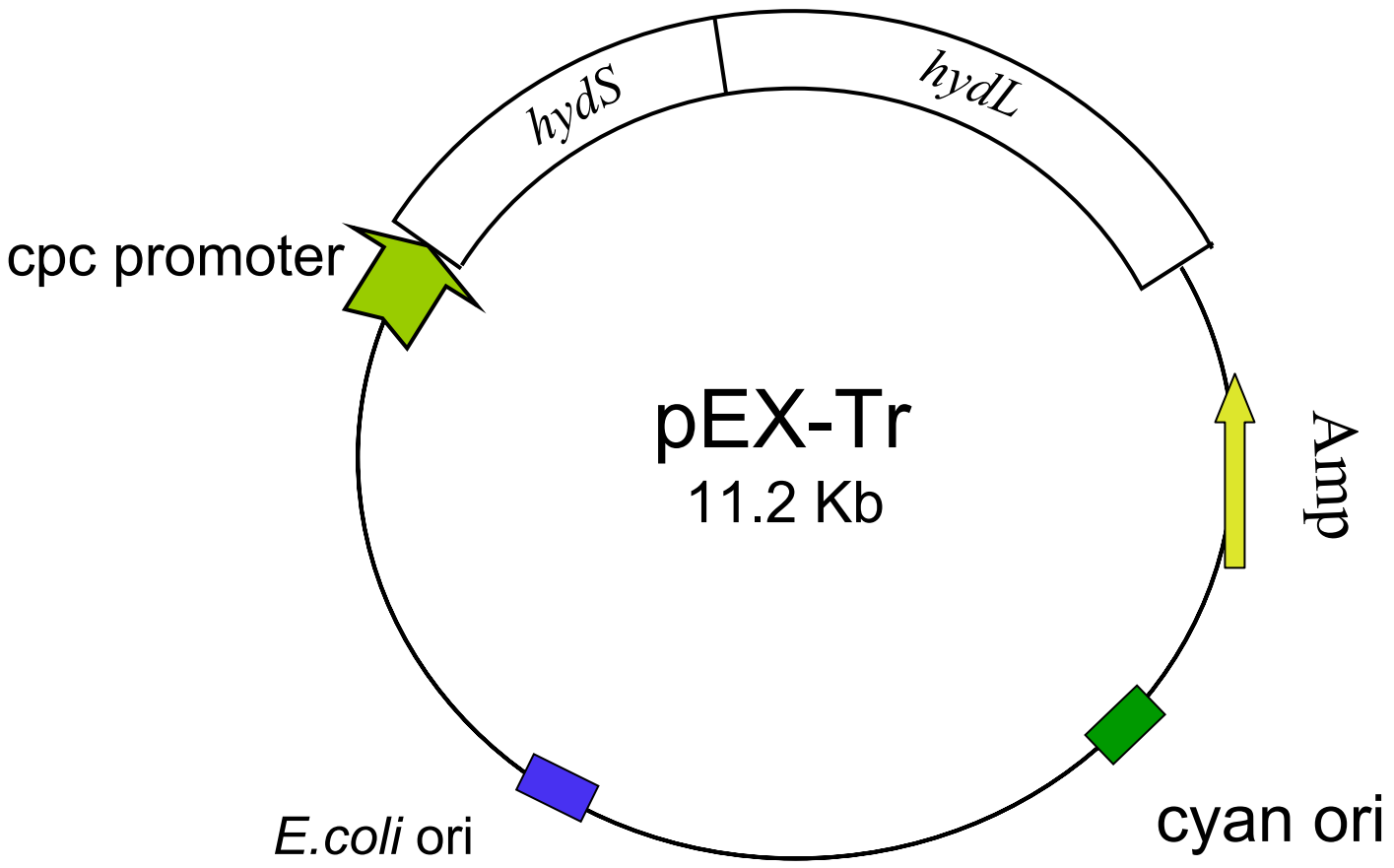
Catalytic region: HydS + HydL

Electron-transfer elements: Isp1 + Isp2

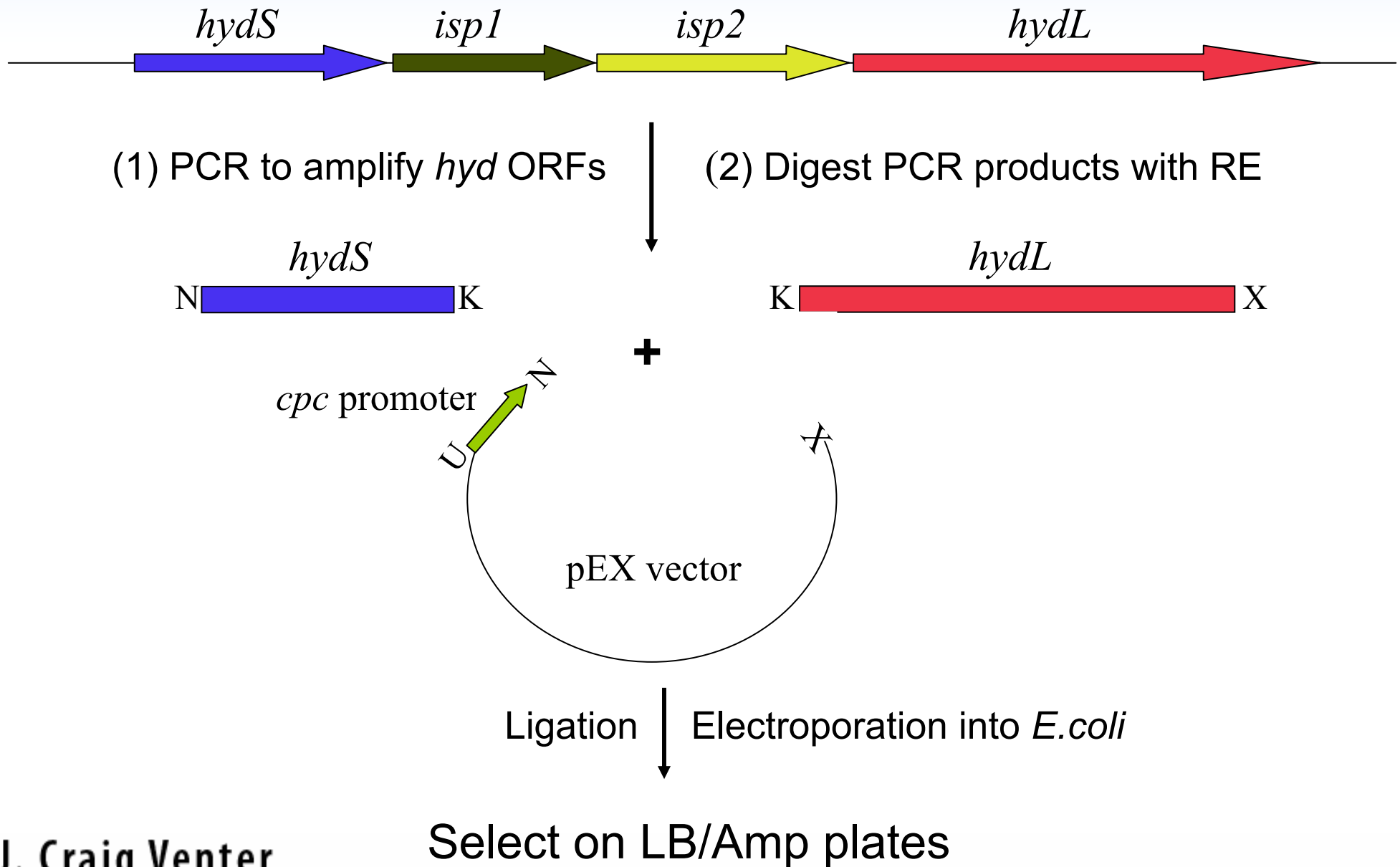
Purified functional hydrogenase from *T. roseopersicina* contains two subunits HydS and HydL.



# Introduction of *hyd* genes into cyanobacteria using a shuttle vector



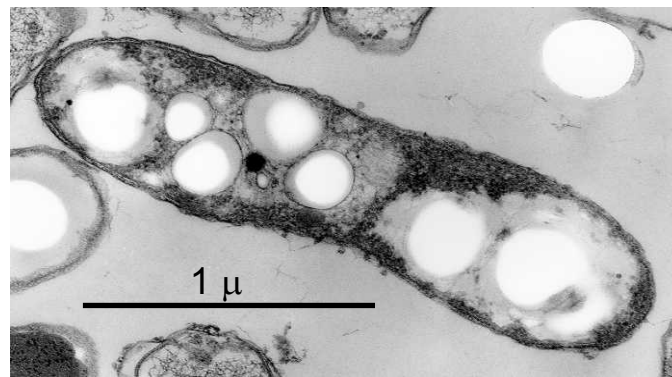
# Construction of pEX-Tr



# *Technical Approach 1.2 (NREL)*

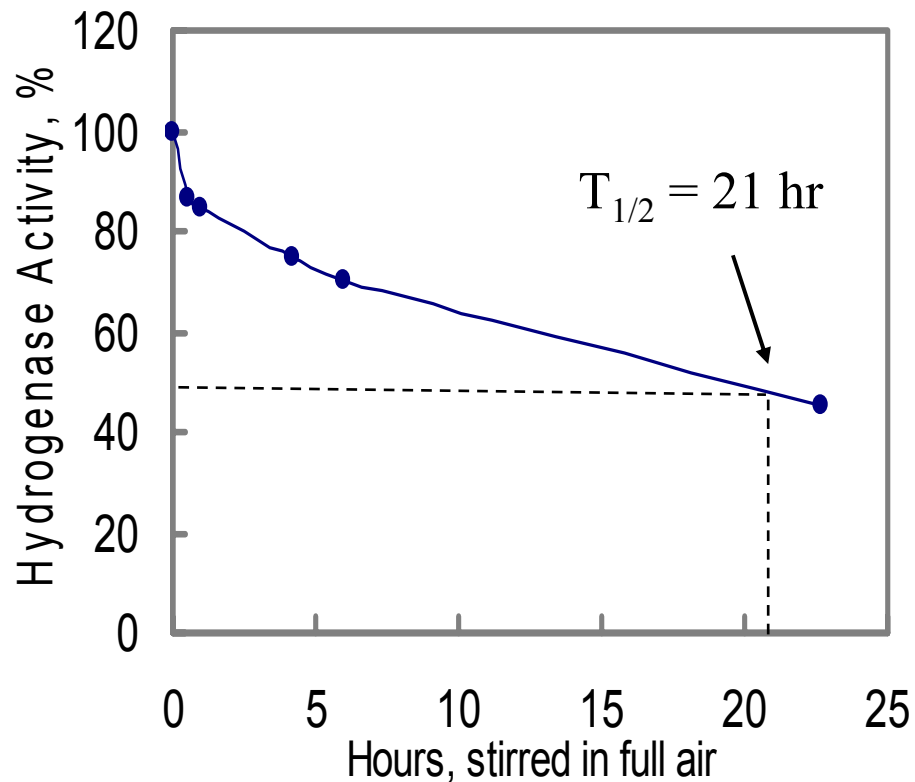
- Goal: Transfer the O<sub>2</sub>-tolerant hydrogenase from *Rubrivivax gelatinosus* CBS into the cyanobacterial hosts *Synechocystis* and *Synechococcus*

***Purple non-sulfur photosynthetic bacterium Rubrivivax gelatinosus CBS was isolated from soils in metro Denver area***

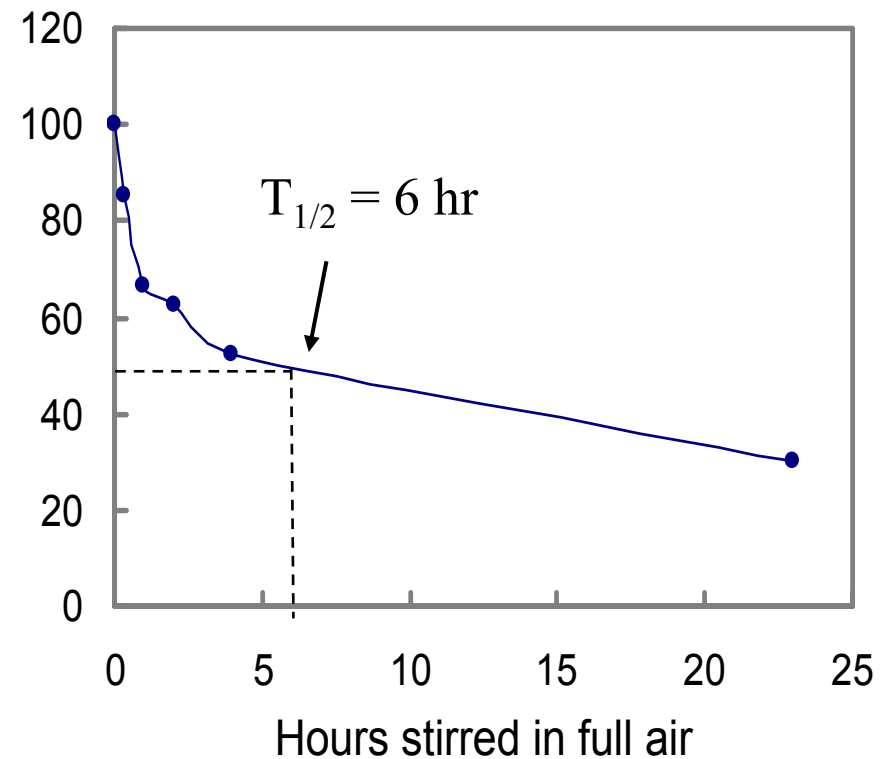


# Hydrogenase from *Rubrivivax gelatinosus* Tolerates O<sub>2</sub>

**(A) Whole Cell**

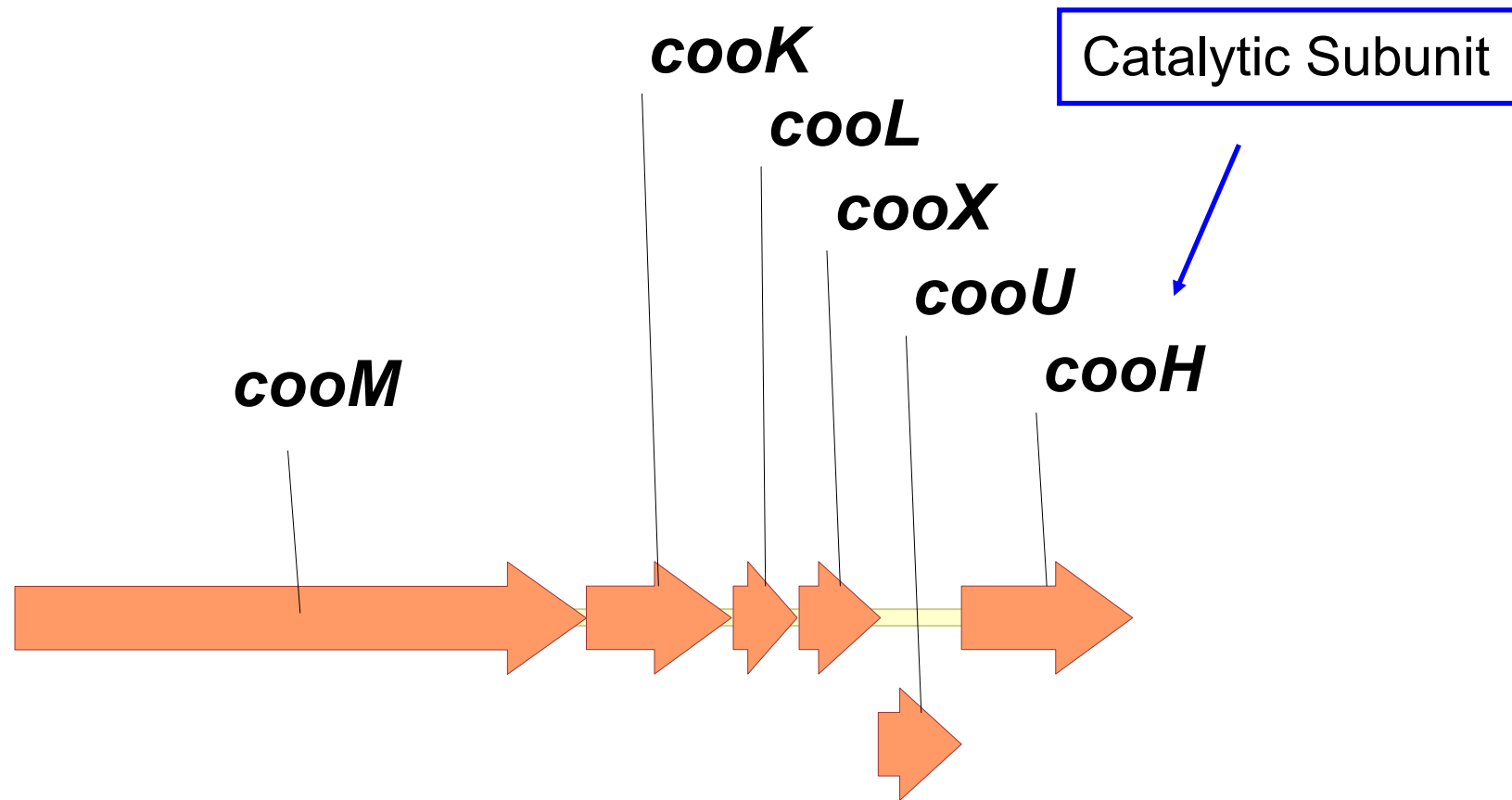


**(B) Purified Hydrogenase**



Maness et al., 2002. *Applied & Environmental Microbiology*, 68: 2633-2636

# Genes Encoding the *Rubrivivax* O<sub>2</sub>-tolerant Hydrogenase are Cloned



- Catalytic subunit: *CooH*
- Electron transfer subunit: *CooL*

# Technical Approach 2 (Venter Institute)

- Identifying novel O<sub>2</sub>-tolerant hydrogenases from environmental microbes and introducing them into cyanobacteria
  - Sample ocean waters, and sequence environmental samples using culture-independent shotgun sequencing approach
  - Construct environmental genomic databases
  - Build HMM models and search putative hydrogenase sequences through the databases
  - Retrieve original DNA samples or DNA library for cloning the genes of novel hydrogenases
  - Express the genes and screening for O<sub>2</sub>-tolerant hydrogenase
  - Transfer the novel O<sub>2</sub>-tolerant hydrogenase into cyanobacteria

# Technical Accomplishments/ Progress

## Sorcerer II Expedition: Ongoing International Water Sampling Project at the Venter Institute



**J. Craig Venter**

I N S T I T U T E

# Technical Accomplishments/ Progress

## The Sargasso Sea Sampling Project- A Pilot Study for VI's International Water Sampling Project

- Generated 1.045 billion base pairs of nonredundant sequences
- Found 1800 genomic species, including 148 new bacterial species
- Identified 1.2 million new genes, including 782 new rhodopsin-like photoreceptors

**Environmental microbes have a lot of potential !**



# Responses to Previous Year Reviewers' Comments

- This new project starts from May 1, 2005 and has not been reviewed previously

# Future Work

- **Remainder of FY2005:**
  - Purify O<sub>2</sub>-tolerant hydrogenases (Milestone)
  - Identify sequences of marine hydrogenases from international waters
  - Identify and clone the genes of putative novel hydrogenases identified from environmental samples
- **FY2006:**
  - Verify hydrogenase functionality in oxygen
  - Determine electron mediator requirements
  - Transfer and express O<sub>2</sub>-tolerant hydrogenases in cyanobacteria
  - Express and characterize novel hydrogenases identified from environmental samples

# Publications and Presentations

- Publications:

- **Environmental Genome Shotgun Sequencing of the Sargasso Sea.**

J. Craig Venter, Karin Remington, John F. Heidelberg, Aaron L. Halpern, Doug Rusch, Jonathan A. Eisen, Dongying Wu, Ian Paulsen, Karen E. Nelson, William Nelson, Derrick E. Fouts, Samuel Levy, Anthony H. Knap, Michael W. Lomas, Ken Nealson, Owen White, Jeremy Peterson, Jeff Hoffman, Rachel Parsons, Holly Baden-Tillson, Cynthia Pfannkoch, Yu-Hui Rogers, and Hamilton O. Smith. ***Science* 2004 Vol.304, 66-74.**

# Hydrogen Safety

- The most significant hydrogen hazard associated with this project is: 10% H<sub>2</sub> gas cylinder (balanced with N<sub>2</sub> or Ar).
  - 10% H<sub>2</sub> is a flammable gas.
  - It is frequently used in the Lab for keeping anaerobic environment in the anaerobic gloveboxes, and as standard H<sub>2</sub> gas for H<sub>2</sub> assays.

# Hydrogen Safety

Our approaches to deal with this hazard are:

- Strictly follow the Venter Institute's safety guidelines
- Perform H<sub>2</sub>-related experiments in our fermentation Lab that is specially designed for hazard gases
- Install an O<sub>2</sub>/H<sub>2</sub> gas detector in the Lab
- Do not mix 10% H<sub>2</sub> by ourselves and always order pre-mixed H<sub>2</sub> gas from commercial vendors