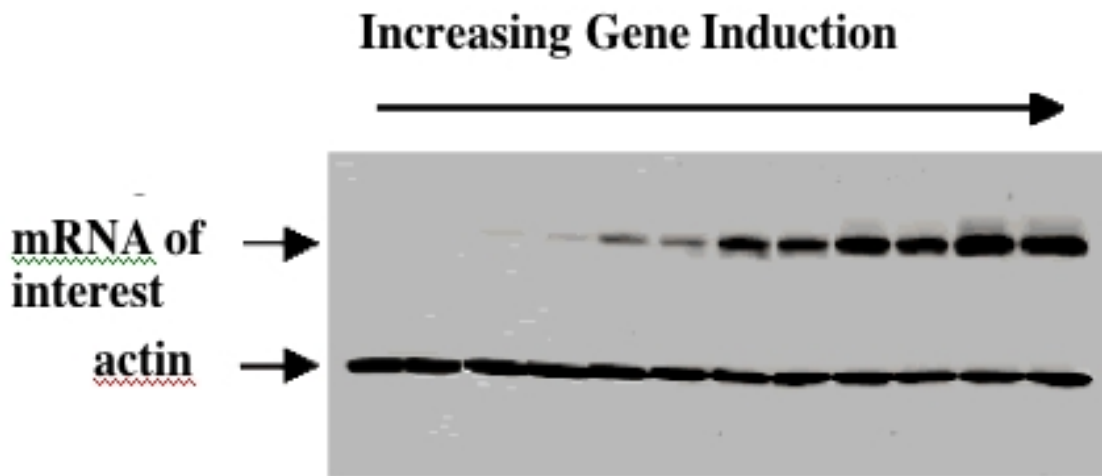


## Introduction

We began this experimental module considering the limited usefulness of sequence information. Life is evidently more complicated than base-pair chemistry since even a perfect cataloging of an organism's genetic information does not allow us to build a cell from scratch. Furthermore, two cells in a multicellular organism can have identical genomes but different physiologies. Even at an organismal level, we see that identical twins do not exhibit identical traits. Moreover, minimal differences in DNA code can separate species. With human DNA sequence less than 1.25% different from that of chimpanzees, it appears too simple to believe, "...our fate is in our genes" (as Nobelist Jim Watson told TIME magazine in 1989, "We used to believe our destiny was in the stars; now we know in large measure our fate is in our genes.")

Rather than genetic content it may be changing expression patterns of a genome that explain cellular differentiation and development. A single cell develops into a differentiated multicellular organism by varying gene expression in groups of cells as they divide. A liver cell must express the parts of the genome related to liver function, while a skin cell uses the parts of the code for making skin related proteins. Scientists are trying to describe other "-omes," such as the "transcriptome" (the complete RNA content of a cell or organism) and the "proteome" (its total protein content), to complement the cataloging of an organism's total DNA content. Fortunately, techniques for detecting the RNA and protein output of a cell abound. Older methods have been used productively for decades and newer techniques offer increased sensitivity and higher throughput methods. Because of their widespread use, several fundamental techniques in gene expression analysis will be considered in detail.

One classic technique for monitoring gene expression is Northern analysis. In this approach, an RNA sample is electrophoresed through a polyacrylamide matrix and then transferred ("blotted") from the gel to a solid support, usually made of nitrocellulose or nylon. The blot is then probed with radiolabelled DNA, or less often RNA, and then exposed to X-ray film. Hybridization of the probe to the blot is detected as a darkened area of the film and the signal gives information about the size and concentration of that RNA in the original sample. Valid Northern analysis data includes re-probing the blot with a "loading control" to demonstrate that each sample was equally loaded on the agarose gel and evenly transferred to the blot. Typical loading controls are 18S rRNA and actin mRNA since these are abundant transcripts in most cell types and are seldom affected by experimental conditions.



### Sample Northern gel

An alternative to Northern analysis is q-PCR (quantitative-Polymerase Chain Reaction, sometimes also called RT-PCR which can stand for either Reverse Transcriptase-PCR or real time-PCR ... RT RT-PCR??). You gained experience with “end point PCR” in the DNA Engineering module when you used the final product of that amplification in cloning. With q-PCR, quantitative information is gleaned from the early stages of the PCR cycling protocol. A specialized thermal cycler is used as well as a fluorescent dye to monitor the amount of double stranded DNA in each reaction at each step of the PCR protocol. RNA is isolated from cells of interest (as you did last time) and converted to DNA using an enzyme called reverse transcriptase (as you will do today). This DNA serves as the template in the q-PCR reactions. After a limited number of PCR cycles, the amount of PCR product can be sensitively detected by its fluorescence and quantitatively reflects the amount of transcript in the original sample.

Gene expression can also be assessed by measuring the protein product of a gene. Convenient enzymatic assays are available for some proteins, like beta-galactosidase, and this is how you will assess protein activity. You’re also familiar with assessment of fluorescent proteins which can be measured using flow cytometry, a technique that is both quick and quantitative. What about the proteins with no convenient enzymatic activity to test? What about the labs that can’t afford FACS machines? The most widely applicable technique for measuring proteins is Western analysis, which is similar to Northern analysis in that the cell’s contents (its proteins in this case) are separated by electrophoresis and subsequently moved to a filter. The filter is then probed with an antibody that recognizes the protein of interest and a secondary antibody is used to detect the first. Antibodies to many proteins are commercially available, and other companies are in the business of making custom antibodies.

By the way, where did all these directional techniques, like “Northern” and “Western” come from? The founding member of the geographical collection is the “Southern” in which DNA, most often digested genomic DNA, gets separated through a gel and then blotted to a filter. The blot is then probed with radiolabelled DNA of choice. Hybridization of the probe to the blot reveals the presence, copy number and size of the sequence of interest. This technique was first described in 1975 by Ed Southern. The Western and Northern are humorous derivatives of Professor Southern’s name, and while there is no “Eastern” technique, permutations such as the “Far Western” and the “NorthWestern” now exist.