the Lew Wentz Foundation

Evaluating the Effect of Promoter Strength on the Complementation of Deletion Mutants in *Synechocystis* sp. PCC 6803

Avery Dutcher, Neil Miller, and Dr. Robert Burnap

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078

OKLAHOMA STATE UNIVERSITY

Introduction

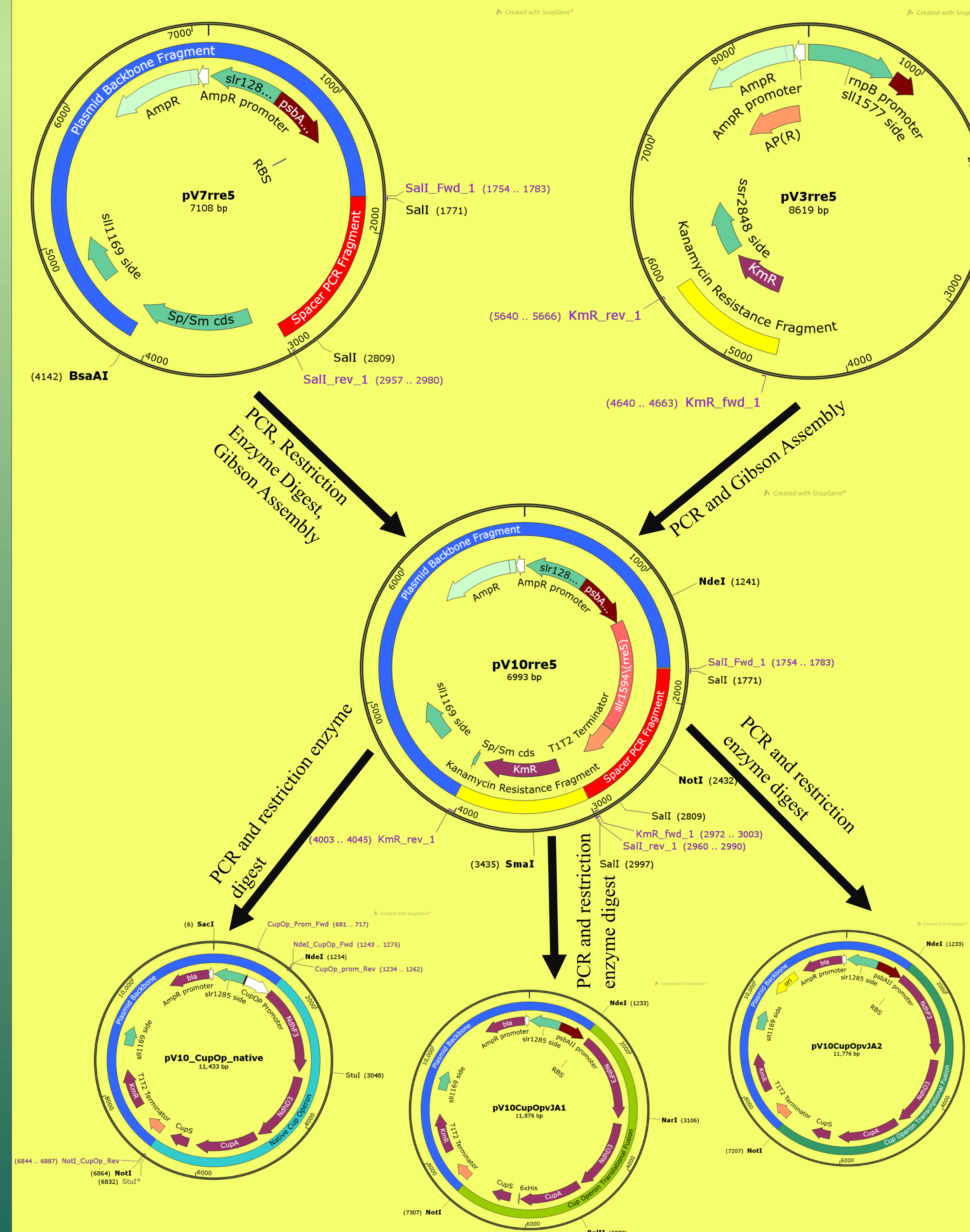
- The oxygen rich atmosphere we depend on was largely generated by numerous Cyanobacteria in the early stages of life on earth
- Cyanobacteria possesses unique CO_2 uptake systems, allowing them to efficiently scavenge inorganic carbon
- Cyanobacteria possesses an inorganic carbon concentrating mechanism including two CO_2 specific uptake systems. One is constitutive and one is low-CO_2 inducible. By limiting the CO_2 availability, the bacteria will express the inducible CO_2 concentrating mechanism to survive
- These systems are NDH-1_3, the inducible complex and NDH-1_4, the constitutive complex
- Previous work demonstrated we can complement deletions with the operon, however expression may not be optimal for future studies, and different expression systems produce different amounts of protein.

Objectives

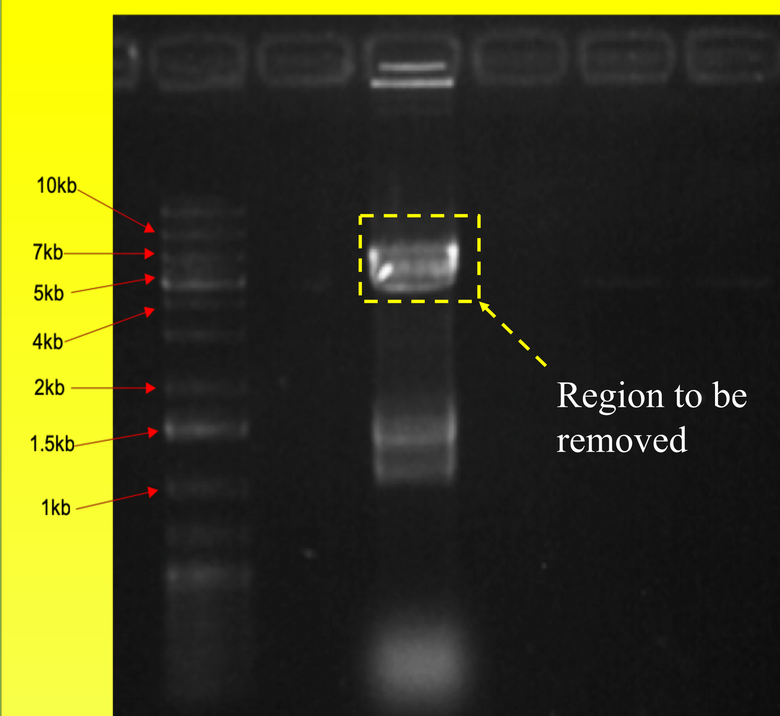
- The goal of our project is to develop a system for expressing NDH-1_3 operon in sufficient amounts for our future experiments
- The experiments here will test the effects of different gene expression control systems on the ability of deletion mutants complemented with the NDH-1_3 operon to grow in limiting CO_2

Methods

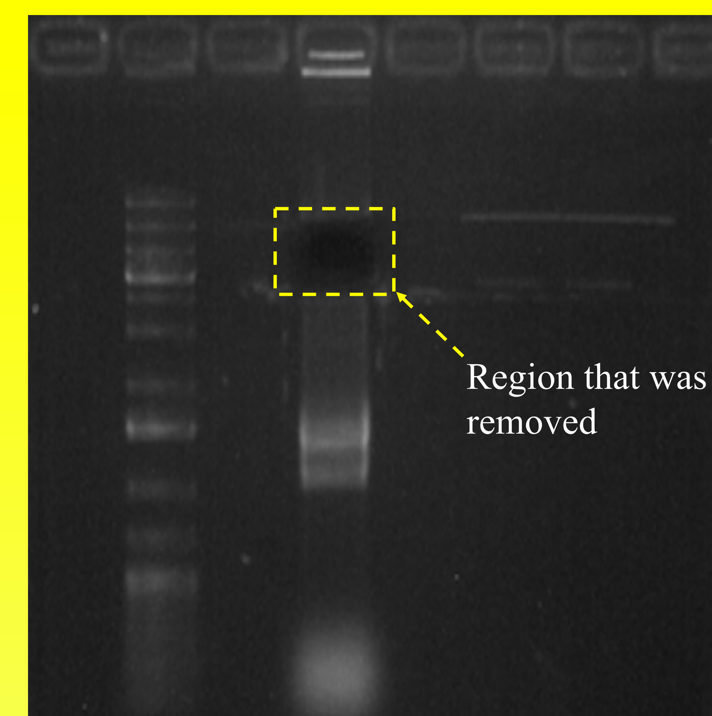
We began our experiment by constructing a new plasmid that contains antibiotic resistance (pV3) and a backbone fragment (pV7). We then created 3 different complementation plasmids each with different promoters in order to analyze promoter strength. The 3 promoters include native promoter, transcriptional fusion non-native promoter, and translational fusion.



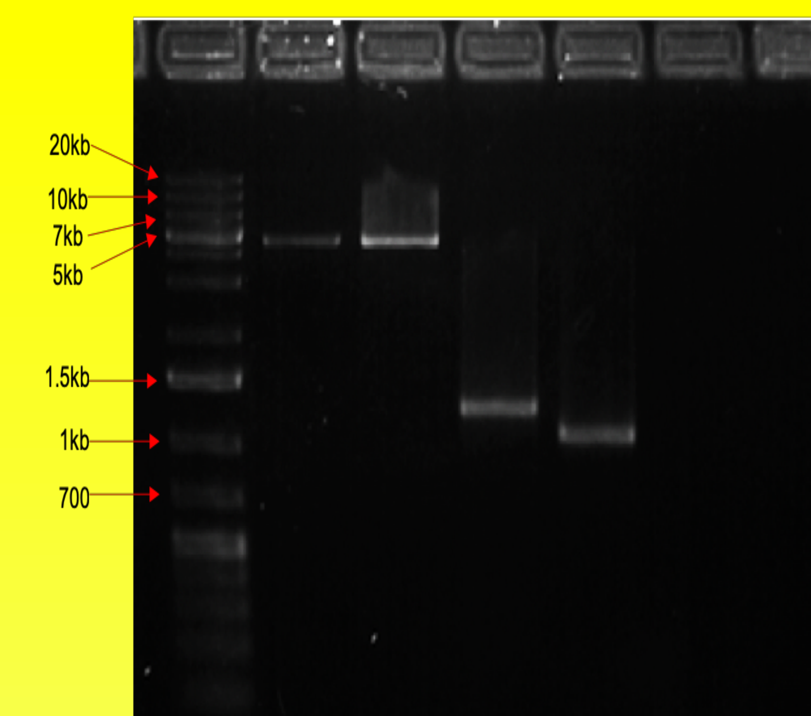
Methods (continued)



Agarose gel stained with gel green. Digestion of backbone product (blue). Backbone is shown as the larger and brighter band

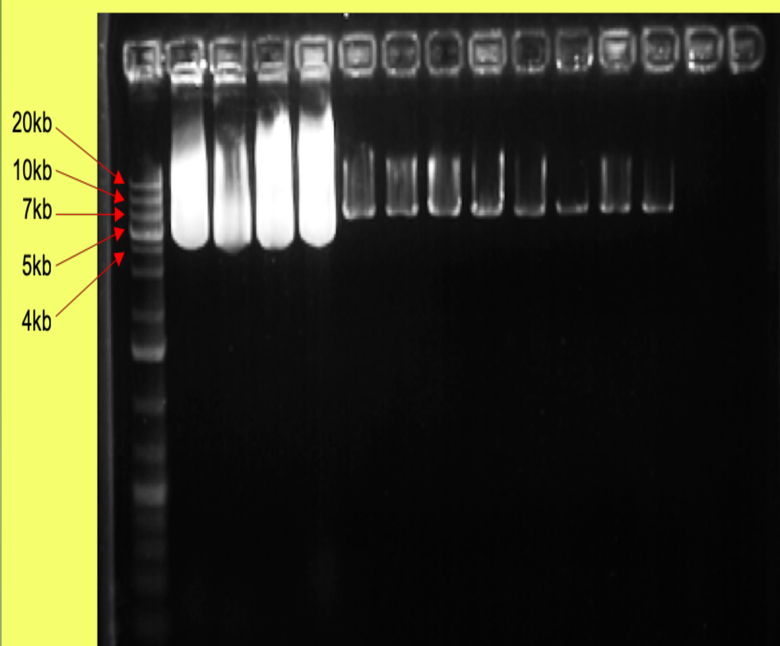


Agarose gel demonstrating the backbone product being cut out



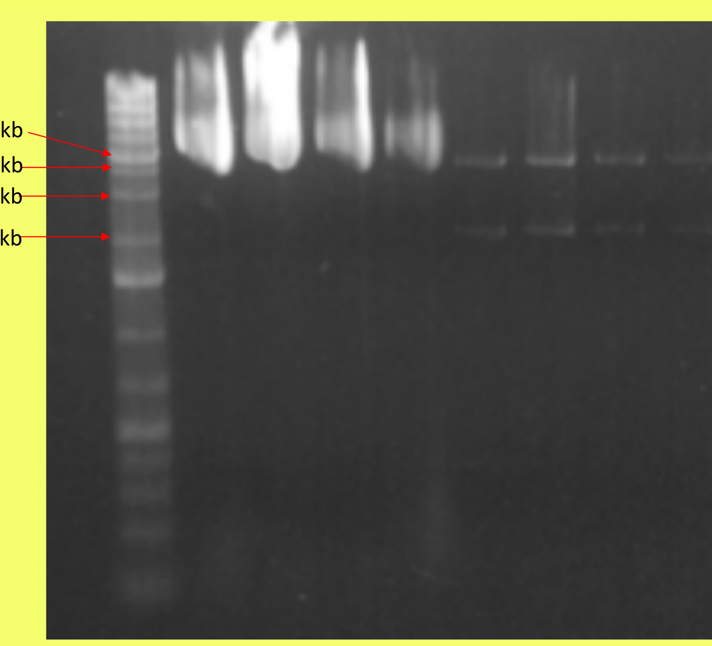
Agarose gel stained with gel green. PCR used to clone out Kanamycin Cassette (yellow) from pV3, spacer (red), and restriction digestion to provide the pV7 backbone (blue)

- Lane 1 &2 : pV7 backbone
- Lane 3: Spacer Fragment
- Lane 4: Kanamycin Cassette

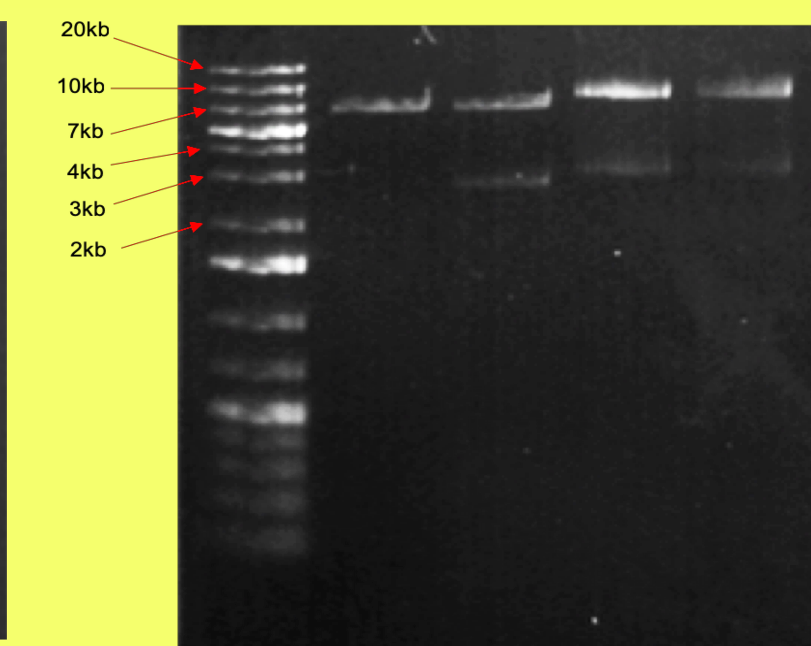


Agarose gel stained with gel green.

- Lane 1-4: raw plasmid, each isolated from a single colony
- Lane 5-12: checking activity of restriction enzymes that will be used to test each of the four isolates

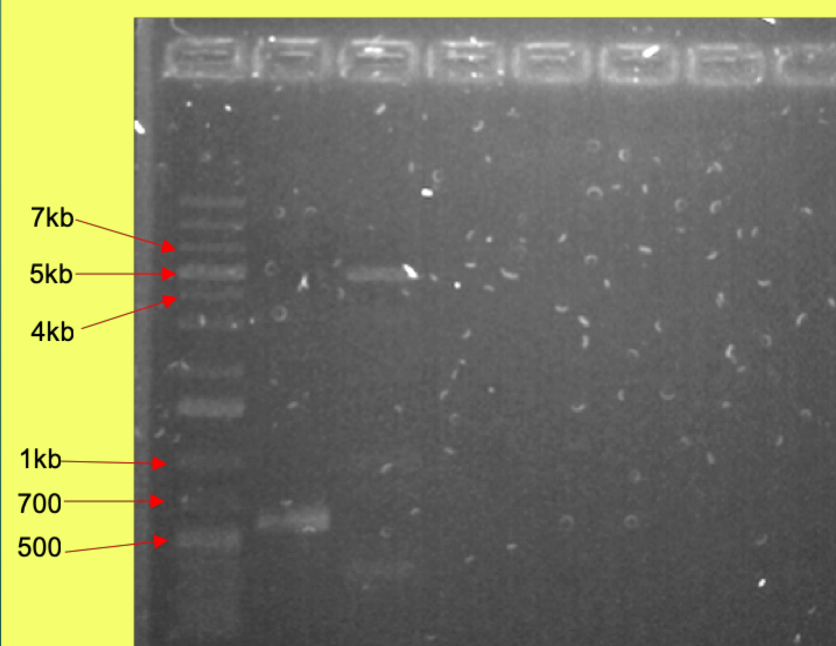


Agarose gel stained with gel green. Demonstrating correct construction of pV10. All fragments are presented in the gel



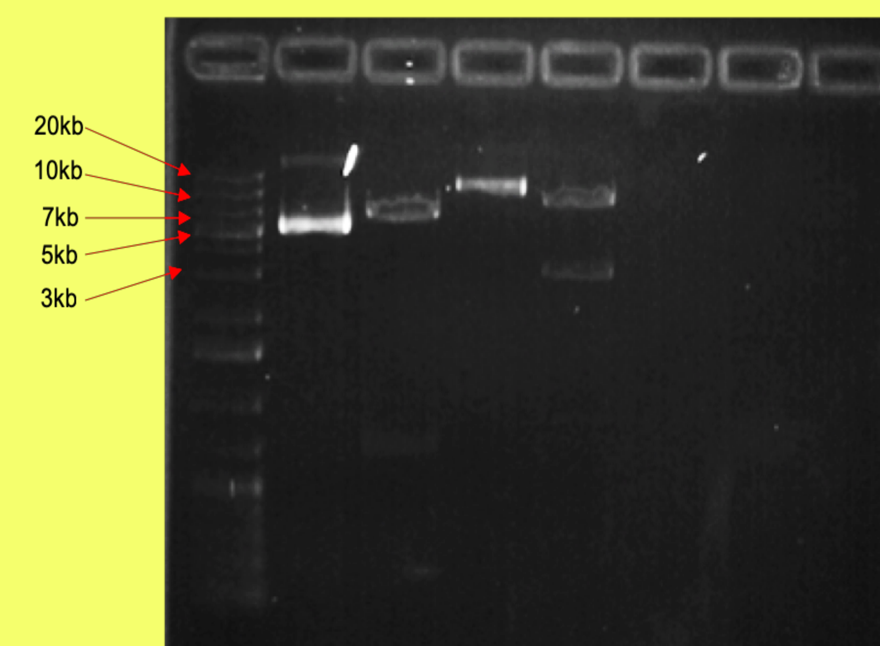
Agarose gel stained with gel green.

- Lane 3: pV10V1 (translational fusion digested with PST1)
- Lane 4: pV10V2 (transcriptional fusion digested with PST1)



Agarose gel stained with gel green. PCR

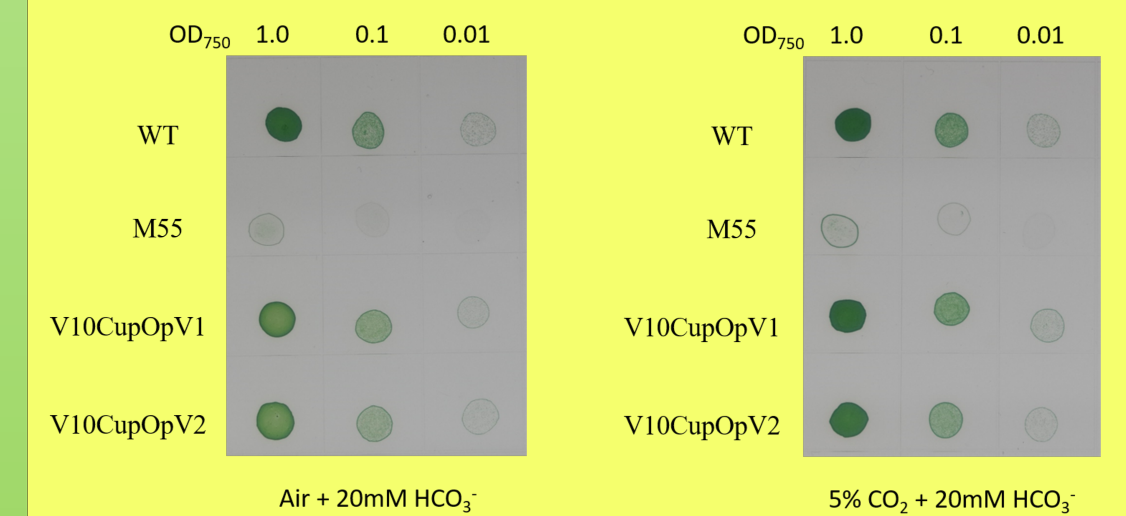
- Lane 1: Native CupOp promoter
- Lane 2: Entire CupOp



Agarose gel stained with gel green.

- Lane 1 & 2: pV10 rre 5
- Lane 3&4: pV10 digested with diagnostic restriction enzymes (Assembled version is shown in the gel to the left)

Results



- A series of spot assays were completed in order to demonstrate the phenotypic characteristics of each complement versus the WT and M55 (negative control)
- The spot assays shown above analyzed 2 different promoters, pV10CupOpV1 (transcriptional fusion) and pV10CupOpV2 (translational fusion)
- Results were not recorded for the native promoter due to contamination issues
- Growth was compared under two conditions, 5% CO_2 and Air
- No significant change in growth was noted between the promoters and growth conditions

Conclusion

- The growth in air phenotype was restored in both complementation strains
- The differences between the promoters had no effect on the growth in air phenotype
- Either strain could be used to study NDH-1_3 complexes further.

Future Research

- Repetition of spot assay with the native promoter for further analysis
- Deeper analysis of the carbon concentrating mechanism
- Analyze this expression system with the same set of genes in various organisms

Acknowledgments

- Many thanks to the Wentz Scholarship program for an amazing learning experience. Also, a special thanks to my mentor, Dr. Burnap, and graduate student mentor, Neil Miller, for the laboratory training, teaching and assistance provided.
- This research was funded by the U.S. Department of Energy.

DEPARTMENT OF ENERGY · UNITED STATES OF AMERICA