E. hydro Express

Streamlining Bacterial Production of Hydrogen Gas

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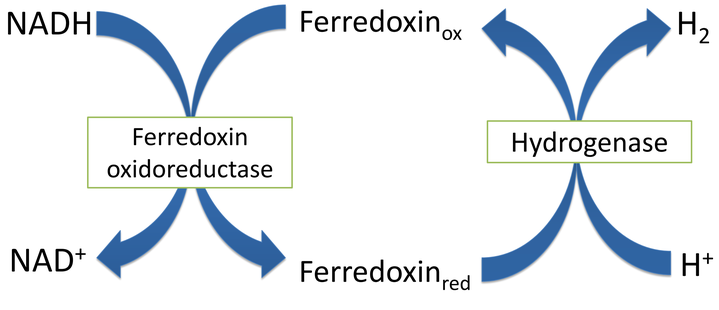
Abstract

To exploit the fermentative capabilities of *Escherichia coli* to produce hydrogen gas, we performed P1 transduction on strain FMJ39 from JW1228-1 to produce the desired triple mutant with the necessary metabolic flux to hydrogen production. In the fermentation process *E. coli* converts glucose into various intermediate states to generate energy. The transduction of the adhE knockout found in JW1228-2 to FMJ39 will produce a triple mutant with the following genes deleted: ldhA, pflB, and adhE. From these deletions insertions of mhpF, pyruvate decarboxylase, and ferredoxin oxidoreductase will result in a more direct metabolic line towards hydrogen production.

Introduction

- Hydrogen can be used as an alternative source of energy since it has the potential to eliminate the problems associated with fossil fuel.
- Most hydrogen gas is currently produced using thermochemical reformation of fossil fuels, resulting in carbon dioxide bi-products.[6]
- Biohydrogen production presents an environmentally-friendly conversion of hydrogen energy for the future.[4]
- One emerging area of focus involves taking advantage of the dark fermentation process in microorganisms.
- We recognized key elements from existing systems that can modified into a new pathway that may optimize bacterial production of hydrogen gas towards the ideal ratio.
- Our project focuses on producing hydrogen gas from *Escherichia coli* by knocking out then inserting select components to maximize hydrogen production through the dark fermentation process.



Materials & Methods

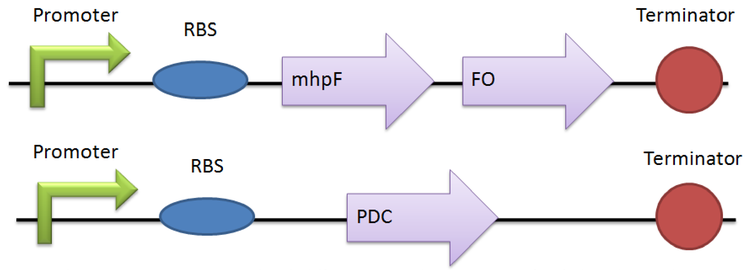


Figure 1. The composite part mapped with different BioBrick components. (Top) The mhpF and FO are parts that we submitted to the Registry of Standard Biological Parts. (Bottom) The PDC is a part taken from the Registry.

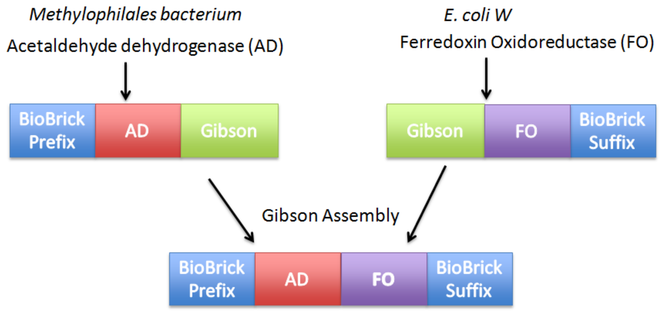


Figure 2. Combining the BioBrick standard of prefix and suffix along with Gibson Assembly yields a composite part that includes our own design without scar during ligation.

Results

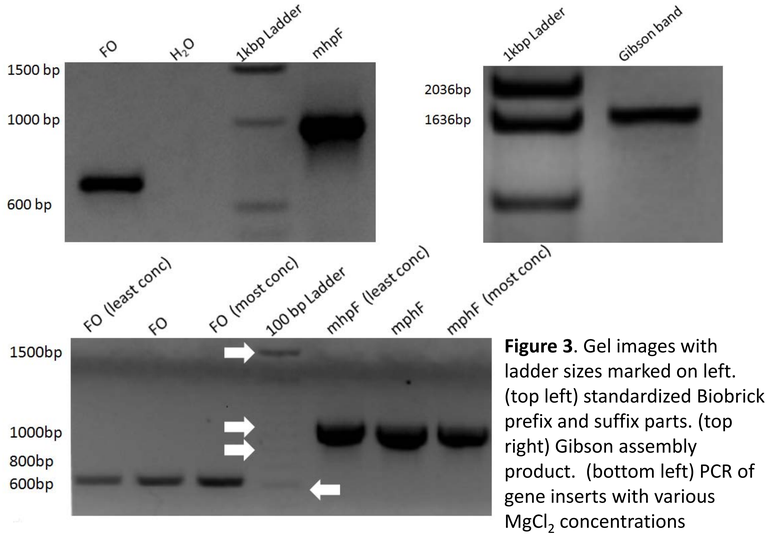


Figure 3. Gel images with ladder sizes marked on left. (top left) standardized Biobrick prefix and suffix parts. (top right) Gibson assembly product. (bottom left) PCR of gene inserts with various $MgCl_2$ concentrations

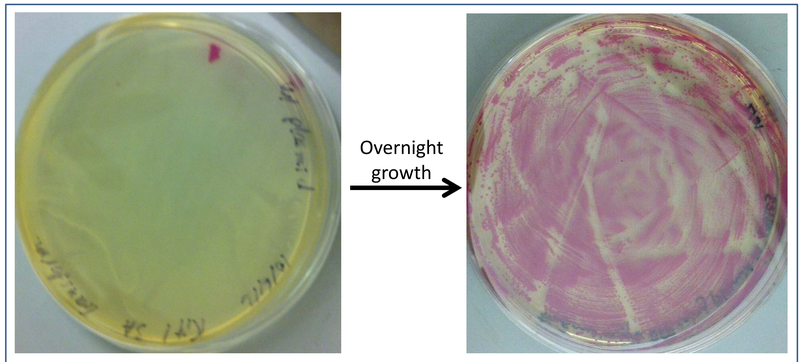


Figure 4. FMJ competent cells were successfully transformed using a reporter Biobrick containing red fluorescence protein (RFP). (left) Single transformed colony plated on streptomycin and chloramphenicol. (right) After smearing and overnight growth, the transformed cells grew abundantly.

Future Projects

- Test the fusion protein with acetaldehyde dehydrogenase and ferredoxin oxidoreductase in the FMJ39 *E. coli* strain.
- Test each of the two separate genes for activity.
- Test other fermentative pathways for comparative analysis.
- Design a photo-fermentation pathway and pair with the dark-fermentation pathway designed here. Photo-fermentation is capable of breaking down small organic acids to potentially produce more hydrogen.
- Design a pathway for efficient breakdown of cellulose to glucose. Inclusion of this step will yield a complete system capable of producing hydrogen from raw cellulose.

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Acknowledgements

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