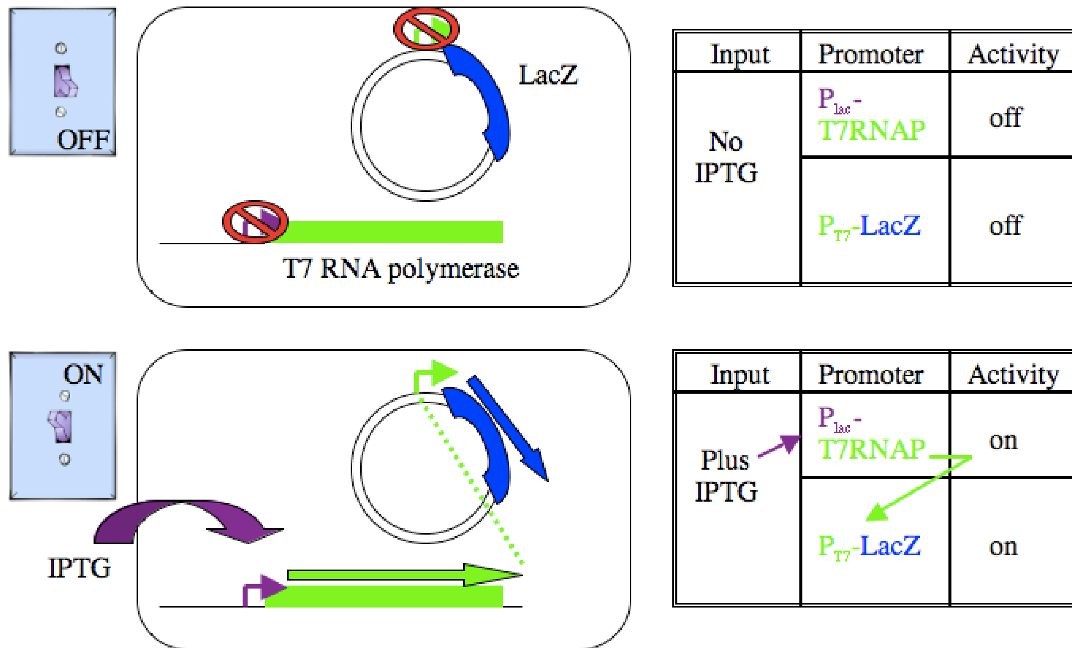
Introduction

Cells can be considered miniature machines. They use energy to make things, record events, move around, and modify their environment. They differ from standard, mechanical machines in ways that will be further explored in the 20.109:Systems engineering experimental module, but for today consider the remarkable and useful fact that you're working with a protein-making factory. Under the right conditions, the majority of each cell's protein output can be the protein you're most interested in. Though each cell is only microns in size, it's easy to grow lots of them to yield microgram quantities of protein. Most astonishing of all is the ease with which experimental conditions can be changed to affect the protein output in rational and defined ways. The protein of interest can be turned on or off, modified or not. You did this when you added IPTG with or without an unnatural amino acid to your growth media. Today you will assess the consequences of these manipulations.

The cells you are using carry a plasmid that expresses beta-galactosidase when IPTG is added to the growth media. This expression method was originally described in 1986 (Studier and Moffatt *J. Mol. Biol.* Volume 189 (1): 113-130 pubmed) and then commercialized by a company called Novagen. Two specialized components are used. First is an expression plasmid in which the gene of interest, in this case the LacZ gene that encodes beta-galactosidase, is downstream of a special bacterial promoter. The promoter is not normally active since it's recognized and used only by an RNA polymerase from the T7 bacteriophage. The gene for the T7 RNA polymerase has been added to the genome of the cells you're using. Ironically enough, it's the promoter from the LacZ gene that's regulating the T7 RNA polymerase expression. Without inducer, the LacZ promoter is off, so there is no T7 RNA polymerase made and so no LacZ is expressed from the plasmid. With IPTG, the lacZ promoter is de-repressed, leading to expression of the T7 RNA polymerase, which transcribes lots of LacZ, and voila…lots of beta-galactosidase. High levels of expression can be expected since the T7 RNA polymerase promoter is strong and each cell maintains many copies of the expression plasmid.



Methods for protein overexpression are of more than academic interest since a number of human diseases arise from defects in protein production. Type I diabetes, for example, occurs when pancreatic cells are unable to make insulin. Diabetic patients must regularly treat themselves with shots of insulin to provide the missing protein. Until the 1970s, insulin was isolated from slaughtered pigs (the pig and human versions of this protein differ by only one amino acid). This method for insulin production was laborious, inefficient, and unreliable. One of the earliest examples of DNA engineering came in the 1970s when the human insulin gene was inserted into a bacterial expression vector, allowing the human gene to be expressed by the bacteria. Now most diabetics use insulin purified from such over-producing bacteria.

Today you will assess the over-production and modification of beta-galactosidase in the three samples you induced last time (no IPTG, plus IPTG, plus IPTG and plus some unnatural amino acid). First, you will perform a beta-galactosidase assay to compare the enzyme activity in these cells. This assay will be familiar to you from last time, though you'll see there are some modifications that must be made to the protocol and calculations when whole cells are used instead of purified proteins. Second, you will examine the protein expression profile in each of your samples using an acrylamide gel to separate proteins in a way similar to the way agarose gels separate DNA. Finally, you harvest some cells to store for next time when you will try to purify the beta-galactosidase enzyme.