Background and Motivation

- Synechococcus sp. PCC 11901 is an attractive host for the metabolic engineering and synthesis of D-lactate due to natural transformability, short doubling time, and the ability to thrive with high light intensities and a wide range of salinities.
- Deletion of the recJ gene has been studied to increase the transformability of Synechocystis sp. PCC 6803 two-fold.
- It is hypothesized that recJ deletion in PCC 11901 will accelerate its engineering for increased D-lactate synthesis.

Methodology

Two recJ genes present within the genome

- Case 1 deletion:
  - recJ
  - 2001 nt
- Case 2 deletion:
  - recJ
  - 2,286 nt
- Homologous recombination replaces recJ genes with streptomycin resistance (SmR) and CRISPR (Cpf1) genes, for both cases.

Results

- Initial designed method was unsuccessful

New Method

- Sequencing results came back positive for the desired plasmid design

Future Work

- Transform the recJ deletion plasmids into PCC 11901 and confirm genetic segregation
- Construct and transform a second set of plasmids for the purpose of D-lactate production
- Compare the D-lactate production in recJ deleted strains vs strains with no recJ deletion using HPLC
- Our PCC 11901 sample is currently contaminated with PCC 7002, replating and DNA analysis is currently ongoing for strain isolation

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