

Lab - Transformation of *E. coli* Bacteria with the pGLO plasmid

I. INTRODUCTION:

Green Fluorescent Protein is used extensively in biology research and work. It is one among several fluorescent proteins that allow DNA to be sequenced automatically by lasers. It is used quite frequently to label cells for studying how organisms develop as embryos and it is a key part of ongoing stem cell research. The protein itself does not glow but it can be made to fluoresce with a green light when exposed to ultraviolet (UV) light.

Bacteria grow well with the food source Luria Broth (LB). You can grow them either in liquid LB or on the surface of a petri dish with a mixture of LB and agar that solidifies into a thick gel. Antibiotics are sometimes also added to the agar to help control the growth of bacteria that are unwanted at the end of the experiment. Most antibiotics either kill the bacteria or keep them from reproducing. We will be using an antibiotic called ampicillin (amp).

Lab groups will prepare bacterial cells using calcium chloride to help the small plasmid circles of DNA get through the membrane of the cell. Then, you will get a plasmid into half of these cells by weakening the membrane further with heat shocks (cold, hot cold). A small percentage of the millions of bacteria in your sample should successfully take in plasmids.

Each plasmid contains a gene coding for green fluorescent protein (GFP), a gene coding for an enzyme (beta-lactamase) that breaks down the antibiotic ampicillin and a region that is like a lock or on/off switch for the green fluorescent protein gene. The on/off switch or lock is called an "inducible operon" that is usually in the "off" or locked position. The sugar arabinose (ara) is the key that opens the lock and turns on the GFP region of the plasmid.

You will put your transformation mixes (and your control sample) onto petri dishes to compare what happens with ones that have the plasmid to ones that have not received the plasmid. After the bacteria have had a chance to grow we will look at them under normal and ultraviolet light. It will take at least a day to show results.



Pre-lab Questions

1. What is a plasmid?
2. What is a gene?
3. What is luria broth (LB)?

Wards Transformation Of E Coli Lab Answers

M Tight



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Biology ,1999 *Biotechnology* Holt Rinehart & Winston,Holt, Rinehart and Winston Staff,1998 Annual Report,
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Lectures Held at the 8th DECHEMA Annual Meeting of Biotechnologists, May 28-30, 1990, Frankfurt Am

Main, F.R.G.: Biochemical methods for water analysis (GDCh-Workshop), Presentation of cell culture technology laboratories, Microbial principles in bioprocesses applied genetics, Microbial material deterioration, Environmental biotechnology Dieter Behrens, Peter Krämer, 1990 **Cumulated Index Medicus**, 1993 **ICRDB Cancergram**, 1986 **Learning to Save the World** Betsey Behr Brada, 2023-02-15 Learning to Save the World provides an innovative analysis of how individuals inhabit, refuse and reconfigure the contours of global health. In 2001, Botswana's government, faced with one of the highest HIV prevalence rates in the world, committed itself to sub-Saharan Africa's first free public HIV treatment program. US-based private foundations and medical schools offered support to demonstrate the feasibility of public HIV treatment in Africa. Given US interest and investment in global health, this support created opportunities for US physicians and medical trainees to interact with local practitioners, treat patients and shape health policy in Botswana. Although global health has emerged as a powerful call to planetary moral action, the nature of this exhortation remains unclear. Is global health a new movement for social justice or is it neocolonial, creating new dependencies under the banner of humanitarianism? Betsey Behr Brada shows that global health is a frontier, an imaginative framework that organizes the space, time and ethics of encounter. Learning to Save the World reveals how individuals and collectivities engaged in global health, visiting experts as well as local clinicians and patients, come to regard themselves and others in terms of this framework.

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