ACCELERATING THE ENGINEERING OF CYANOBACTERIA VIA *RECJ* KNOCKOUT FOR D-LACTATE PRODUCTION

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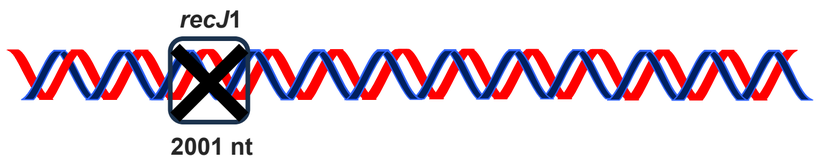
Background and Motivation

- 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**

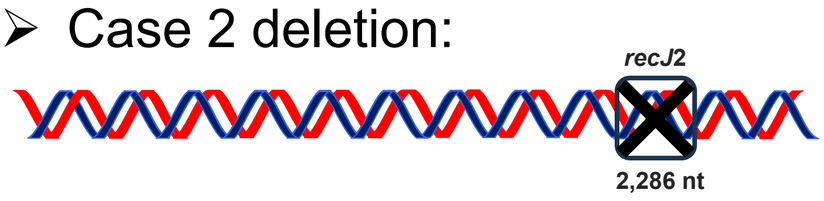
Methodology

Two *recJ* genes present within the genome

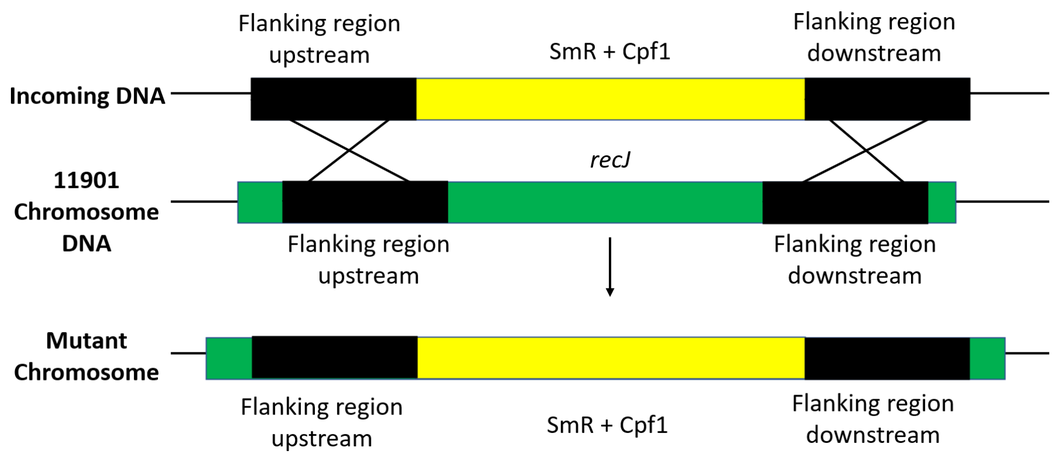
- Case 1 deletion:



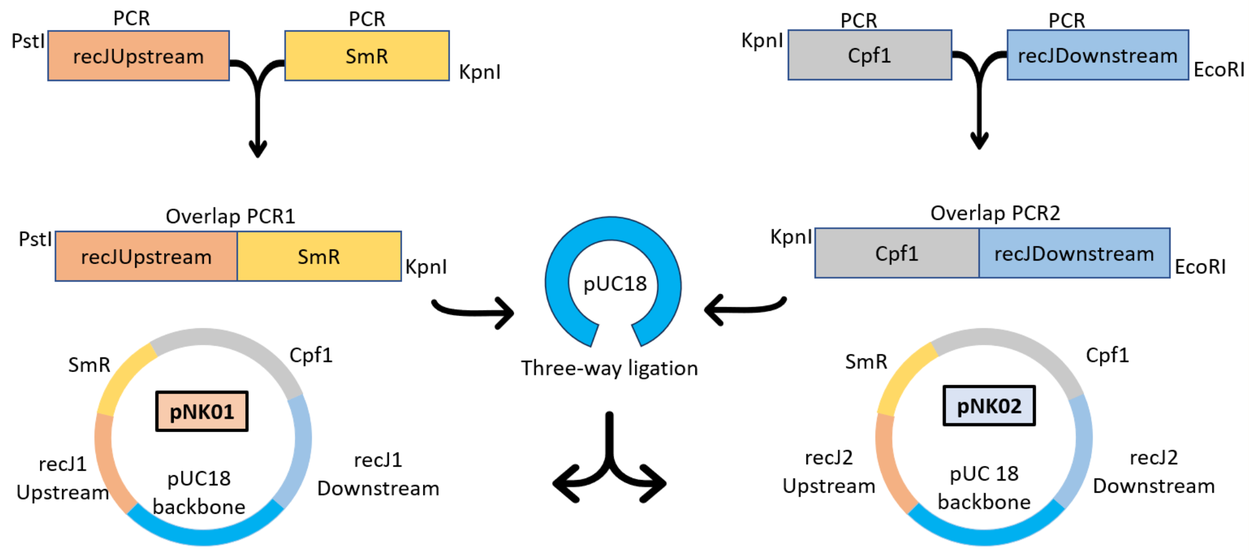
- Case 2 deletion:



- Homologous recombination replaces *recJ* genes with streptomycin resistance (SmR) and CRISPR (Cpf1) genes, for both cases



- Plasmid construction scheme for carrying out the recombination shown above

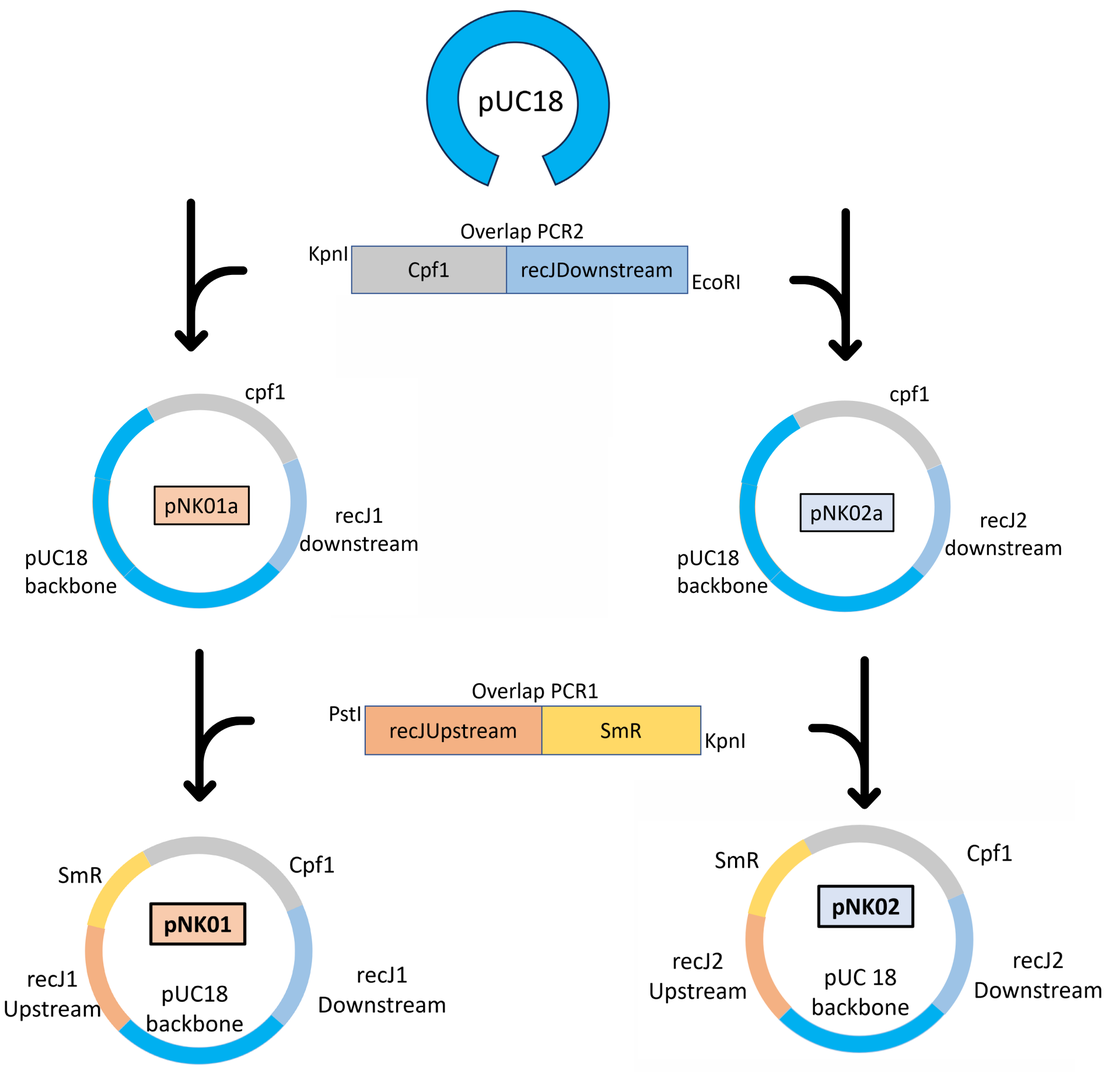


- Plasmids are intermediately transformed into E. Coli cells for cloning

Results

- Initial designed method was unsuccessful

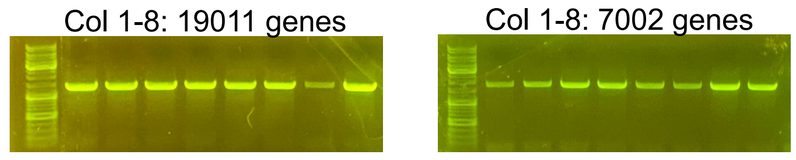
New Method



- **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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