GENE EXPRESSION AND EXPERIMENTAL READOUTS ON THE TURNER BIOSYSTEMS FAMILY OF INSTRUMENTS

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INTRODUCTION

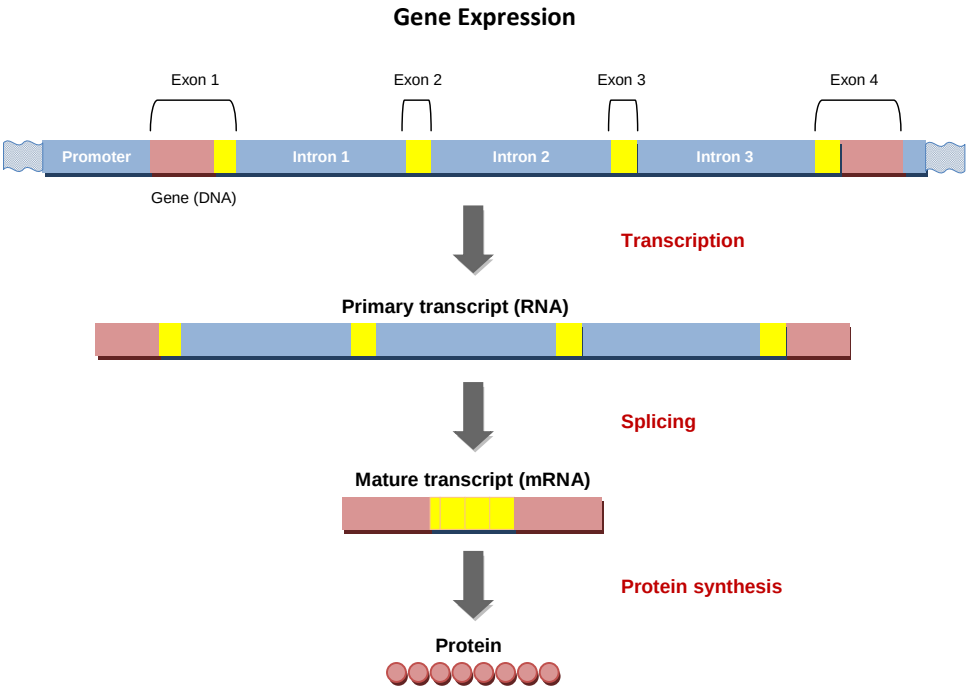
Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. Several steps in the gene expression process may be modulated, including the transcription and translation steps as well as the post-translational modification of a protein.

Gene expression occurs in two major stages with the first being transcription. In this process, the gene is copied to produce an RNA molecule (a primary transcript) with essentially the same sequence as the gene. Most human genes are divided into exons and introns where only the exons carry information for protein synthesis. Most primary transcripts are processed by splicing out the intron sequences generating messenger RNA or mRNA that only contain exons.

The second stage is translation or protein synthesis where information is translated from the genetic level to the protein level. A sequence of three nucleotides encode for one of a possible twenty amino acids with a chain of amino acids folding up to generate the final three-dimensional structure giving the protein its functionality.

Differential gene expression is achieved by regulating transcription and translation. All genes are surrounded by DNA sequences that control their expression with transcription factors which are proteins binding to these sequences switching them on or off. Gene expression is controlled by the availability and activity of different transcription factors and any disruption in this process can lead to disease.

By manipulating this well know cellular process, scientists have been able to alter the gene expression pathway to generate a means of measuring the many cell signaling pathways that have been well studied. This technology plays an important role in the understanding of disease as well as a means of developing drugs to minimize or alleviate the effects of disease.

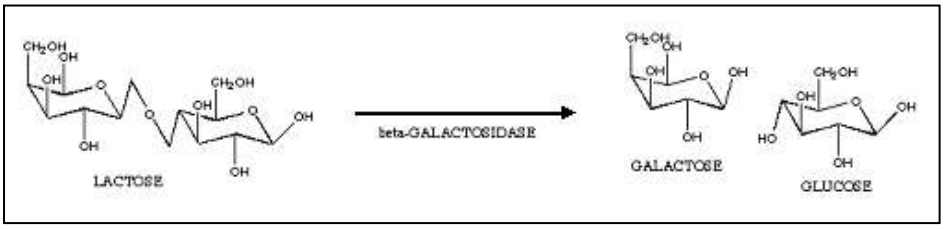


Simplified overview of gene structure and expression. A protein-coding gene is defined by the extent of the primary transcript. The gene is first transcribed to yield a primary transcript, which is processed to remove the introns. The mature transcript (messenger RNA, mRNA) is then translated into a sequence of amino acids, which defines the protein.

COMMON ASSAY METHODS FOR MEASURING GENE EXPRESSION

β-galactosidase (β-Gal):

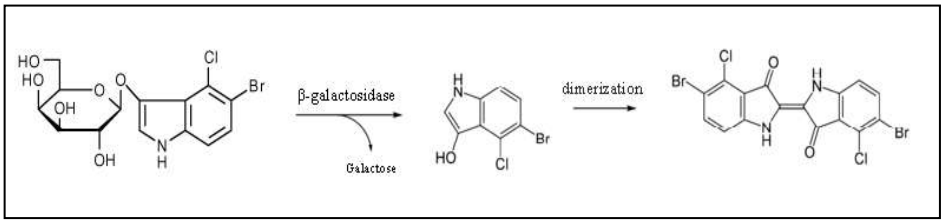
Early experiments in gene expression were conducted using a combination of genetic and biochemical experiments with one such experiment utilizing the *lac* operon of E.coli. Using the *lac* operon of E. coli which includes three genes, one of which is the lacZ gene that encodes for β-galactosidase when transcribed, is a commonly used method for measuring gene expression. β-galactosidase is used by E.coli as a means of metabolism by cleaving the disaccharide lactose into the monosaccarides galactose and glucose. E. coli β-galactosidase has a high turnover rate, hydrolyzing D-galactose from various β-galactosides, which allows for very low level detection in common cell types. β-Galactosidase substrates can also be used in conjunction with galactosidase-conjugated secondary detection agents in enzyme linked immunosorbent assays (ELISA) formats and in immunohistochemical techniques.



β-galactosidase is an enzyme that is both stable and resistant to proteolytic degradation and is commonly used as a reliable marker for measuring gene expression. When expressed in transfected cells, β-galactosidase cleaves a substrate that can be measured via absorbance, luminescence or fluorescence depending on selection of assay format.

Measuring β-galactosidase via the Absorbance method:

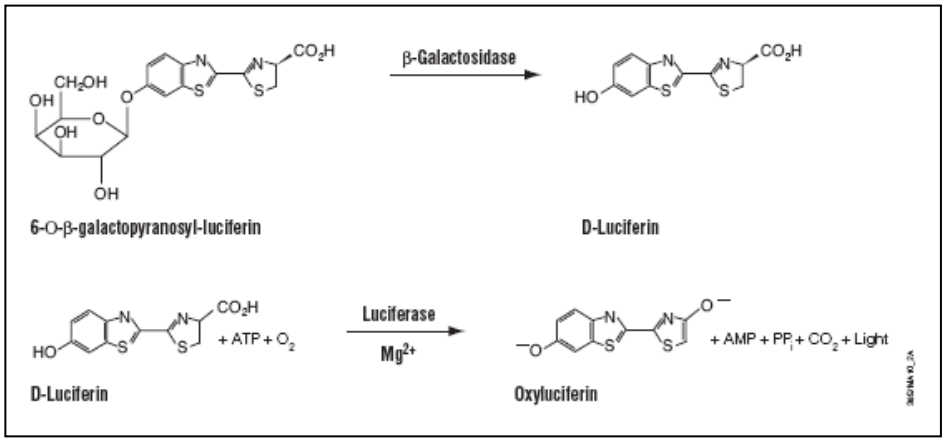
The colorless substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) is hydrolyzed by β-galactosidase into an insoluble highly blue product. This method provides a simple and robust colorimetric assay that can be measured via absorbance at 595nm.



Although chromogenic assays of ß-galactosidase activity (i.e. X-Gal) are useful, recent applications using chemiluminescent 1,2 Dioxetane substrates, which emit visible light upon enzyme catalysis, provide rapid results with very low background and high intensity signal are available for use. The Chemiluminescent lacZ ß-Galactosidase Detection Kit available from Marker Gene Technologies Inc. allows for the quantitative measurement of beta-galactosidase enzyme activity in mammalian, yeast, and bacterial cells.

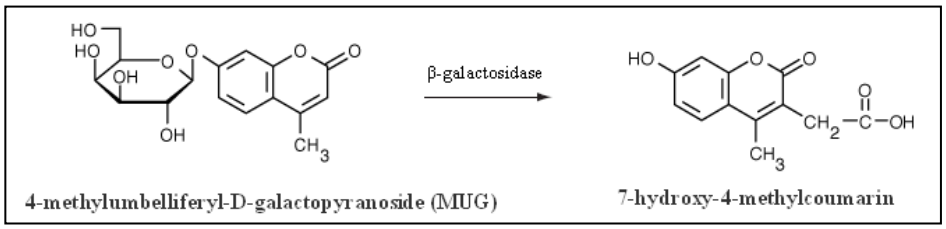
Measuring β-galactosidase via the Luminescence method using the Beta-Glo® Assay System from Promega:

The amount of β-galactosidase present in a sample correlates with the amount of luminescence generated by that sample. In the reaction, 6-O-β-galactopyranosyl-luciferin is cleaved by β-galactosidase to yield luciferin, which is then catalyzed by luciferase in the presence of cofactors to yield light. The Beta-Glo® Assay System from Promega offers a system that is both highly sensitive and produces a stable signal over several hours.



Measuring β-galactosidase via the Fluorescence method:

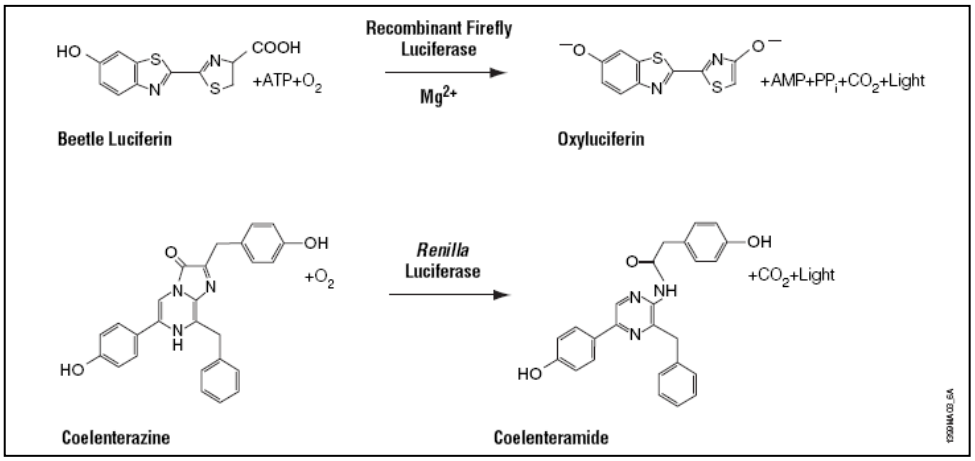
In an article published by Vidal-Aroca et al, they describe a fast, fluorescence based method that makes β-galactosidase determination as straightforward and simple as any other method. With the use of 4-methylumbelliferyl β-D-galactopyranoside (MUG) as a substrate, β–galactosidase is capable of cleaving the substrate into a product that is fluorescently active. Once cleaved, the reduced 7-hydroxy-4-methylcoumarin molecule is free to fluoresce when excited at 360nm emitting light at 460nm.



Measuring Gene Expression via Firefly Luciferase:
Firefly luciferase is one of the most commonly used bioluminescent reporter systems. A monomeric enzyme of 61kDa catalyzes a two-step oxidation reaction to yield light that is capable of being measured via a Luminometer. The first step of the process is the activation of the luciferylcarboxylate by ATP to yield a reactive mixed anhydride. In the second step, this activated intermediate reacts with oxygen to create a transient dioxetane that breaks down to the oxidized products, oxyluciferin and CO_2. Upon mixing with substrates, firefly luciferase produces an initial burst of light typically in the 550–570nm range that decays over about 15 seconds to a low level of sustained luminescence.

Measuring Gene Expression via *Renilla* Luciferase

Renilla luciferase is a 36kDa monomeric enzyme from the sea pansy *Renilla reniformis* that catalyzes the oxidation of coelenterazine to yield both coelenteramide and blue light with a spectral peak of 480nm.



Utilizing both Firefly and *Renilla* Luciferase to measure Gene Expression using the Dual-Luciferase® Reporter Assay System from Promega.

The Dual-Luciferase® Reporter (DLR) Assay System contains two different luciferase reporter enzymes that are expressed simultaneously in each cell. Typically, the experimental reporter is correlated with the effect of specific experimental conditions, while the activity of the co-transfected "control" reporter gene provides an internal control, which serves as the baseline response. Normalizing the experimental reporter gene to the activity of an internal control minimizes the variability caused by differences in cell viability and transfection efficiency. Thus, dual reporter assays allow more reliable interpretation of the experimental data by reducing extraneous influences. The experimental and control luciferase enzymes used in the Dual-Luciferase® Reporter (DLR) Assay have distinct evolutionary origins. The firefly luciferase and the *Renilla* (sea pansy) luciferase can discriminate between their respective bioluminescent substrates and do not cross-activate.

The firefly and *Renilla* substrates have been developed specifically to maximize the sensitivity of the assay reagent. This system is widely used in the life science research because of the superior light generation and high signal to noise ratio.

CONCLUSION

We described three different methods of measuring gene expression via fluorescence, luminescence and absorbance that are capable of being measured on the Turner BioSystems family of instruments. Depending of the throughput of your needs, we offer both single tube and microplate instruments that are capable of measuring gene expression assays in all three detection modes. Our instruments offer superior sensitivity with an ease of use that is unmatched allowing Scientists more time to perform research rather than being trained on the use of an instrument.

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The Modulus™ and Modulus™ II Multimode Microplate Readers are skillfully designed for today's life science laboratory. In addition to having performance on par with single-mode instruments, the Modulus™ and Modulus™ II Microplate instruments blend user-friendly operation with easy data handling and flexible purchasing options. The result of this design is an instrument with superior performance that is easy to use, is affordable, and can be customized to your laboratory's specific needs.

The Modulus™ II Multimode Microplate Reader combines the superior performance expected from single-mode instruments with the functionality of multiple modes. To achieve industry-leading performance, the Modulus™ II Microplate is designed with optical channels dedicated to each individual technology. Unlike other multimode systems, readings taken with the Modulus™ II Microplate are not degraded by indirect fiber-optic transmission or crowded optical channels. Dedicated optical channeling ensures that the Modulus™ II Microplate provides sensitivity and dynamic range on par with the highest performing single-mode instruments.

The Veritas™ Microplate Luminometer provides superior sensitivity and precision for all luminescent assays. Low-noise circuitry and an advanced photon-counting photomultiplier tube (PMT) provide unmatched signal-to-noise ratios. With a detection limit of 3 x 10^{-21} moles of luciferase, the Veritas™ is the most sensitive microplate luminometer available.

With its innovative detector design, the Veritas™ Microplate Luminometer features a dynamic range of greater than nine logs. This measurement technology spans the full range of virtually all chemiluminescent and bioluminescent assays, eliminating the need to dilute samples or manage detector-driven gain changes. To achieve this extra-large reading range, the Veritas™ is capable of simultaneously measuring samples of varying brightness. The PMT automatically adjusts for the optimum reading of bright or dim samples. This means that the Veritas™ is capable of achieving 2 – 3 logs more reading range than competing luminometers.

The 20/20n Luminometer provides exceptional sensitivity for all luminescent assays. Proprietary circuitry and an advanced photon-counting photomultiplier tube (PMT) produce unmatched signal-to-noise ratios. The 20/20n has a detection limit of 1 x 10-21 moles of luciferase, making it one of the most sensitive tube luminometers available. With greater than 8 logs of linear dynamic range, the 20/20n Luminometer can measure both dim and bright samples without dilution. The extended dynamic range of the 20/20n spans the full range of virtually all chemiluminescent and bioluminescent assays, eliminating the need to dilute samples or manage detector-driven gain changes.

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