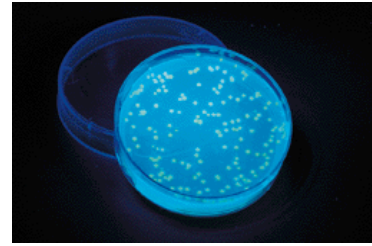


Transformation of pGlo into *Escherichia coli*

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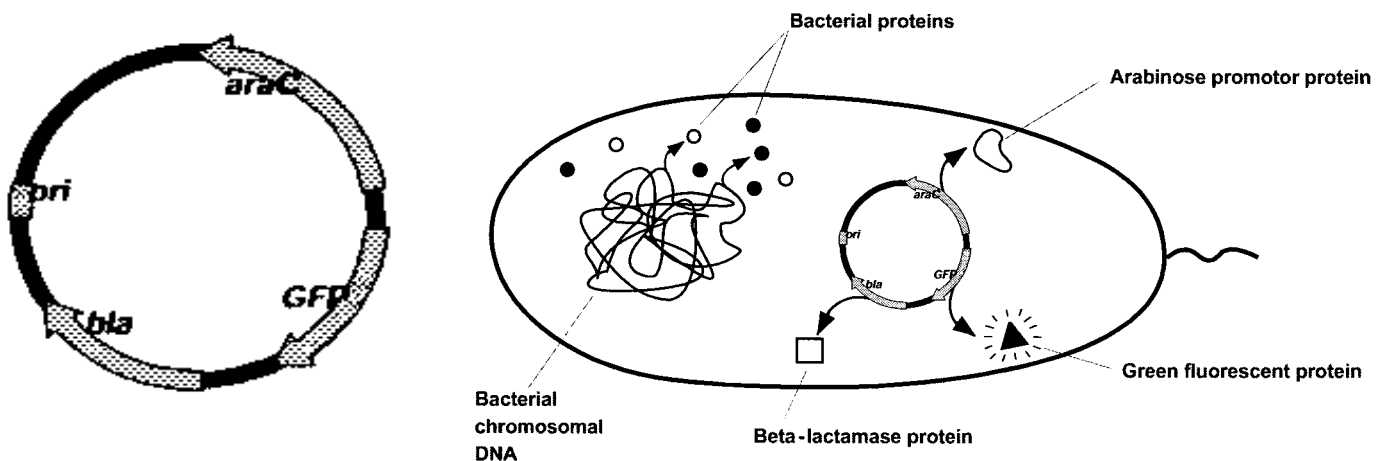
1. Introduction

We did the experiment to learn about the transformation of the *GFP*-gene. It's transformed with the help of pGlo-plasmid into *E. coli*.

2. Theory and Methods

The gene for the GFP was originally isolated from the luminescent jellyfish *Aequorea victoria*. The 3-D structure of the GFP causes it to resonate when it's exposed to ultraviolet radiation and gives off energy in the form of visible light. The *GFP*-gene was inserted into pGlo plasmid. Unique pGlo plasmid encodes the gene for the GFP and a beta lactamase, which provides resistance to the antibiotic ampicillin.

pGlo also incorporates a special gene regulation system which can be used to control expression of the fluorescent protein in transformed cells. The gene can easily be switched on in transformed cells by adding the sugar arabinose to the nutrient medium (positive control of transcription). Selection for cells that have been transformed with pGlo DNA is accomplishing by growth on selective media containing the antibiotic ampicillin. Transformed cells will appear white on plates which do not contain arabinose and fluorescent green under UV light when arabinose is included in the nutrient agar. To do the transformation the cells must be competent. This can be done with CaCl_2 .



The pGlo-plasmid in detail and the experimentation in *E. coli*. The arabinose promotor protein needs arabinose to change its conformation and bind to the DNA. The RNA polymerase is now allowed to begin transcription.

Cells of an “over night” culture are centrifuged and the supernatant poured off. Then they are made competent with CaCl_2 and cooled down on ice for 15 minutes. Then they are centrifuged again and put on ice for another 10 minutes. In the +DNA culture we put in pGlo. In the –DNA culture we put in nothing. They are for the negative control. Both are cooled down on ice for another 10 minutes. We prepared four different Agar plates:

- LB+Amp (*E. coli* with DNA pGlo plasmid)
- LB+Amp+Ara (*E. coli* with DNA pGlo plasmid)
- LB+Amp (*E. coli* without DNA pGlo plasmid)
- LB (*E. coli* without DNA pGlo plasmid)

Both cultures are put into 42°C hot water, incubated and then cooled down on ice. This shock is important for the success of the transformation. The cultures are mixed with liquid LB and incubated for 10 minutes at room temperature. Then they are spread onto the appropriate plates and incubated over night at 37°C.

3. Results

	+DNA with pGlo-plasmid	-DNA without pGlo-plasmid
LB	nd	Strong growth, no fluorescence
LB + Amp	Colonies are existent (some cells are transformed by pGlo-plasmid), no fluorescence	No growth (no resistance)
LB + Amp + Ara	Colonies are existent, they have fluorescence when they are exposed to ultraviolet radiation	nd

nd: experiment not done

4. Discussion

The –DNA culture:

The LB-plate has lawn colonies. The non-transformed *E. coli* were grown under optimised conditions. They don't fluoresce because they don't have the *GFP*-gene.

The LB+Amp plate has no colonies, because without the pGlo-plasmid there is no resistance against ampicillin.

The +DNA culture:

The LB+Amp plate allows only the transformed bacteria to grow, because they received the ampicillin-resistance-gene. That's the reason why there are not so many colonies on the plate.

They don't glow, because the plate has no arabinose.

The LB+Amp+Arabinose plate has the same quantity of colonies like the LB+Amp plate. But they are glowing because of the expression of the *GFP*-gene.

The results are compatible to the expectations.

5. Literature

BBOM 10th Edition: 10.6, 8.5, 20.8, 31.1

Other questions we could ask

We could look how many bacteria are transformed.