WEB REPORT

Introduction

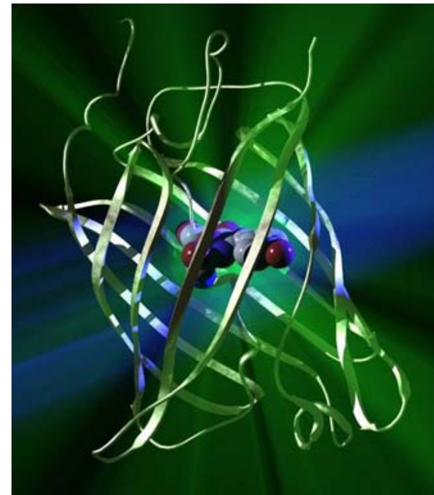

Figure 1. Structure of the Green Fluorescent Protein (GFP).

The green fluorescent protein (GFP) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to UV light. The wild-type GFP (wtGFP) from *A. victoria* has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm which is in the lower green portion of the visible spectrum.

GFP has rapidly become a standard tool for investigating a variety of cellular activities, and has served as a model system for understanding spectral tuning in chromophoric proteins**.** As a noninvasive fluorescent marker in living cells, it allows for a a wide range of applications where it may function as a cell lineage tracer, reporter of gene expression or as a measure of protein-protein interactions. It is also worth mentioning that the availability of GFP and its derivatives has thoroughly redefined fluorescence microscopy and the way it is used in cell biology and other biological disciplines.

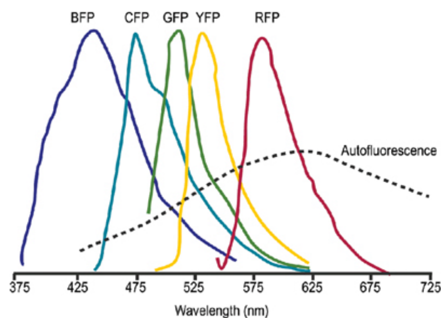

Figure 2. Emission spectra of several different fluorescent proteins including GFP, BFP and dsRed.

Random and directed point mutations of the residues in the active site of the fluorophore result in various changes in fluorescent behavior. The **Blue Fluorescent Protein (BFP)** is one of these GFP mutants. Another commonly used distant homolog of GFP is found in coral reef: **dsRed**, which is different from GFP not only in that it fluoresces in the red spectrum, but also in that it requires oligomerization into tetramers in order to function.

The specific aim of our project was to make ***E.coli*** **Top 10** strain bacteria that produce fluorescent proteins - GFP, BFP and dsRed. We did this in order to learn more about fluorescence and fluorescent proteins, especially as it relates to their usage in the biomedical sciences, and to learn some of the fundamental methods of experimental molecular biology, such as bacterial transformation, DNA agarose electrophoresis and SDS-PAGE protein electrophoresis.

Materials and methods

We used isolated plasmid DNA of three separate plasmids encoding for GFP (carries kanamycin resistance), dsRed (kanamycin resistance) and BFP (ampicilin resistance) proteins to transfect *E. coli* strain TOP10, which was already resistant to the antibiotic streptomycin. We grew bacteria in agar media containing streptomycin to exclude the presence of any other bacteria. Using the $CaCl_2$ heat shock method, we made bacteria competent to receive new plasmids which we added. LB agar plates with kanamycin and ampicillin (in addition to streptomycin) were used to select for the bacterial colonies that took up our plasmids.

Using a commercially available plasmid isolation kit, we lysed the bacteria and precipitated the genomic DNA, in order to obtain pure plasmid DNA from our transformed bacteria. We ran the isolated plasmid DNA on a **DNA agarose gel** (DNA electrophoresis). Negatively charged DNA molecules traveled towards the positive electrode and separated according to size due to different velocities of the fragments. After staining the DNA with ethidium bromide, we compared our transformed samples to the non-transformed bacterial control and pure initial plasmid DNA in order to prove that the plasmids were indeed taken up by the bacteria.

Similarly, we used SDS detergent combined with heating to lyse the bacteria and obtain a whole cell denatured protein sample. In this technique, called **SDS-PAGE** (SDS-Poly-Acrylamide Gel Electrophoresis) proteins are separated analogously to DNA electrophoresis. We again compared the transformed and the non-transformed bacterial protein samples to detect the expression of the fluorescent proteins of interest.

Finally, bacteria were grown and observed under UV light to visually confirm the presence of fluorescent proteins.

Results and discussion

Bacterial transformation was successfully preformed judging by the growth of bacterial colonies on selective agar plates. Bacteria transformed with the GFP, dsRed and BFP plasmids were plated on plates containing either streptomycin and kanamycin or streptomycin and ampicillin, in the case of the BFP plasmid. In all three cases we observed bacterial colonies, indicating that our bacteria were successfully transformed. The control consisted of bacteria to which no DNA

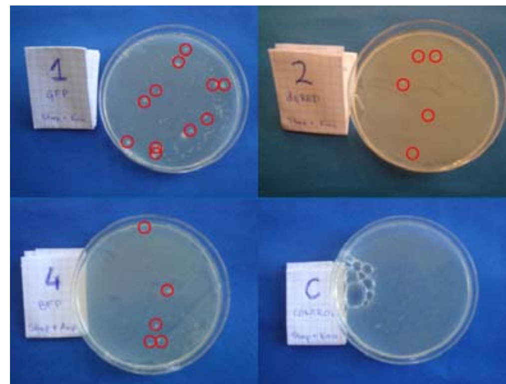

Figure 3. Transformed bacterial colonies on selective agar plates.

was added, and this sample was plated onto agar containing streptomycin and kanamycin. As expected, there were no bacterial colonies observed, as there was no kanamycin resistance gene present.

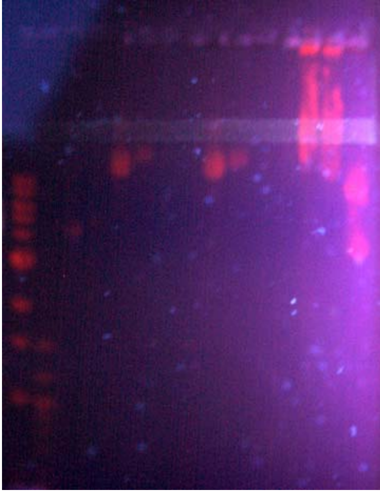

Figure 4. DNA electrophoresis suggestive of successfull transformation.

DNA electrophoresis strongly suggested presence of fluorescent protein plasmids in transformed bacteria compared to the untransformed controls. Under the UV light, we could see DNA fragments stained with ethidium bromide, and we noticed DNA bands in transformed bacteria that did not appear in control samples. The size of the fragments seemed to be between 5000 – 6000 bp, which is consistent with the expected plasmid sizes.

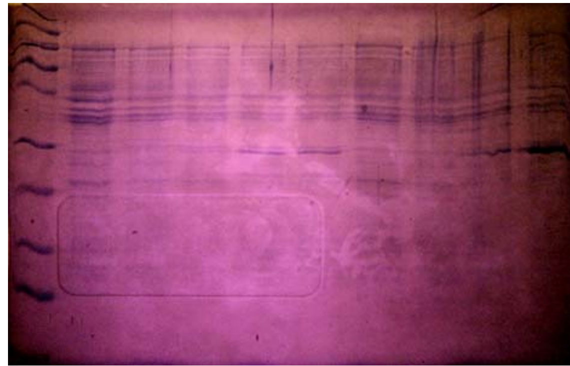

Figure 5. SDS-PAGE gel showing the presence of flourescent proteins in transformed cells

Similarly, SDS-PAGE protein gels suggested that transformed cells were expressing fluorescent proteins. After putting the gel in a dye, we saw protein bands in a position in which there were none in the control sample. This difference was particularly evident in the case of the dsRed.

Most importantly, we managed to show, using both visible light and UV illumination that our bacteria were successfully transformed and expressing their respective fluorescent proteins.

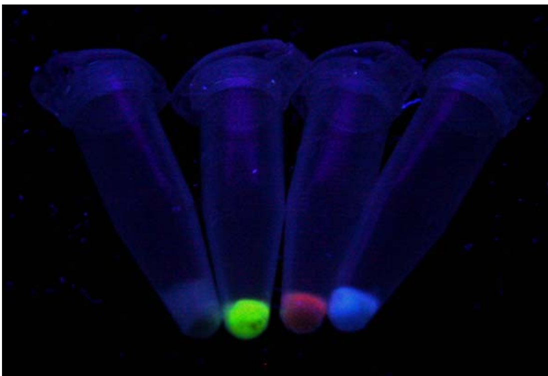

Figure 6. Bacteria expressing flourescent proteins observed under UV light.

Resources and references

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