

## Module 5. General Background for Protein Expression in *E. coli*

An operon is a group of linked genes that are transcribed together, often producing a single message which is then translated into several proteins. The operon structure and the “polycistronic” message allows prokaryotes to coordinately regulate genes that share a common function. In *E. Coli*, three genes that code for enzymes involved in lactose metabolism, beta-galactosidase (*lacZ*), permease (*lacY*), and transacetylase (*lacA*), are produced by the *lac* operon. These enzymes are normally present at very low concentrations in cells; but when lactose is the sole carbon source, they are induced. After the available lactose is metabolized, the level of RNA transcript and protein returns to a low level. The *lac* operon is under the control of the lactose repressor protein, encoded by a “cis-acting” regulatory gene, (*lacI*). The *lac* repressor asserts negative regulation by keeping the *lac* promoter inactive in the absence of an inducer. The repressor functions as a tetramer and binds to a portion of the promoter called the *lac* operator. Allolactose acts as the inducer in nature. When allolactose is present it binds to the repressor and prevents the protein from binding to the operator, thus opening up the *lac* promoter to RNA polymerase.

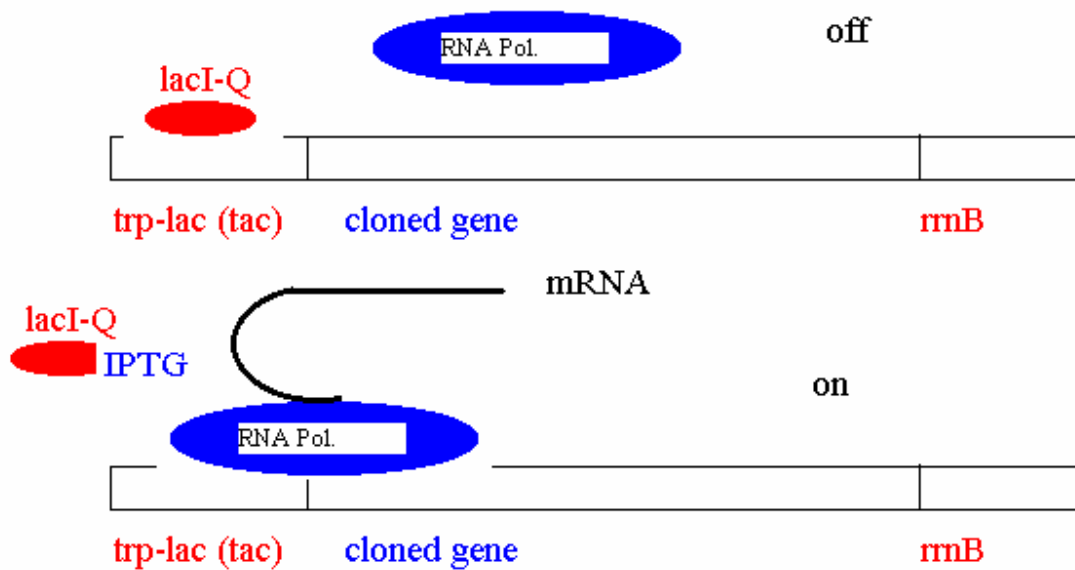


Figure 1. Diagram of negative repression imparted by the *lac* repressor (*lacI*) in the pTTQ series of vectors.

The *lac* operon is a widely studied genetic regulatory system and has been adapted as a tool for many applications in Molecular Biology and Biotechnology. In Module 1 you used a non-metabolized inducer, isopropyl beta-thiogalactoside (IPTG) and a substrate (Xgal) that turns blue when hydrolyzed by beta-galactosidase. This system allowed you to distinguish *E. coli* colonies carrying vector+insert (white) from those carrying only the vector (blue). In this module, we will use a vector that exploits the *lac* operon regulatory system to control the production of protein in *E. coli*. The pTTQ series of plasmid vectors use a strong allele of the *lac* repressor (*lacI<sup>Q</sup>*) and a promoter constructed out of portions of two bacterial promoters. The “tac” promoter (not to be confused with TAC libraries or *taq* polymerase) was constructed using

tryptophane (*trp*) –35 sequences and the lacUV5 operator sequences. This hybrid promoter is 5-10 times stronger than the lacUV5 promoter and is regulated by *lacI*<sup>Q</sup>.

In Module 5 we will extract protein from *E. coli* cultures carrying the pTTQ18 vector in which an enzyme was cloned into the EcoRI and BamHI sites of the poly-linker. Sequences from the *Thermus aquaticus* DNA polymerase were amplified with primers that anneal to opposite ends of the coding sequence and carry an EcoRI restriction site at the 5' and a BglII restriction site at the 3' end. The enzymes BamHI (5'-GGATCC) and BglII (5'-AGATCT) create compatible ends, but ligation creates a site 5'-GGATCT that is not recognized by either enzyme. The cloning strategy created a gene that uses the *tac* promoter to initiate transcription and a fusion protein where the first two amino acids of the *T. aquaticus* enzyme (Met-Arg-...) are replaced by (Met-As-Ser...). The goals of this lab are to 1) learn how to isolate protein and 2) compare proteins in *E. coli* cultures that have been induced with IPTG to non-induced cultures.

## Methods Background

Increases in the salt concentration make less water available to keep a protein soluble, and precipitates form when there are not enough water molecules to interact with protein molecules. The use of increasing salt concentrations to precipitate a protein is known as “salting-out”. Ammonium sulfate is widely used for salting-out because it has high solubility in water and is not expensive.

When salting out enzymes attention must be paid to the possible loss of activity due to denaturation. In addition, care must be taken to insure that proteases do not destroy the target enzymes. For this reason, phenylmethoysulfonyl fluoride (PMSF), a potent protease inhibitor is often added. PMSF is a VERY dangerous neurotoxin, and 4-(2-amino-ethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF) is sometimes substituted as a less toxic alternative (although AEBSF is STILL toxic).

Precipitation with ammonium sulfate may be performed by either solid salt or by dilution from a saturated solution [approx. 4.1M in distilled water]. For solid salt precipitations performed on ice, the formula  $G = (533 (S2 - S1) / (100 - 0.3 S1))$  is used as a guide, where  $S1 = \% \text{ saturation in starting solution}$ ,  $S2 = \% \text{ saturation in final solution}$  and  $G = \text{grams of solid ammonium sulfate to be added per litre}$ . Reproducible results will only occur if care is taken to keep the temperature constant.

To do a “cut”, you first need to measure the volume of the starting protein solution and determine how much ammonium sulfate is required to go from a starting saturation of 0% to a final saturation of 35%. So, using the formula  $(533[35-0]) / (100 - 0.3 \times 0) = 186.55 \text{ gm per L}$ . For 50 ml of protein solution one would add 9.3 gm. Note that tables in reference books often differ in how they present the amounts to be added (per L vs per 100 ml). The formula above assumes you are precipitating protein on ice.

Purified proteins can be stored as ammonium sulfate precipitates at 4 C for extended periods, which can be advantageous if the protein is known to be sensitive to freezing. An added

advantage of storing proteins as ammonium sulfate precipitates is that the high salt concentration is an effective inhibitor of bacterial growth.

**References:**

Stark, M. J. 1987. Multicopy expression vectors carrying the lac repressor gene for regulated high-level expression of genes in *Escherichia coli*.  
*Gene*. 51:255-267.

Engelke, E. R., Krikos, A., Bruck, M. E., and Ginsburg, D. 1990. Purification of *Thermus aquaticus* DNA polymerase expressed in *E. coli*. *Analytical Biochemistry* 191:396-400.

<http://sbio.uct.ac.za/Sbio/documentation/Protein%20Concentration.html>

<http://www.glue.umd.edu/~nsw/ench485/lab6a.htm>