Enhanced Production of Organic Materials Under Light-limited Conditions Through CRISPR Engineering of Cyanobacterial Hydrogenase

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Background

With global warming worsening, the demand for sustainable biofuels is urgent. This study aims to boost organic material production in cyanobacteria under low-light conditions by using CRISPR to enhance hydrogenase enzymes. Specifically, we'll modify the genes of cyanobacteria like *Synechococcus* 11901 to ramp up hydrogenase activity using powerful promoters. We'll then compare the growth, chlorophyll content, and metabolic reactions of these modified strains to wild types when exposed to different hydrogen levels. The results could transform biofuel production sustainably and help fight climate change.

Research Aims

Boost cyanobacteria biomass output during low-light phases by amplifying the expression of the cyanobacterial hydrogenase enzyme. This endeavor seeks to elevate the energy efficiency of cyanobacterial cultivation, presenting a viable and eco-friendly biomanufacturing strategy.

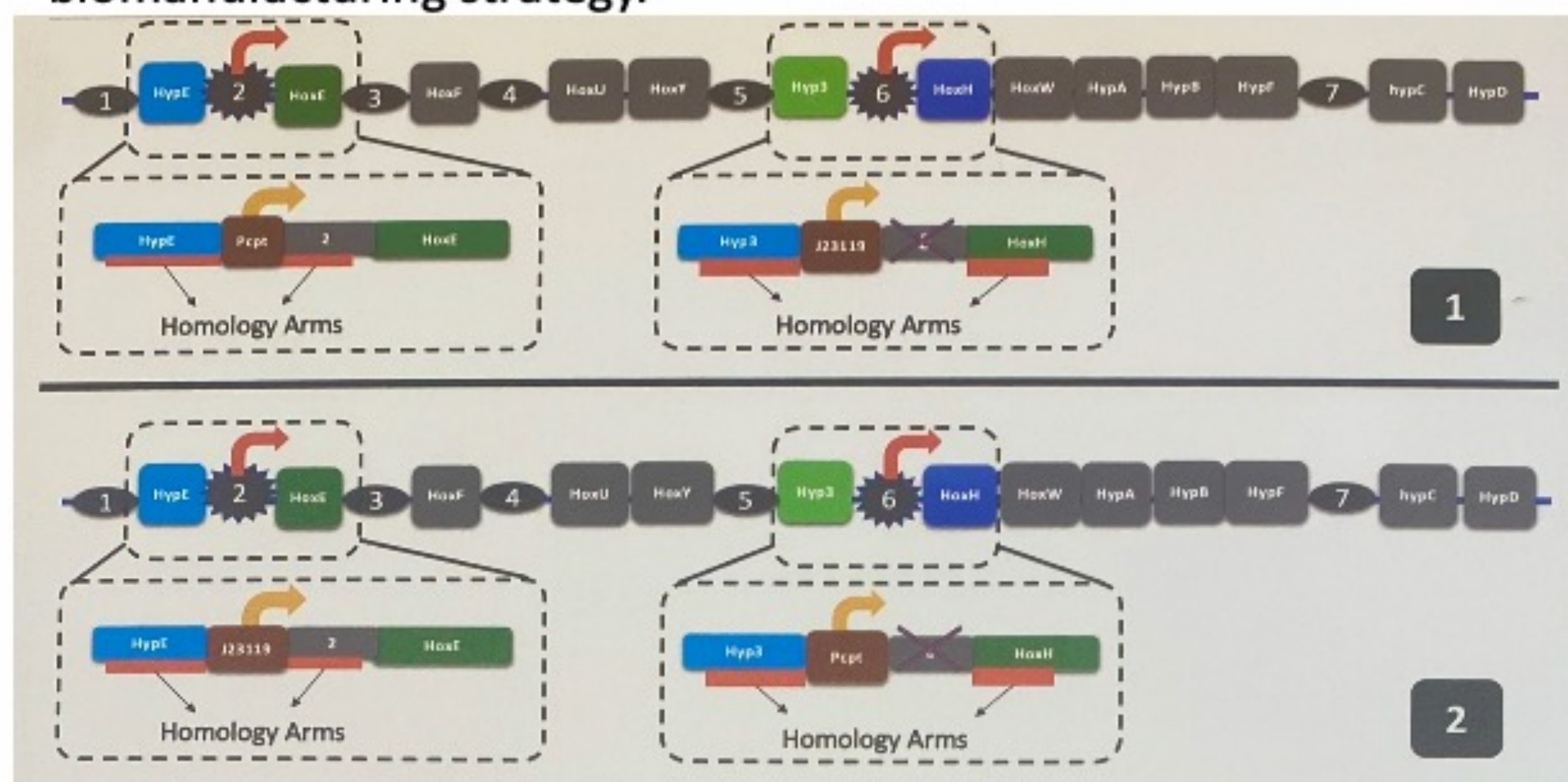


Figure 1. Gene Overexpression

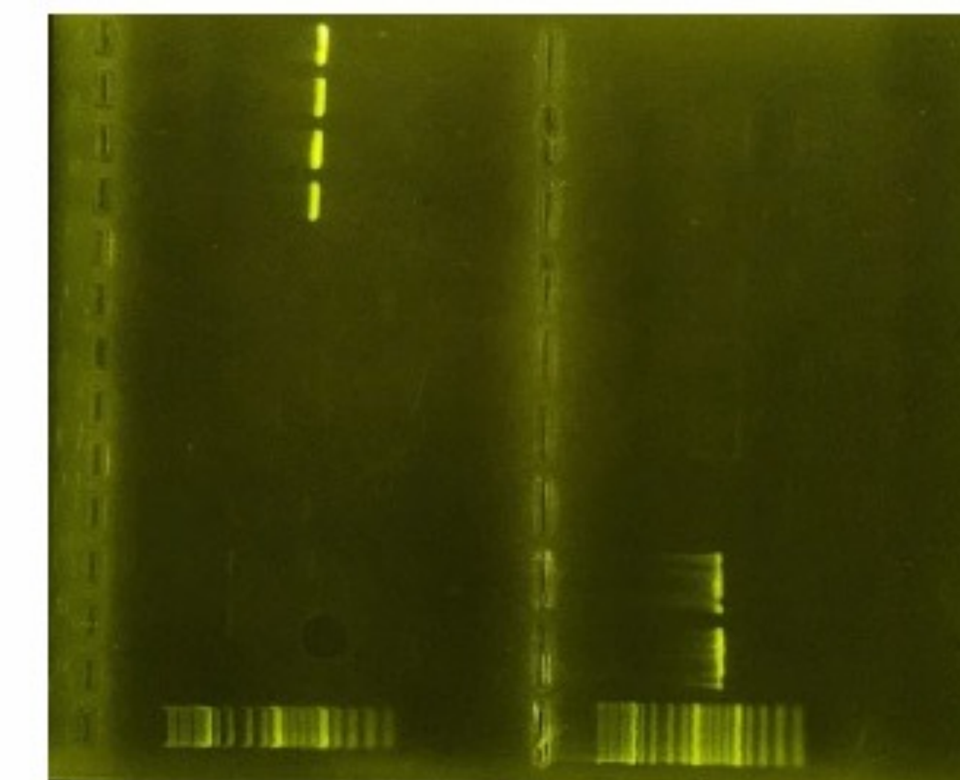
Challenges

Their productivity in biomass encounters constraints particularly in the absence of sunlight, impeding their viability as dependable sources for sustainable bioproduction. This limitation during periods of darkness poses a challenge to fully harnessing their potential in various applications requiring consistent output

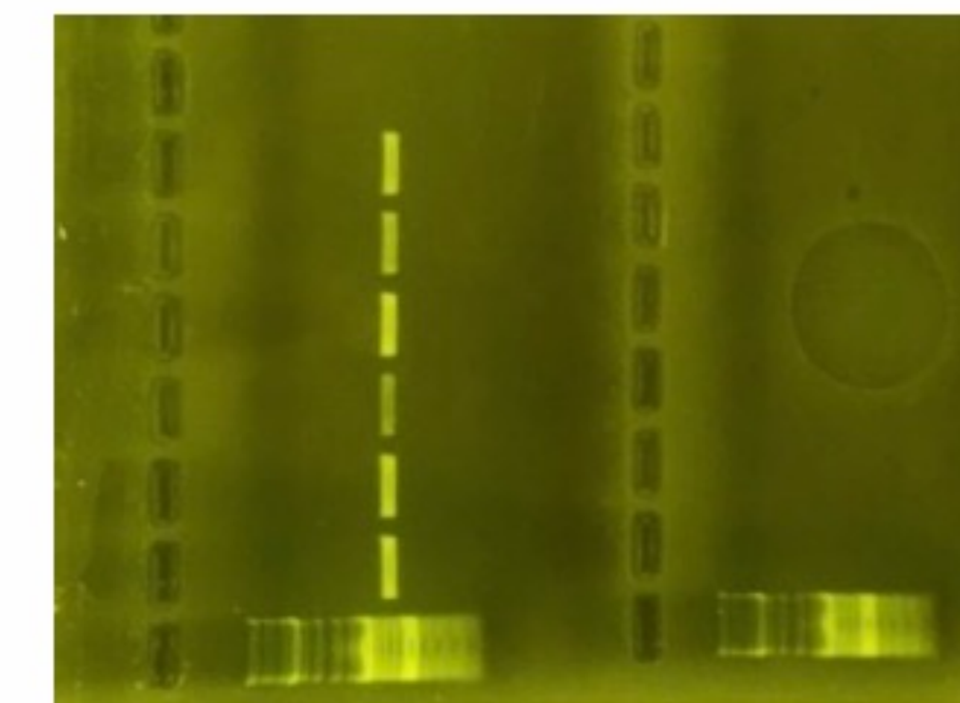
Approach

1. Choosing the cyanobacterial strain *Synechococcus* sp. PCC 11901, known for its swift replication rate and remarkable growth potential.
2. **Gene Overexpression :** Leveraging the potency of two synthetic promoters, Pcpt and J23119, we will substitute the native promoter of hydrogenase genes (HoxE and HoxH). This manipulation will drive the heightened expression of the hydrogenase complex.
3. **Transfiguration :** Engineered plasmids harboring the targeted promoters and genes will facilitate the metamorphosis of *Synechococcus* 11901.
4. **Segregation :** The modified strains will be grown on plates containing antibiotics to confirm the effective integration of the artificial promoter into their genetic makeup.
5. **Comparative Assessment:** The modified variants will be compared to the original strain regarding chlorophyll levels, biomass generation across different light strengths, and growth under varying supplies of H_2 and CO.

Results



a)



b)

Figure 2. Gel Electrophoresis results for fully segregated integration of promoters in a) pVh7 and b) pVh12

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