BioPharmaceutical Technology Center
I N S T I T U T E

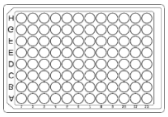
Measuring β-Galactosidase Expression

Field Trip Background

Background Information

What do cow milk, spider silk, and human insulin have in common? They all contain recombinant **proteins** that can be made inside of bacterial cells! Since 1977, bacteria have been used as cell factories for making proteins used in scientific research, agriculture, therapeutics and more. The ability for bacteria to make these proteins will often depend on the environment that they are in, such as a liquid **media** containing at least a **carbon source** and salts, necessary for the bacteria to grow.

In this field trip, we will be using a specific strain of ***Escherichia coli*** bacteria to produce a protein called **beta-galactosidase (β-gal)**. We will experiment with different carbon sources to see how they affect the growth of the bacteria and the protein expression of β-gal. We will use β-gal to cleave a **substrate** that creates a color change, which we will be able to measure. You will use microplates, multichannel pipettes, and a spectrophotometer, ending the lab with data analysis to better understand the impact of different carbon sources on your protein expression system.



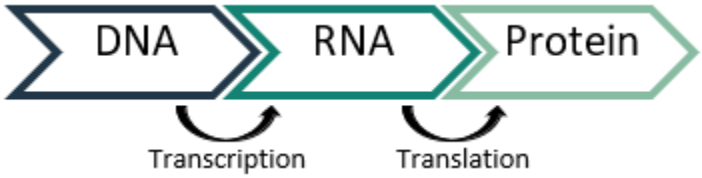
A 96-well microplate

Escherichia coli K-12 Strain W3110 Bacteria

Escherichia coli K-12 or ***E. coli K-12*** is a type of bacteria that is non-pathogenic and does not survive well in the environment. It is one of the most frequently used strains of *E. coli* in laboratories and every letter of its genetic sequence is known. We will be using strain W3110, however, any strain that is able to use lactose as a carbon source (Lac^+) could be substituted.

Genetic Information Processing: Protein Expression

E. coli contains one circular chromosome of DNA. This DNA is transcribed into RNA, and then the RNA is translated into the proteins for the bacteria, which are made up of amino acids. In this lab, the gene we are most interested in expressing and tracking is β-gal, which is encoded in the Lac operon. We want to know which conditions cause our bacteria to express β-gal better or worse.



Media

E. coli bacteria grow optimally at 37°C, double every ~20 minutes, and are inexpensive to use. Growth of W3110 is also **prototophic** so it is able to synthesize all the nutrients needed for growth in minimal **media**. Media is liquid or gel that is made for the bacteria to grow in or on. A **minimal media** contains only the essentials, like a carbon source and salt. The bacteria then need to make DNA nucleotides, amino acids, and other metabolites on its own. A **rich** or **complex media** provides "pre-made" nutrients so the bacteria use less energy making what they need.

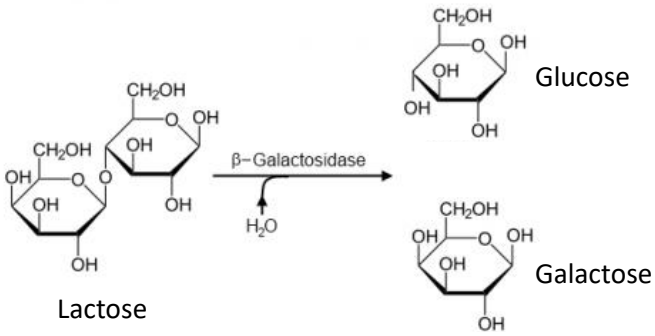
Media (continued)

We will use liquid **[Lysogeny] Luria Broth (LB)**, which is a rich medium containing complex additives (yeast extract and hydrolyzed protein to provide amino acids, vitamins, minerals, etc.) and salt (sodium ions). However, this does not provide a carbon source, like sugar, to help the bacteria generate ATP for energy. In this lab, we will explore the effects of adding different sugars to the LB on bacteria cell growth (measured at 600nm for cell density) and β-gal protein expression (measured at 405nm for yellow).

Beta-Galactosidase and the *Lac* Operon

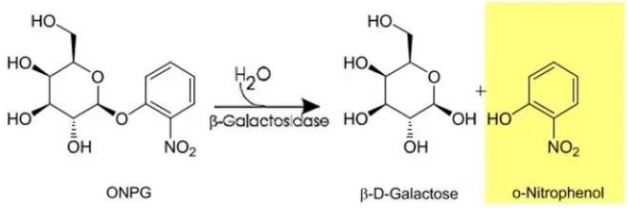
Beta-galactosidase (β-gal) is an **enzyme** that is encoded by the *LacZ* gene in the *lac* operon. The *lac* operon is a unit of linked genes (*LacZ, LacY, LacA*) and noncoding regions (CAP site, promotor, operator) involved in lactose metabolism. β-gal breaks down lactose to simpler sugars: galactose and glucose.

Image from Bio-physics-wiki



β-gal can also convert lactose to allolactose, which is the natural inducer for the *lac* operon. The *lac* repressor normally blocks transcription of the *lac* operon because bacteria will only utilize lactose if glucose is unavailable.

Image from https://microbiologie-clinique.com/ONPG-test-en.html



After allowing the cells to grow and express our protein, we will lyse them and provide a substrate: O-Nitrophenyl-β-D-galactopyranoside (ONPG). ONPG is structurally similar to lactose, and therefore, β-gal can break down or cleave ONPG. ONPG is colorless, but one of the products after cleavage, O-nitrophenol, is yellow, which allows us to measure the product with a spectrophotometer. We will equate the color development to the expression and activity of the β-gal protein. Depending on the sugars we provide our bacteria, we may or may not get expression of β-gal due to the control of the *lac* operon.

If you have any questions or would like more information before coming your field trip, please contact us at btci@btci.org. Alternatively, bring your questions along and we can discuss them during the lab.
We look forward to seeing your group on your scheduled field trip day.
Thank you for your interest in the BTC Institute's Biotechnology Field Trips program!

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