Unlocking the *Synechocystis* pCC5.2 plasmid for high-yield production in cyanobacteria

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Synechocystis sp. PCC 6803 harbors three small cryptic plasmids (pCA2.4, pCB2.4, and pCC5.2) in addition to its chromosomal DNA and four larger plasmids. The natural role of these plasmids within the cell remains largely unknown. Nevertheless, prior studies have employed them as templates for expression vectors in cyanobacteria despite their low copy numbers which may limit their potential for high-yield production of target products. In the Laboratory of Photosynthesis (Roman Sobotka, Martin Tichý), a high-copy (HC) variant of pCC5.2 was identified, characterized by a significant increase in plasmid copy number resulting from a deletion upstream of the putative CyRepA2 primase-helicase open reading frame, a protein involved in pCC5.2 replication. This deletion was also associated with enhanced *cyrepA2* transcription in HC pCC5.2. Based on these findings, the current study aims to modify the pCC5.2 plasmid into an inducible high-copy shuttle vector designed for efficient expression of therapeutic peptides. A critical aspect of this effort involves characterizing the plasmid's replication mechanisms by investigating both the structural and functional attributes of the recombinantly expressed CyRepA2 protein, as well as identifying the origin of replication region in pCC5.2. These analyses will elucidate the regulatory elements necessary for replication, providing a foundation for refining and enhancing the vector’s performance.