Accelerating the Engineering of Cyanobacteria via *recJ* Knockout for D-Lactate Production

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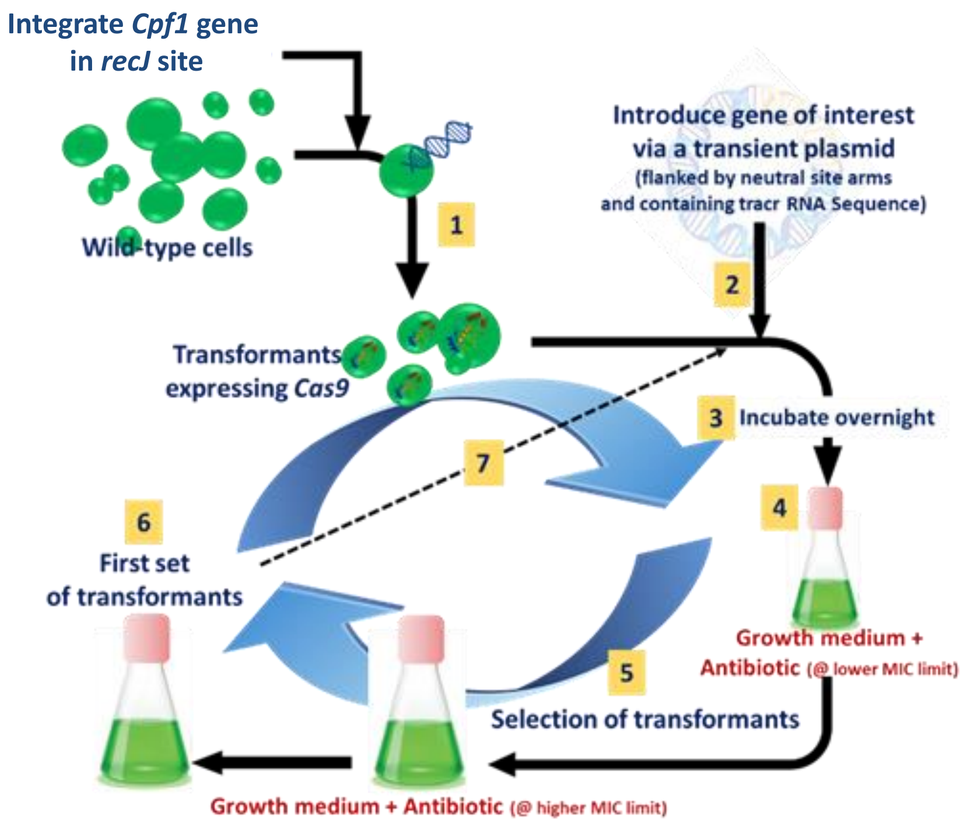
Background and Motivation

- Synechococcus sp. PCC 11901 is an attractive host for the metabolic engineering and synthesis of D-lactate due to its 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**

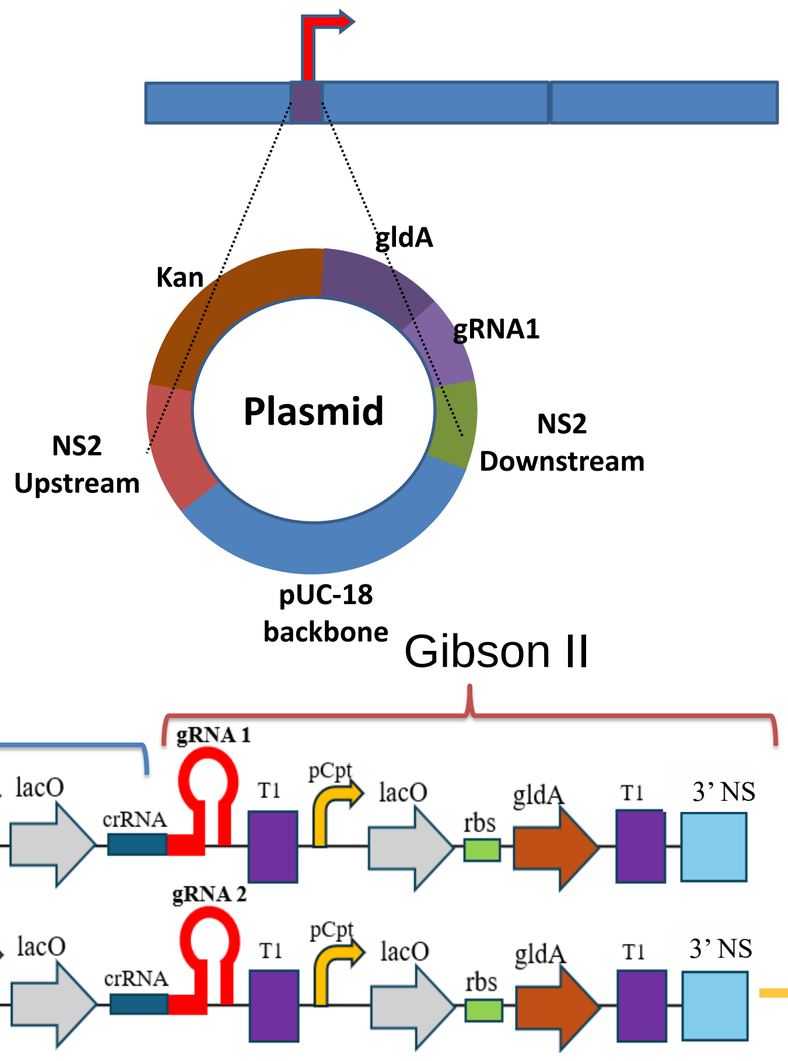
Methodology

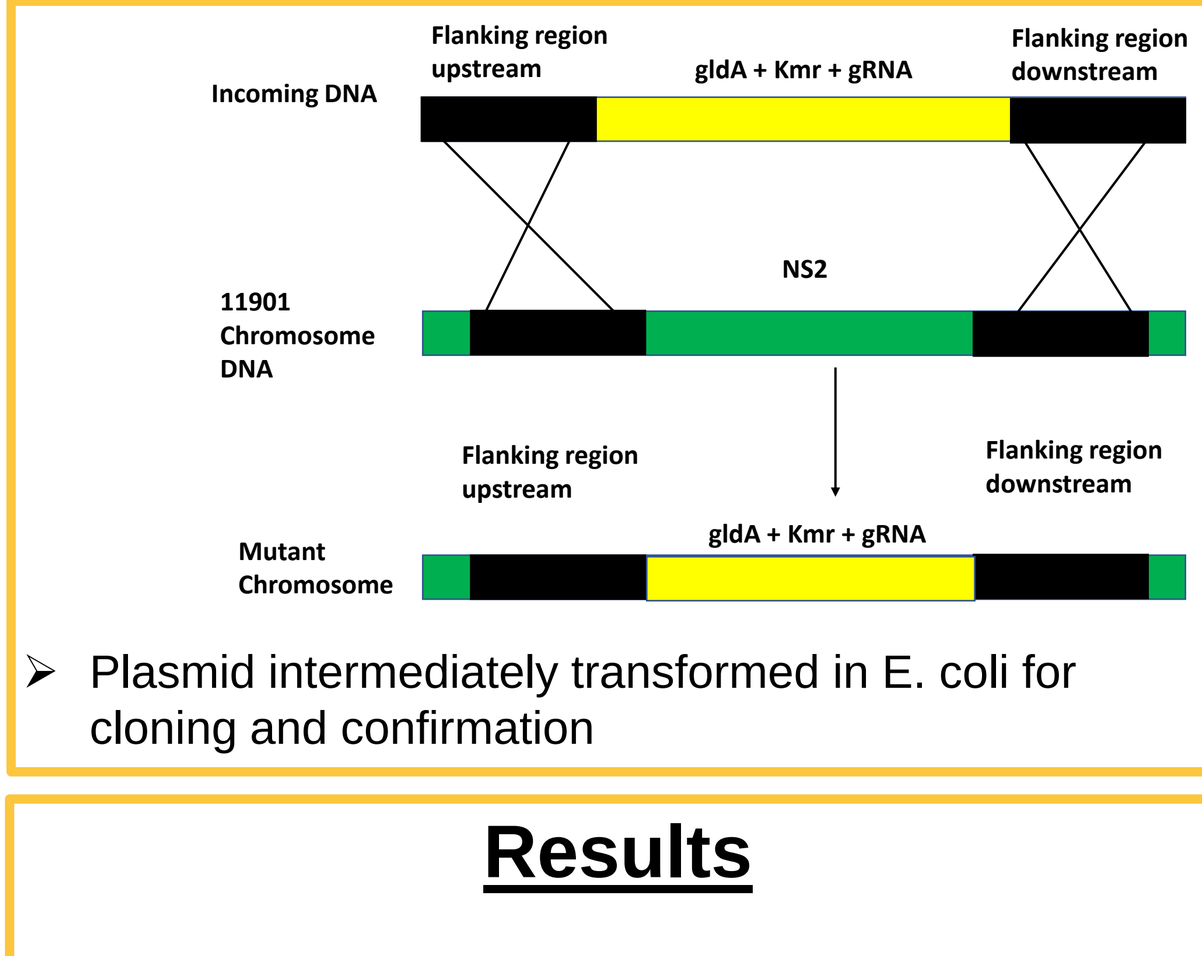
- Natural transformation of 11901 for CRISPR mediated integration or knockout of genes



- Neutral site (NS2) is to be replaced with D-lactate encoding genes

Gibson method used for plasmid construction

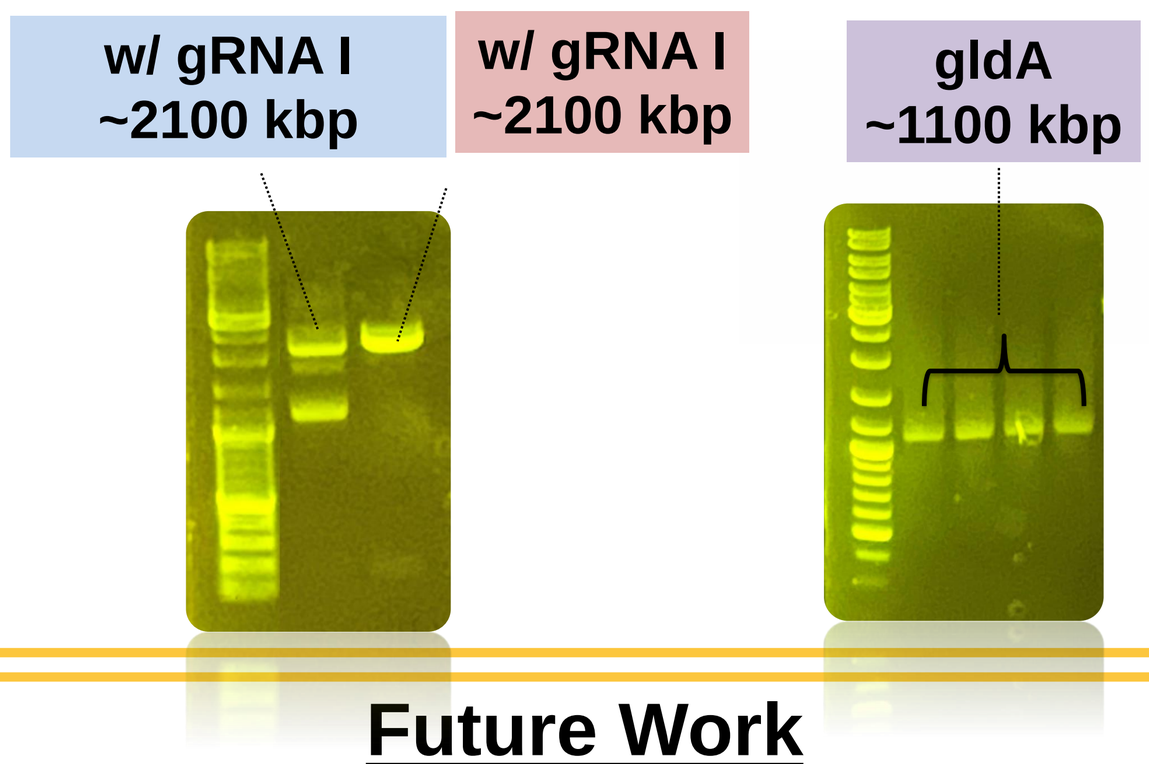




- Plasmid intermediately transformed in E. coli for cloning and confirmation

Results

- Plasmid digestion for several colonies show successful construction of Gibson II plasmid



Future Work

- Construct Gibson-1 DNA fragment and full plasmid
- Transform the *recJ* deletion plasmids into PCC 11901 and confirm genetic segregation
- Transform the 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 an analytical D-lactate kit

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