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

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Project ID # PDP31

This presentation does not contain any proprietary or confidential information

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_2 photo-production

Partners

- Pin-Ching Maness, National Renewable Energy Laboratory

Objective

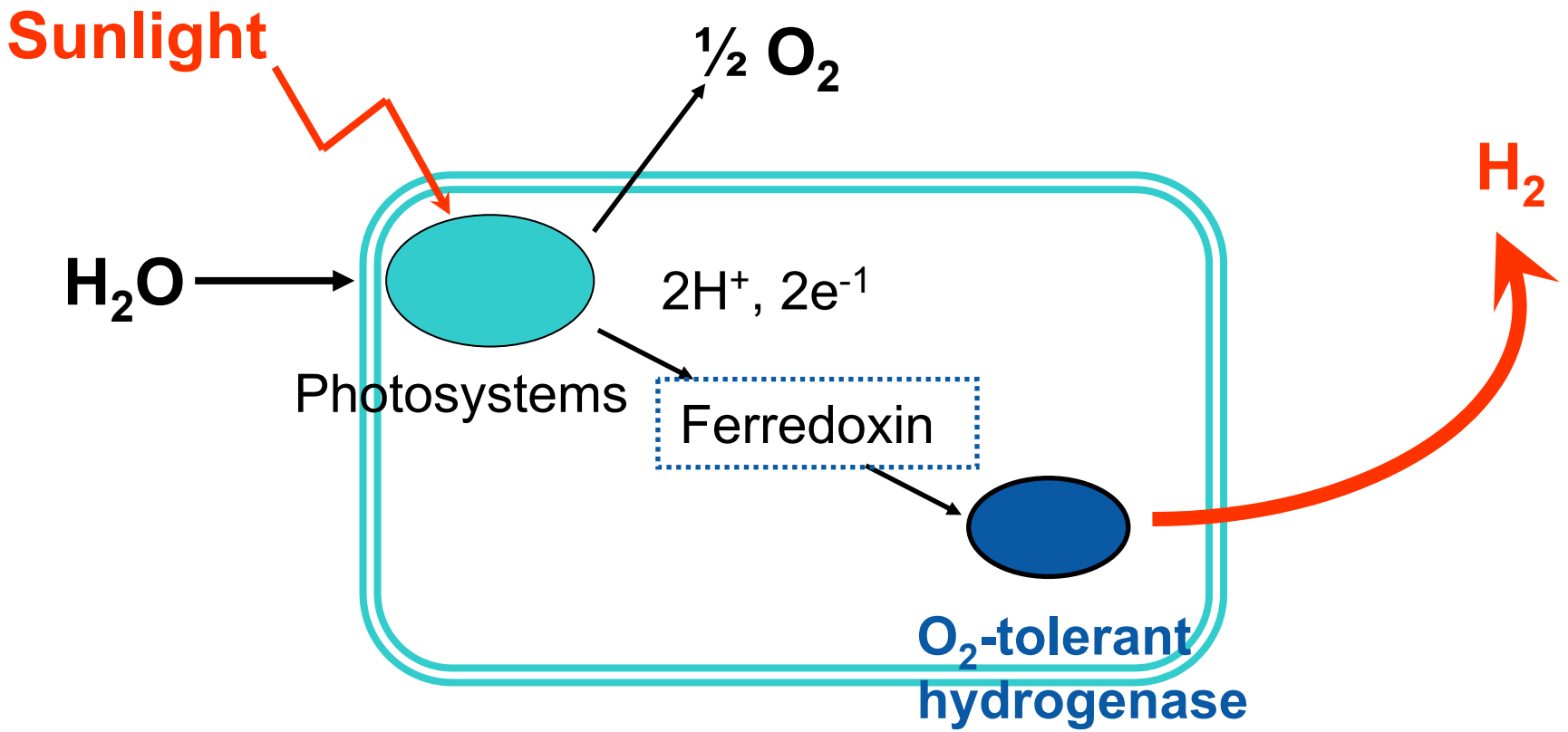
- Develop an O_2-tolerant cyanobacterial system for sustained and continuous light-driven H_2-production from water
  - Transfer and express known O_2-tolerant hydrogenases in cyanobacteria
  - Identify novel O_2-tolerant hydrogenases from VI's ongoing sampling in international waters and transfer them into cyanobacteria

Approach to address hydrogenase O_2-sensitivity barrier

- *Problem:* Cyanobacteria have the ability to split water photolytically into O_2 and H_2, but their hydrogenases are highly O_2-sensitive. In contrast, a few anoxygenic photosynthetic bacteria have O_2-tolerant H_2-evolving hydrogenases, but they can not use water as the electron donor.

- *Approach:* Use genetic techniques to transfer O_2-tolerant H_2-evolving hydrogenases from anoxygenic bacteria into cyanobacteria

Cyanobacterium Transformed with an O_2-tolerant [NiFe]-Hydrogenase

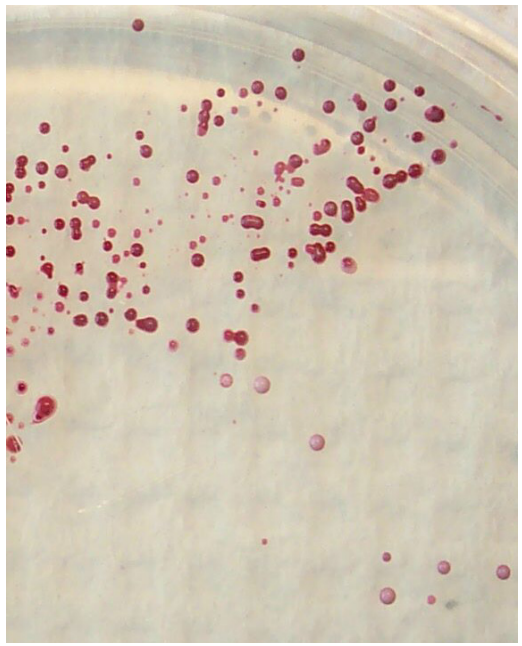


- The overall goal is to produce a cyanobacterial recombinant to produce H_2 continuously

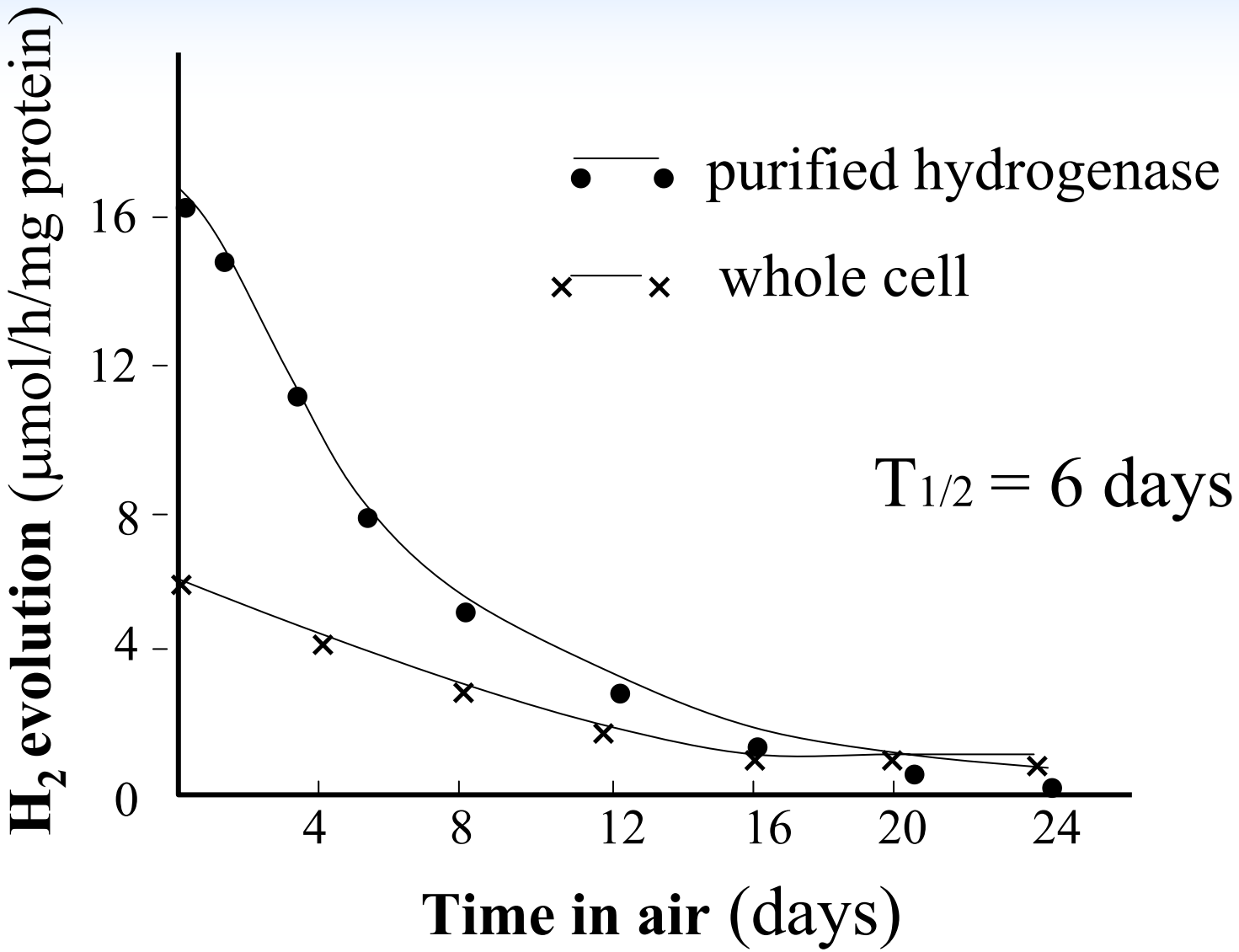
Technical Approach 1.1 (Venter Institute)

- Goal: Introducing a known O_2-tolerant hydrogenase from *Thiocapsa roseopersicina* into cyanobacteria (*Synechococcus and Synechocystis)*

Purple sulfur photosynthetic bacteria Thiocapsa roseopersicina

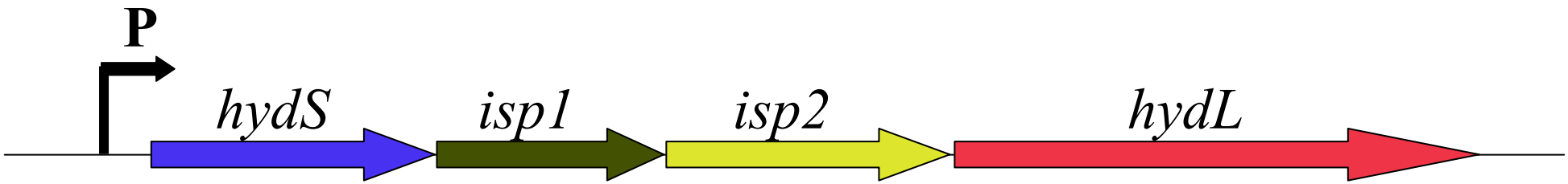


O_2-tolerant hydrogenase Hyd from *T. roseopersicina*



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

The gene locus of *T. roseopersicina* O_2-tolerant hydrogenase

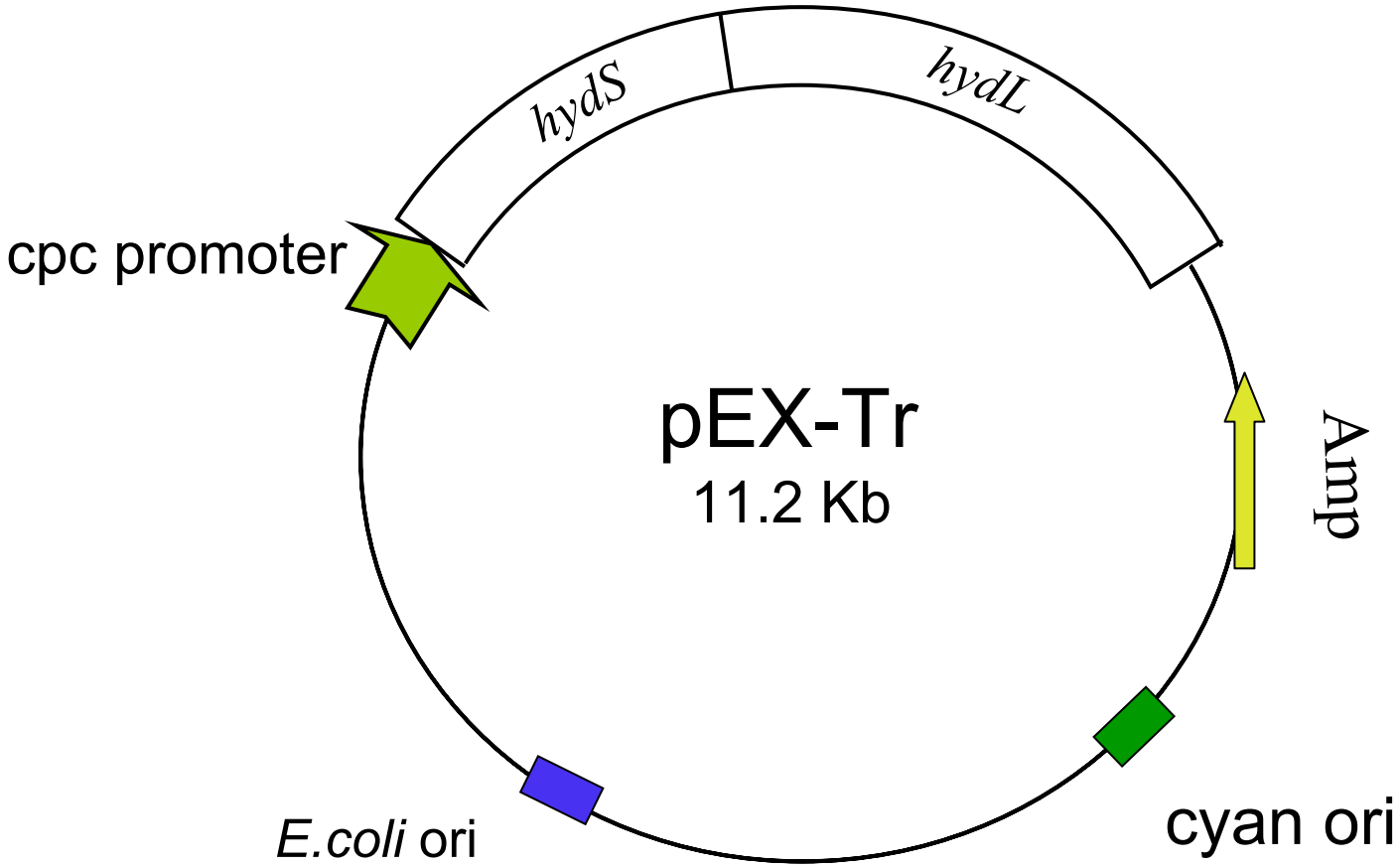


Catalytic region: HydS + HydL

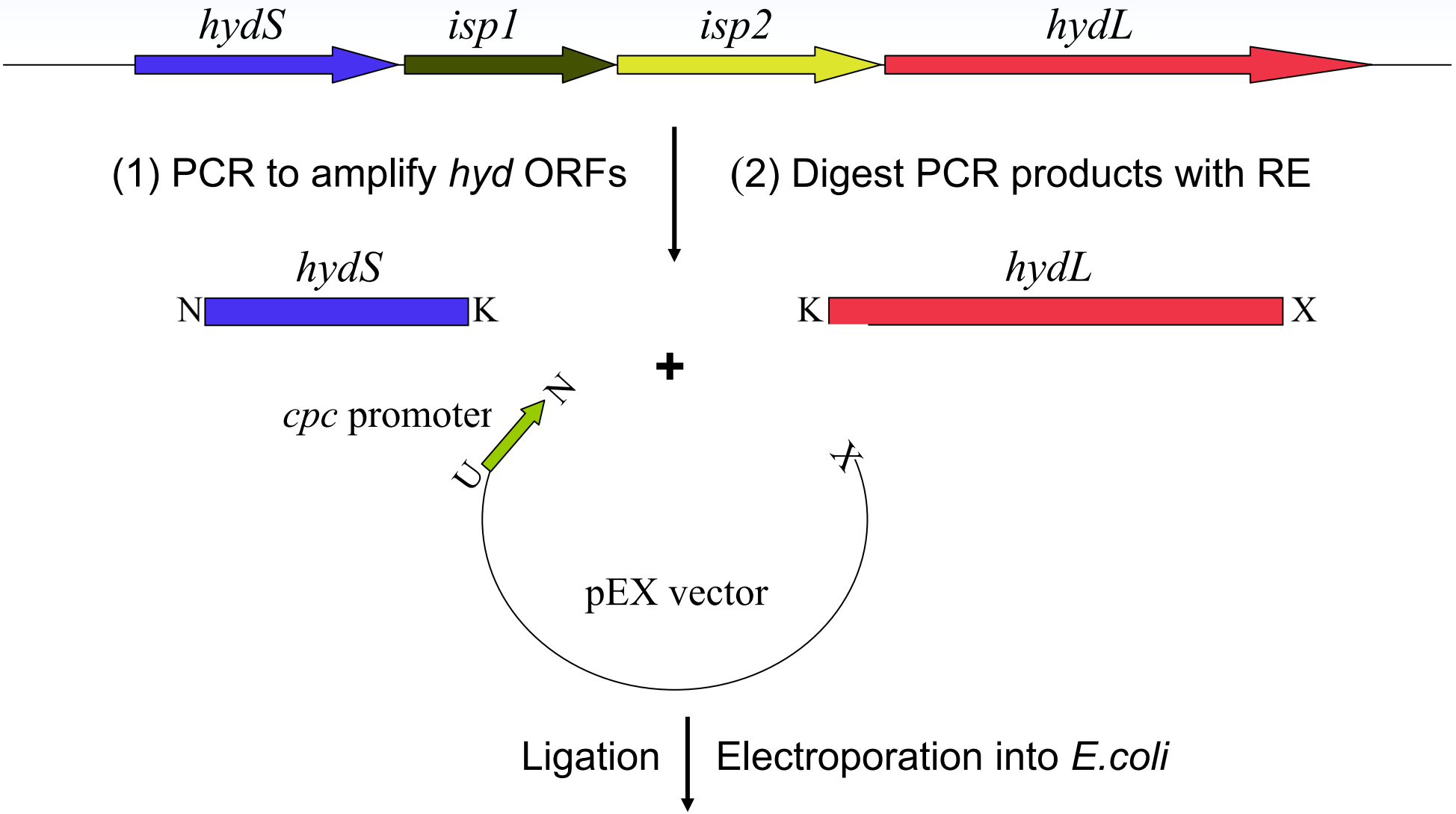
Electron-transfer elements: Isp1 + Isp2

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

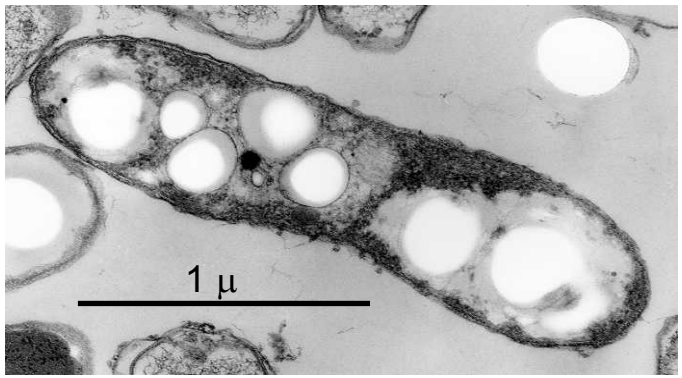


Select on LB/Amp plates

Technical Approach 1.2 (NREL)

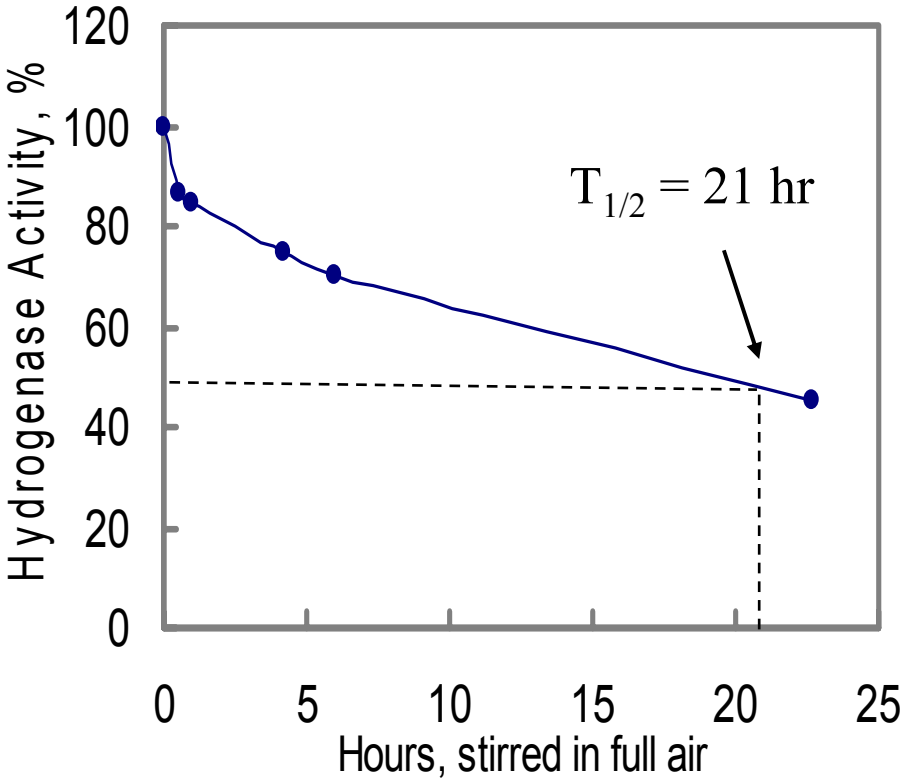
- Goal: Transfer the O_2-tolerant hydrogenase from *Rubrivivax gelatinosus* CBS into the cyanobacterial hosts *Synechocystis* and *Synechococcus*

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

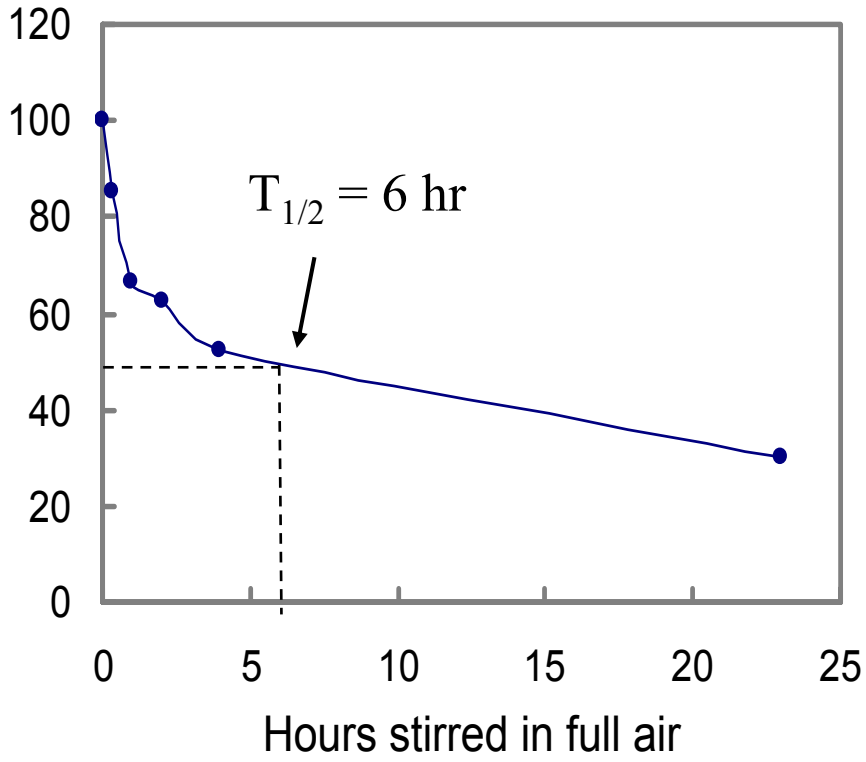


Hydrogenase from *Rubrivivax gelatinosus* Tolerates O_2

(A) Whole Cell

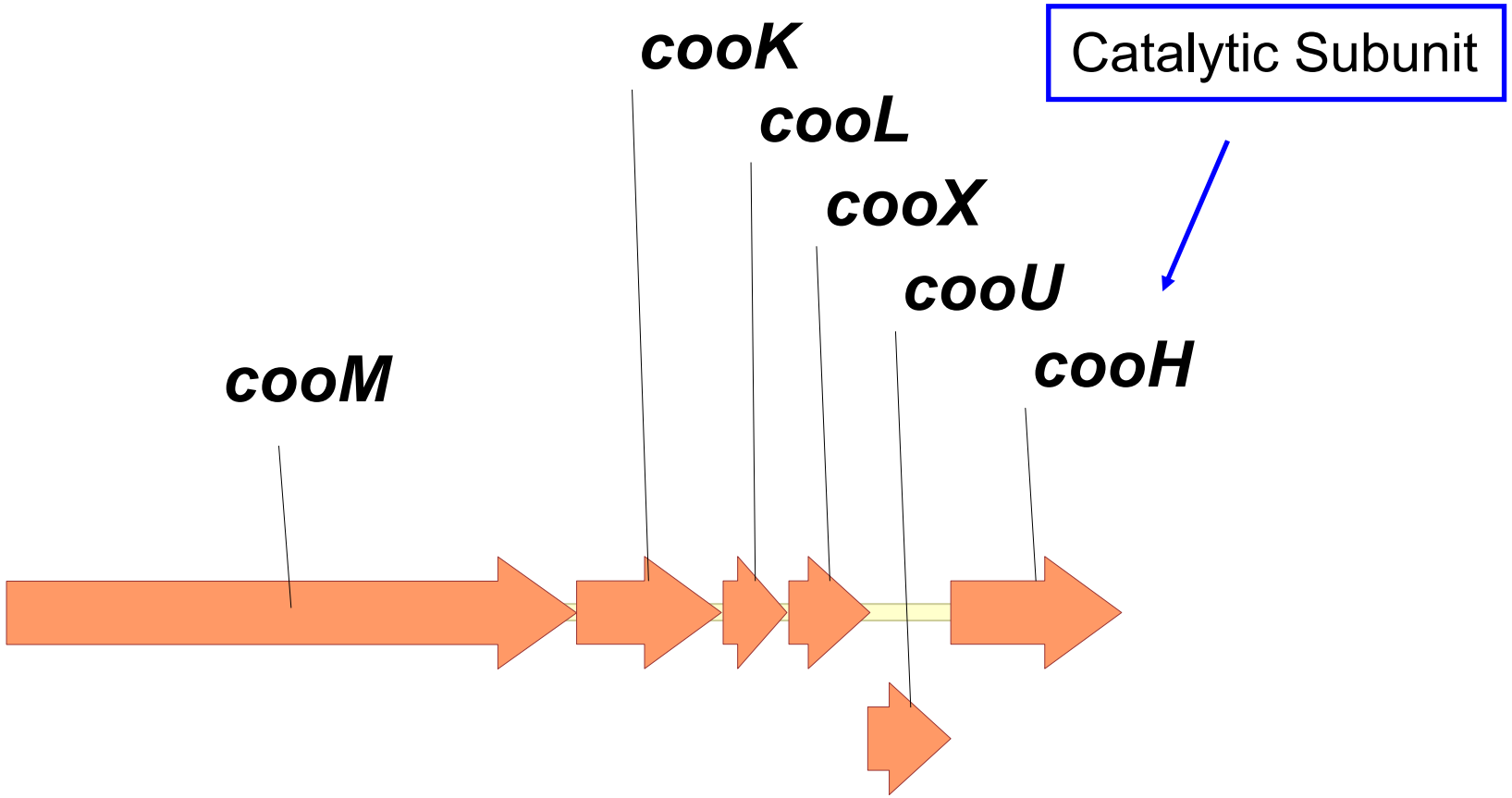


(B) Purified Hydrogenase



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

Genes Encoding the *Rubrivivax* O_2-tolerant Hydrogenase are Cloned



- Catalytic subunit: CooH
- Electron transfer subunit: CooL

Technical Approach 2 (Venter Institute)

- Identifying novel O_2-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_2-tolerant hydrogenase
  - Transfer the novel O2-tolerant hydrogenase into cyanobacteria

Technical Accomplishments/ Progress

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

J. Craig Venter
INSTITUTE

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_2-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 O2-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_2 gas cylinder (balanced with N_2 or Ar).
  - 10% H_2 is a flammable gas.
  - It is frequently used in the Lab for keeping anaerobic environment in the anaerobic gloveboxes, and as standard H_2 gas for H_2 assays.

Hydrogen Safety

Our approaches to deal with this hazard are:

- Strictly follow the Venter Institute's safety guidelines
- Perform H_2-related experiments in our fermentation Lab that is specially designed for hazard gases
- Install an O_2/H_2 gas detector in the Lab
- Do not mix 10% H_2 by ourselves and always order pre-mixed H_2 gas from commercial vendors