# Enhancing Natural Genetic Transformation in Synechococcus sp. PCC 7002 via Multimeric Integration


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## Introduction

*Synechococcus sp. PCC 7002* is a marine strain of *cyanobacteria* that is naturally capable of foreign DNA uptake. Replicating plasmids are typically delivered to this strain via bacterial conjugation, however this research establishes a protocol for an alternatively faster and more scalable gene delivery technique. Utilizing plasmid multimers for natural transformation will increase transformation scalability and further improve biotechnological applications for this strain of cyanobacteria.

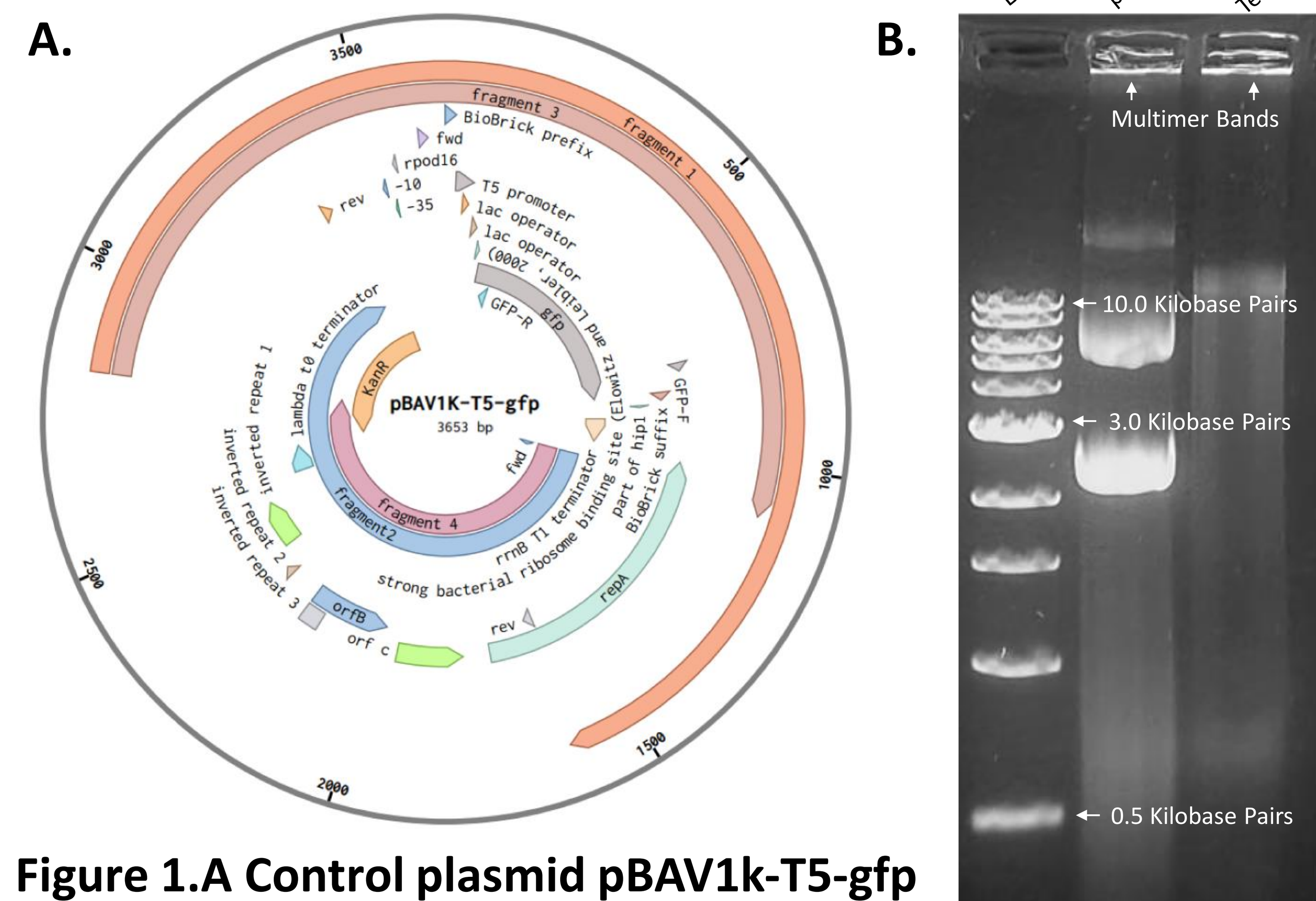


**Figure 1.A Control plasmid pBAV1k-T5-gfp**

**Figure 1.B Gel Electrophoresis of pBAV1k-T5-gfp multimers**

**1.A** This research utilized pBAV1k-T5-gfp, a broad host range plasmid as a positive control. Its transformation capabilities have been experimentally verified in *Synechococcus sp. PCC 11901, 7002,* and *Synechocystis sp. PCC 6803*, a freshwater strain of cyanobacteria. However, this is a novel use of pBAV1k-T5-gfp in *cyanobacteria*. **1.B** Multimeric bands are visible at the top of a gel electrophoresis due to their large size. TempliPhi multimer amplification isolated this type of DNA for transformation.

## Results

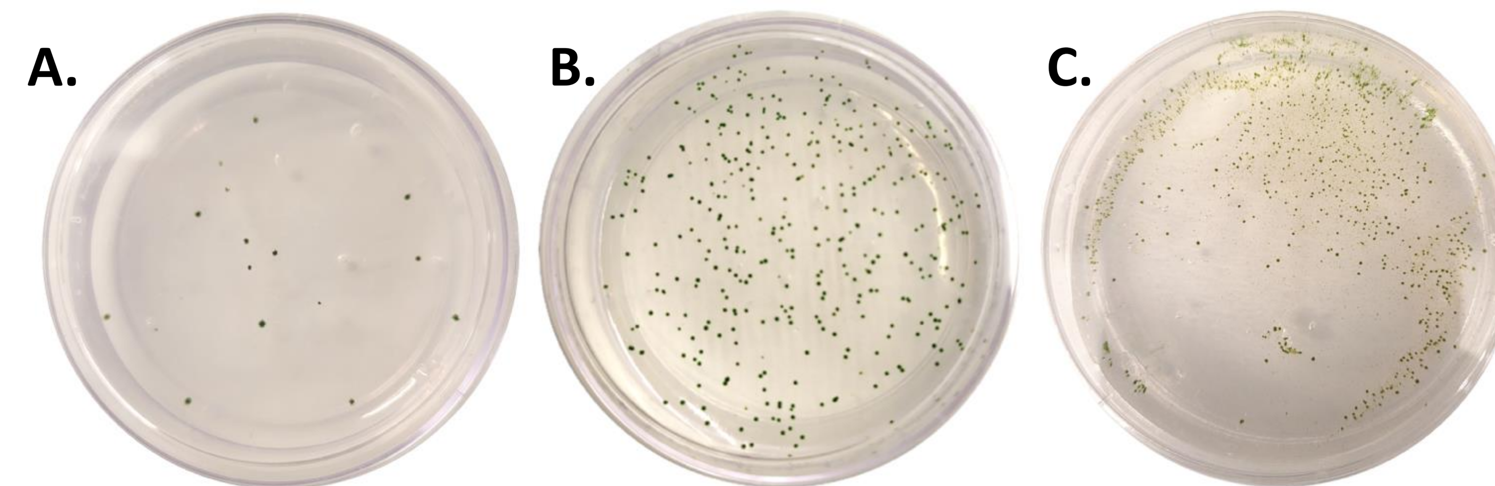


**Figure 3. Comparison of transformation methods for pBAV1k-T5-gfp 3.A** 16 colonies obtained via miniprep in Caron. **3.B** 332 colonies obtained via TempliPhi in Caron. **3.C** 646 colonies obtained via TempliPhi in humidity incubator.

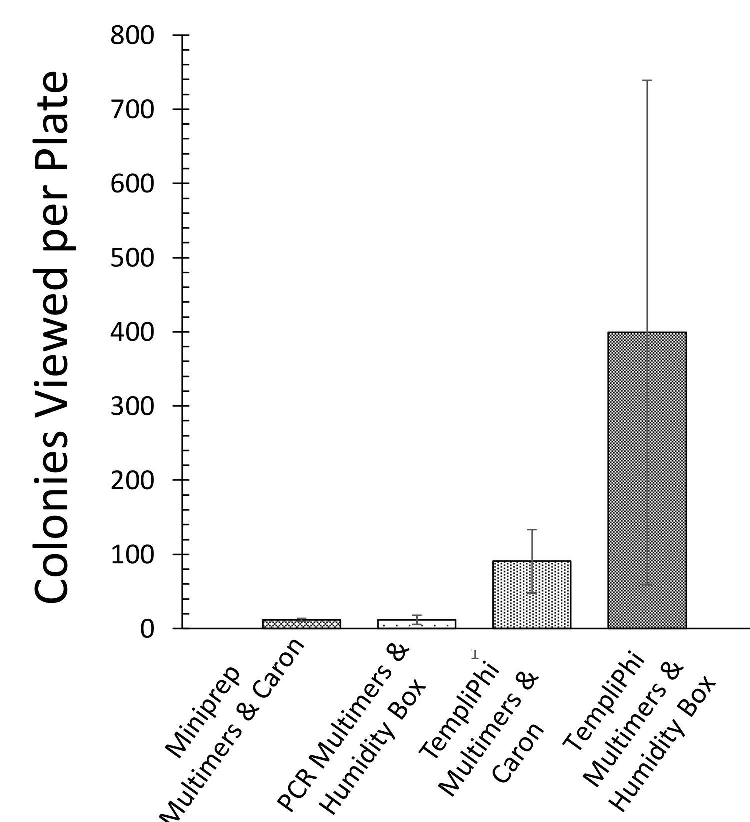


**Figure 4. Comparison of multimer methods and humidity conditions**

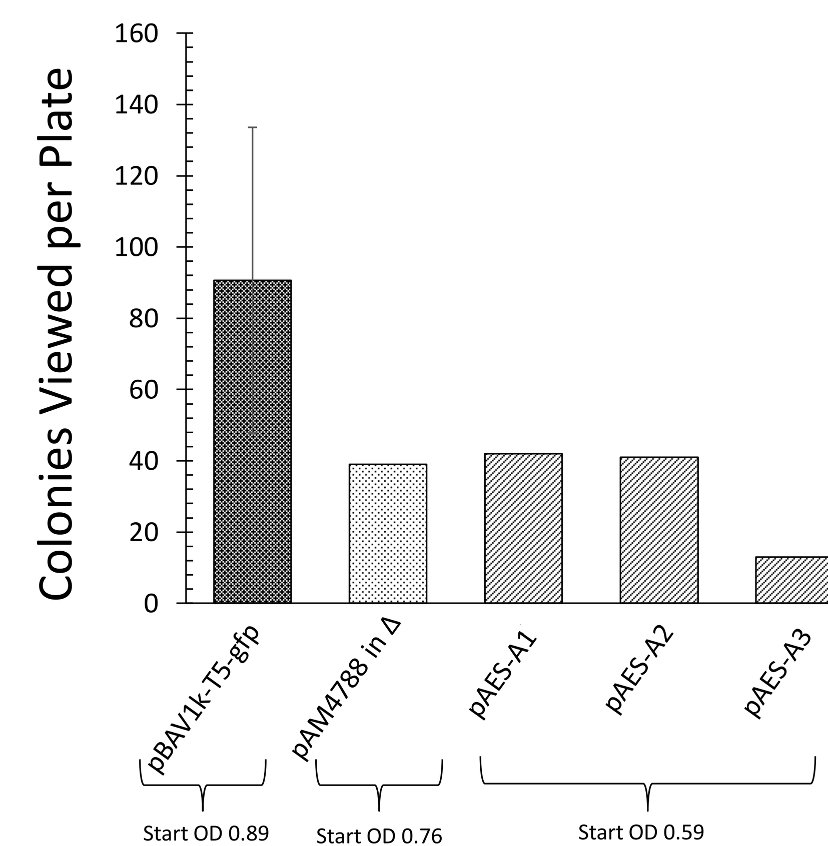


**Figure 5. Testing variant plasmid multimers under standardized protocol**

## Future

It is an objective to reduce the 6 hour recovery period, which can be achieved by altering the nutrient composition of the A+ liquid media. In further experimentation, addition of glycerol, peptone $H_2O$, yeast extract, sodium bicarbonate, or reduction of $MgSO_4$ concentration will be tested. Further, a dsDNA BR Assay Kit will be used to measure multimer concentration upon transforming for quantitative analysis.

## Methodology

Plasmids preassembled from minipreparation contain a small quantity of multimeric plasmid DNA. Additionally, prolonged overlap extension PCR was a method used to obtain multimers using two fragments of the target DNA as primers [1]. A third method utilizing preconstructed plasmids with the Cytiva TempliPhi Amplification kit was found to be the most efficient.

### Optimized Protocol for Multimeric Transformation

Plasmid minipreparation was performed in DH10B Escherichia coli competent cells to isolate and purify the target DNA. The sample was diluted to 2 ng/μL which was then amplified via a polymerase enzyme to form multimers with the Cytiva TempliPhi kit. *Synechococcus sp. PCC 7002* WT cells were inoculated and grown overnight until an OD of 0.6 had been obtained. The entire reaction of plasmid multimers were introduced to the host and transformed over a recovery period of 6 hours. Cells were then plated, and colony data was recorded after one week.

*Synechococcus sp. PCC 11901* is another marine strain of cyanobacteria with a rapid doubling time of two hours [2]. This protocol was tested for this strain, and properly transformed for pBAV1k-T5-gfp, and pBb(RSF1010)1k-GFPuv, another broad host range positive control.

## References


[1] ACS Synth. Biol. 2020, 9, 12, 3228–3235 (2020).

[2] Vogel, A.I.M., Lale, R. & Hohmann-Marriott, M.F. Streamlining recombination-mediated genetic engineering by validating three neutral integration sites in Synechococcus sp. PCC 7002. J Biol Eng. 11, 19 (2017).



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