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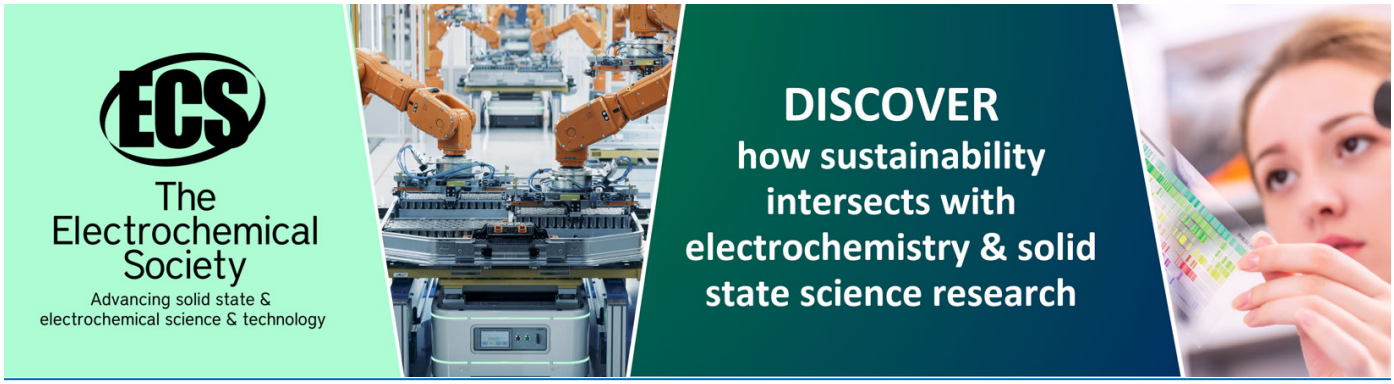

Fluorescent labeling of bacteriophage T7 by CRISPR-Cas9

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Abstract. The objective of this work was to develop a simple method for coupling a fluorescent protein with a bacteriophage capsid. The *Escherichia coli* bacteriophage T7 was chosen as a model one. The method, based on CRISPR-Cas9 system and the λ-Red recombination, allowed to insert the gene of enhanced yellow fluorescent protein into the phage genome.

1. Introduction

Fluorescence is widely used in various biological and biomedical studies. Fluorescence microscopy (FM) allows to observe objects, in most cases inaccessible to optical microscopy. FM is a method of obtaining an improved image using the luminescence of excited atoms and molecules of dyes into organisms pre-stained by special ones. Fluorescent proteins, originally derived from living organisms such as corals and jellyfish, are often used as dyes. The triad of amino acids serves as a fluorophore in such proteins: Ser, Tyr and Gly. The advantage of using fluorescent proteins in FM is their compatibility with living cells (their presence in the body usually does not affect the processes in the cells) and their ability to form a fluorophore group without the involvement of external cofactors and enzymes: they can fluoresce inside the cell by themselves [1].

Enhanced Yellow Fluorescent Protein (EYFP) has a fluorescence spectrum in the yellow-green region, an excitation maximum at a wavelength of 513 nm and an emission maximum at 529 nm [2]. One of its uses is the creation of fusion proteins, which allow using genetic engineering techniques for fluorescent labeling of proteins of interest. Thus, it becomes possible to track *in vivo* the dynamics of synthesis, as well as the location and structures formed by a protein in the cells.

A genetic engineering method CRISPR-Cas, based on the adaptive immune system of bacteria and archaea, is currently popular for genome modifications. The use of such systems for genome editing is a new and rapidly developing area, but most experiments have focused on editing the genomes of multicellular organisms. At the same time, modification of viruses paid a little attention, while such experiments are of considerable practical interest [3].

Bacteriophages are viruses of bacteria that can infect and eliminate entire bacterial populations. Bacteriophages are of great interest as agents for the treatment of bacterial infections. In addition, the study of their interaction with bacteria made it possible to open and use a variety of molecular tools used in various fields, ranging from the food industry to the cloning and editing of living organisms.

As well as, bacteriophages are a promising agent against bacteria that have developed antibiotic resistance [4].

In this work, using the type II CRISPR-Cas system, as well as the λ-Red recombination system [5], a modification of the genome of bacteriophage T7, infecting *Esherichia coli*, was performed to obtain the fusion of the virus capsid protein with EYFP.

2. Materials and methods

The DH5α bacterial strain (*Escherichia coli*) was used in this study.

To implement the modification in the genome of bacteriophage T7, we proposed the following approach. We used the type II CRISPR-Cas system - one of the simplest and most studied CRISPR-Cas systems, the effector complex of which is formed by Cas9 protein and guide RNA, which directs Cas9 to the desired DNA sequence. The λ-Red recombination system was also used, allowing changes in the genome *in vivo* due to homologous recombination. The CRISPR-Cas9 system introduced a double-strand break at the selected insertion site of the EYFP gene during the experiment. After that, homologous recombination and insertion of EYFP gene into phage DNA from the recombination matrix occurs. As a result, the EYFP gene was added to the 3'-end of the capsid gene gp10B of bacteriophage T7 (Figure 1).

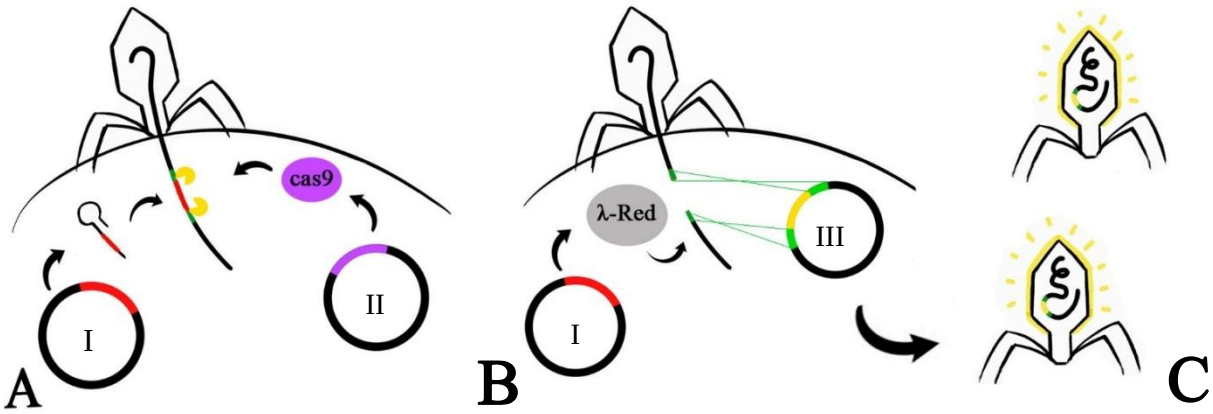

Figure 1. Scheme of bacteriophage T7 genome editing. **A.** During phage infection, the guide RNA expressed from the plasmid I (indicated in red). This RNA contains a spacer which is identical to the bacteriophage T7 genome region. The effector protein Cas9 is expressed from the plasmid II (indicated in purple). Cas9 recognizes and cleaves a region of phage DNA by guide RNA. **B.** λ-Red recombination enzymes are expressed from the plasmid I. Homologous recombination occurs with the plasmid III (indicated in green and yellow) carrying the EYFP gene. **C.** As a result, we obtain modified T7 phage which capsid protein is fused with EYFP.

3. Results and discussion

Plasmids encoding the λ-Red recombination system with guide RNA (pKDsgRNA) and a matrix for recombination (pBadEYFPrecomb) were constructed. To check fluorescence of bacteria containing a plasmid with a fluorescent protein gene it was irradiated at 488 nm (Figure 2).

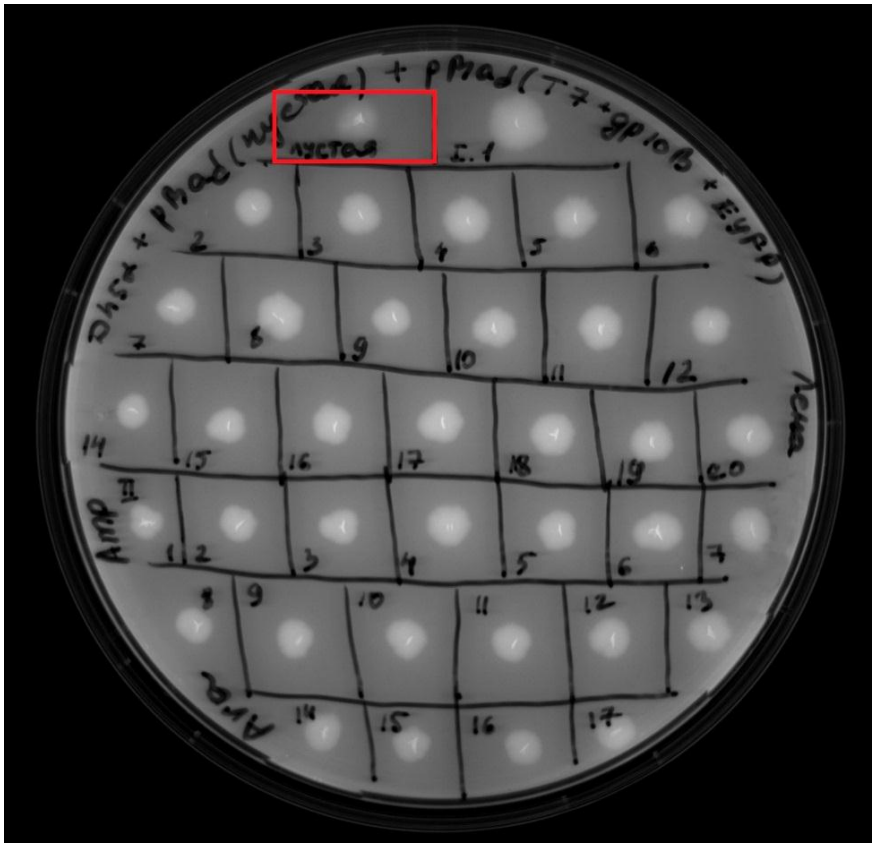

Figure 2. Visual verification for the presence of fluorescence of *E. coli* cells transformed with the plasmid pBADEYFPrecomb, irradiated at 488 nm. A control is *E. coli* transformed with the pBadHisB plasmid that does not contain the fluorescent protein gene were used (highlighted in a rectangle).

Further, *E. coli* bacterial cells, containing the CRISPR-Cas9 system, the λ-Red recombination system and the recombination matrix, were infected with bacteriophage T7. After the phage progeny release verification for the presence of modification was made.

If the modification was successful, the size of the DNA region in the genome of bacteriophage T7, covering the EYFP gene, should be 1137 base pairs (bp).

The PCR results showed that the DNA fragments of randomly selected bacteriophages that have undergone modification have a fragment corresponding to a length of ~ 1137 bp (Figure 3). The sequencing results confirmed the presence of the correct modification in the bacteriophage T7 genome.

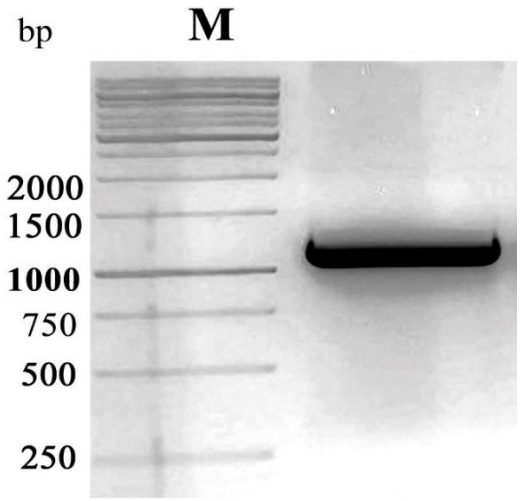

Figure 3. PCR results. pieces of DNA by randomly selected modified bacteriophages, visualized by electrophoresis in 1.5% agarose gel.

The coupling of EYFP and gp10B was verified by Western blot, which allowed to detect the presence of proteins of interest and also to estimate their concentration. The results showed that a protein with a molecular mass corresponding to the molecular mass of the fusion protein present in modified bacteriophages (Figure 4).

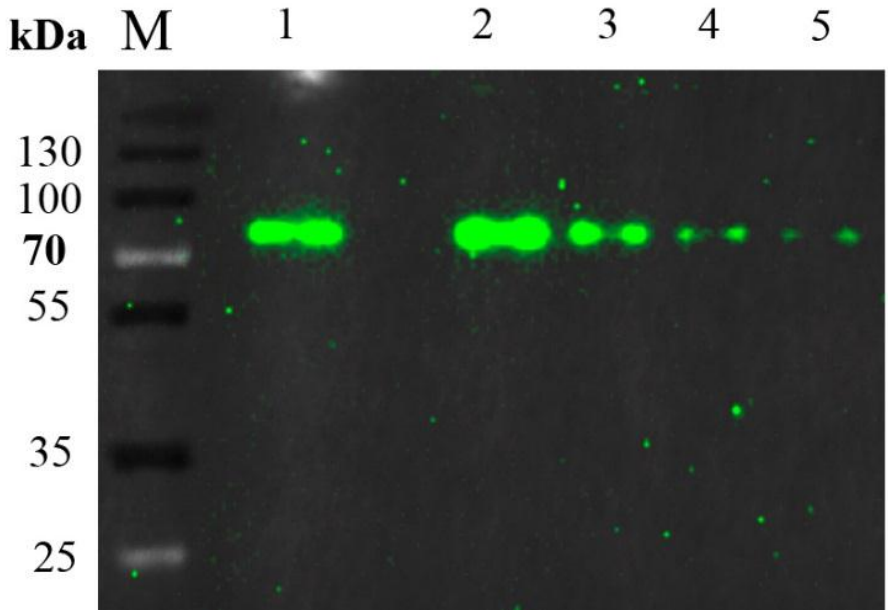

Figure 4. Western blot results. The green bands correspond to the molecular mass of the fusion protein EYFP and gp10B.

4. Conclusions

We were able to insert the fluorescent protein gene into the T7 bacteriophage genome using the method we proposed. This method allows the insertion of various genes into the phage T7 genome directly during infection. The method can be used to introduce various modifications into the genomes

of various bacteriophages, and to select modified viruses. Also, the advantages of this method are simplicity, speed and high efficiency.

References

[1] Nienhaus K and Nienhaus G 2014 Fluorescent proteins for live-cell imaging with super-resolution *Chem. Soc.* 43 1088–1106
[2] Jusuk I Vietz C Raab M Dammeyer T and Tinnefeld P 2015 Super-Resolution Imaging Conditions for enhanced Yellow Fluorescent Protein (eYFP) Demonstrated on DNA Origami Nanorulers *Nature, Scientific Reports* 5
[3] Sharan S K, Thomason L C, Kuznetsov S G and Court D L 2009 Recombineering: a homologous recombination-based method of genetic engineering *Nat. Protoc*. 4 206–223
[4] Pires D P, Cleto S 2016 Genetically Engineered Phages: A Review of Advances over the Last Decade *Microbiol. Mol. Biol*. 80 523-543
[5] Reisch C R and Prather K L J 2015 The no-SCAR (Scarless Cas9 Assisted Recombineering) system for genome editing in *Escherichia coli Nature, Scientific reports* 1-12