Turning Genes On and Off
Regulation of Gene Expression

Introduction

You will be investigating the regulation of genes in *E. coli* responsible for the metabolism of the sugars lactose and arabinose. You will also investigate the regulation of the amylase gene in *Bacillus subtilis*.

Learning objectives
Conceptual
Cells can sense their environment and change in response to it by turning on or off genes.

Practical
By this time in the class you have already been exposed to the plating and sterile techniques that we will be applying in this exercise.

Underlying Science

*E. coli* has an estimated 4403 protein coding genes only a fraction of which are expressed at any one time. This fact seems more obvious in the case of more complex multicellular organisms like yourself. The human genome encodes 50 thousand or more genes, and it is obvious from our complex development, various organs, tissue and cell types that different cells in our body express different genes. Experiments have proven this, for example your liver cells express a different set of genes than the cells of your brain, or any other organ for that matter. This is understandable in that the liver has a wholly different function than the brain. In the case of single cell organisms this is not as obvious because they are not composed of different tissues. However, cells have to deal with changing environmental conditions, and while expressing all of their genes at once may accomplish this, it would require an unnecessary expenditure of energy. Alternatively, if an organism could turn on and off its genes when the appropriate substrates or environmental conditions were either present or absent, such an organism could afford to carry numerous genes for dealing with numerous substrates or environmental conditions. Most of these genes would not be transcribed or translated a majority of the time; instead their expression would be regulated. However some genes need to be expressed all the time. Genes that encode proteins of basic essential functions such as DNA replication, RNA transcription, protein translation, or glycolysis are called housekeeping genes. Typically house keeping genes are always on this is called constitutive expression. It is the genes involved in various responses to changing environmental conditions are regulated. Why? Because for organisms to be successful in nature they must be efficient-- making proteins (transcription and translation) requires energy-- and not all of the substrates that *E. coli* has enzymes to react with are present all the time. Cells do not waste energy by making proteins that they do not need, but they make proteins that they need when they need them. Thus gene regulation allows Organisms to adapt rapidly to changing or new environments making them more competitive in nature.

Protocol
Variables and Controls

**Independent variable**
The type of carbon sources

**Dependent variable**
The conversion of Xgal substrate from colorless to blue
GFP fluorescence
Degradation of starch by amylase

**Control variables**
LB agar plates, Starch plates, plating methods

**Positive controls**
Plating bacteria with substrates as inducer or inhibitor. In these experiments your controls will be internal controls meaning that they are part of the same assay since you will be plating the bacteria on a single plate and applying treated disks containing substrates as inducers or inhibitors at concentrations known to influence gene regulation.

**Negative controls**
Plating bacteria with out the inducer or inhibitor. Also an internal control because parts of the plate will not have the test substrates.

Materials

One of each of the following three cultures.
- *E. coli* transformed with the pGLO plasmid (5ml broth culture grown in LB)
- *E. coli* wild type strain K12 (5ml broth culture grown in LB)
- *Bacillus subtilis* (5ml broth culture grown in LB)

You will need one plate of each of the following agars.
- LB agar with 75 ug/ml ampicillin
- LB agar with 75 ug/ml ampicillin with 0.2% arabinose
- LB Starch agar
- LB agar 40 ug/ml Xgal

Disks each saturated with one of the following
- 2% arabinose
- 100mM IPTG
- 2% glucose
- 2% maltose

Bunsen burner
37 C incubator
Beaker of ethanol
Glass plate spreader

Procedure
1. Collect one of each of the agar plates.
   - LB agar with 75 ug/ml ampicillin
   - LB agar with 75 ug/ml ampicillin with 0.2% arabinose
   - LB Starch agar
   - LB agar 40 ug/ml Xgal

2. Collect one liquid culture of each of the three strains you will that you will be plating
3. Collect the petri plates containing the substrate stocks with saturated disks.

4. Label each plate with the strain
   - K12 will be plated on the LB Xgal plates
   - pGlo will be plated on both the LB amp and the LB amp ara plates
   - B. subtilis (BS) will be plated on the LB starch plate

5. Label the position for each of the four substrate disks with an “A” for arabinose, “I” for IPTG, “G” for glucose, and M for maltose.

![Diagram showing positions: K12, A, I, G, M]

6. Plate 100 ul of each of the liquid cultures by spreading with a sterile glass spreader on the appropriate plate.

7. Using sterile tweezers place substrate disks on the agar above the corresponding labeled position. Sterilize the tweezers using the ethanol and Bunsen burner as you would with plate spreader.

8. Place your plates at 37C and incubate overnight.

9. The next day you will need to come back to lab to make your observations. The pGlo cultures will need to be exposed to UV light to observe the expression of GFP.

10. Record your observations.
    Use the digital camera to record your results.
    **Describe the changes do you observe around each disk on each plate, and speculate as to why? (This could be in table form)**

11. Wrap your plates in parafilm and store them in the fridge for the rest of your lab team mates to observe later.

**Question**
Are you observing rapid evolution? How is gene regulation different than evolution?