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Overexpression of *puc*C improves the heterologous protein expression level in a *Rhodobacter sphaeroides* expression system

L. Cheng, G. Chen, G. Ding, Z. Zhao, T. Dong and Z. Hu

Key Laboratory of Biorheological Science and Technology
Ministry of Education, Bioengineering College, Chongqing University,
Chongqing, China

Corresponding author: Z. Hu
E-mail: huzongli71@163.com / huzongli@cqu.edu.cn

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ABSTRACT. The *Rhodobacter sphaeroides* system has been used to express membrane proteins. However, its low yield has substantially limited its application. In order to promote the protein expression capability of this system, the *puc*C gene, which plays a crucial role in assembling the *R. sphaeroides* light-harvesting 2 complex (LH2), was overexpressed. To build a *puc*C overexpression strain, a *puc*C overexpression vector was constructed and transformed into *R. sphaeroides* CQU68. The overexpression efficiency was evaluated by quantitative real-time polymerase chain reaction. A well-used reporter β-glucuronidase (GUS) was fusion-expressed with LH2 to evaluate the heterologous protein expression level. As a result, the cell culture and protein in the *puc*C overexpression strain showed much higher typical spectral absorption peaks at 800 and 850 nm compared with the non-overexpression strain, suggesting a higher expression level of LH2-GUS fusion protein in the *puc*C overexpression strain. This result was further confirmed by Western blot, which also showed a much higher level of heterologous protein expression in the *puc*C overexpression

strain. We further compared GUS activity in *puc*C overexpression and non-overexpression strains, the results of which showed that GUS activity in the *puc*C overexpression strain was approximately ten-fold that in the non-overexpression strain. These results demonstrate that overexpressed *puc*C can promote heterologous protein expression levels in *R. sphaeroides*.

Key words: Heterologous protein expression; *Rhodobacter sphaeroides* Light-harvesting 2 complex assembly; *puc*C

