STUDY EXPERIENCE OF BACTERIAL TRANSFORMATION BY pGREEN PLASMID

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The acquisition of practical skills in the genetic transformation of microorganisms is an integral part of the training of biotechnology students. The most common study practice is the integration of the green fluorescent protein (GFP) gene from jellyfish and the gene of ampicillin resistance into bacterial cells.

The aim of the work was to adapt the standard procedure of bacterial transformation [1] for educational purposes at the Department of Biotechnology of NAU. Supplying of the pGREEN plasmid was sponsored by BioUkraine initiative. We have used Gram-positive and Gram-negative bacterial cultures available at the Department of Biotechnology of NAU.

Bacterial transformation was performed by the heat shock method. In the first stage, we prepared night cultures of Gram-positive and Gram-negative bacteria in meat-peptone broth. The next stage was the cooling of tested bacterial cultures to the temperature of 4 °C. After the cooling was completed, we added 1 ml of liquid medium containing bacterial cultures to the test tubes, then the content of the test tubes was centrifuged. At the end of the centrifugation, we discarded the supernatant and resuspended the cell sediment in 1 ml of precooled to the 4 °C 0.1 M $CaCl_2$ solution. It should be mentioned that the resuspension of *Bacillus spp.* bacterial cells was difficult. The centrifugation and resuspension processes were repeated two times. Centrifugation was conducted at 3500 rpm for 5 min. At the end of the third centrifugation bacterial cells were resuspended in 120 µl of precooled to the 4 °C 0.1 M $CaCl_2$ solution. Then 10 µl of the solution containing pGREEN plasmid was added to each test tube with bacterial cells. The test tubes were cooled at 4 °C for 10 min. After the cooling test tubes were thermostated at 42 °C for 2 min. Afterward, test tubes were cooled again at 4 °C for 5 min. Then 1 ml of liquid nutrient media was added to each of the test tubes. Test tubes were thermostated for 40 min at the temperature of 37 °C. After this, the content of the tubes was transferred to the solid nutrient medium, containing 100 µg/ml of ampicillin. At the final stage, inoculated Petri dishes were thermostated for 24 h at the temperature of 37 °C.

At the end of the experiment, we observed the bacterial colonies that were resistant to ampicillin and had low fluorescence intensity. The low fluorescence effect can be explained by the fact that tested bacterial cultures have a low level of GFP expression. We recommend using specialized for transformation *E. coli* strains with high levels of recombinant proteins expression to obtain more intensive fluorescence.

Reference:

1. Das S., Dash H. R. Microbial Biotechnology – A laboratory Manual for Bacterial Systems. New Delhi: Springer India, 2015. P. 35-72.
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