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Optimization of Electrotransformation Conditions and Construction of Fluorescent Protein Reporting System for Pseudomonas aeruginosa L10

By

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Abstract

Pseudomonas aeruginosa is an endogenous bacterium with the ability to degrade petroleum pollution and promote reed growth. This study aims to introduce the plasmid pBBR1-MCS5, which confers gentamicin resistance, into this bacterium and optimize the electroporati on conditions to lay the foundation for constructing a genetically engineered strain, thereby enhancing

its application potential. In this study, single-factor experiments were conducted to investigate the effects of OD600nr, value of cell growth state, sucrose concentration in the washing buffer, plasmid addition amount of pBBR1-MCS5, final OD value, electroporati on voltage, and recovery time on electroporation efficiency, to identify the primary influencing factors. Subsequently, Box-Behnken design response surface method was used to optimize these main factors. Additionally, a pBBR1-MCS5 expression vector with a fluorescent gene was constructed, and the expression of fluorescent proteins in Pseudomonas aeruginosa was measured by fluorescence intensity. The results showed that the optimal transformation conditions for Pseudomonas aeruginosa L10 were: OD600nr, value of 0.6 for cell growth state, sucrose concentration of 400 mmol/L in the washing buffer, plasmid concentration of 500 ng, final OD value of 64, electroporati on voltage of 2.5 kV, and recovery time of 3 hours. Under these optimal conditions, further optimization using the response surface method resulted in an electroporati on efficiency of 3.3×10^3 CFU/mu g DNA for Pseudomonas aeruginosa L10, which is 37 times higher than the unoptimized electroporation efficiency. This lays the groundwork for further research into the genomic functions of Pseudomonas aeruginosa.

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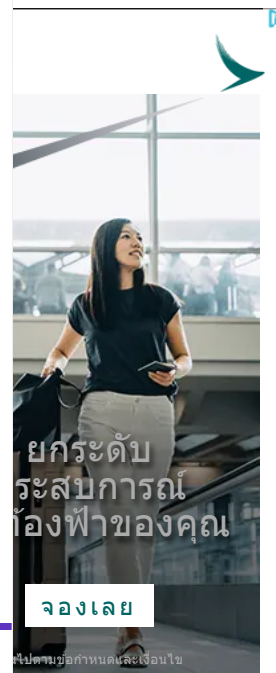You are receiving this email because you requested an alert from **Web of Science. | Unsubscribe**

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