Report from the Front Lines with Green Fluorescent Protein

Abstract: Green Fluorescent Protein, GFP, is being used in numerous research labs across the world to learn about many aspects of other genes. GFP is a perfect candidate for this job because it is a small protein, does not require any other products except UV light for bioluminescence, is very stable and does not break apart or loose its function under various experimental conditions and manipulations.

Introduction:

Every week in your daily paper there is a news article about genetics. It may involve isolating specific genes involved in the mental disease schizophrenia, elucidating the DNA structure of humans, to cloning your family pet. These are all newsworthy stories. But, what is really going on in the research lab of these geneticists and molecular biologists? Once the genes are cloned and isolated, the true work begins for the researcher. Scientist, medical doctors, and drug companies need to know how these various genes in our body are regulated. It is very difficult to directly determine how a specific gene is turned on and off to what stimulants. To understand the regulation of a gene, you need to measure the amount of product being made; either the amount of message (mRNA) or protein. You need to design a system where gene expression can be easily observed.

GFP as a reporter gene:

That is exactly how the green fluorescent protein (GFP) is being used by hundreds of scientists around the world. GFP is the gene of choice for studying the regulation of expression or activity of specific genes. In other words, GFP is being used to “report” on and serves as a reporter gene. This is engineered by inserting the regulatory sequences of an experimental gene upstream from the coding region of GFP. Conditions that result in the expression of the gene of interest will cause the production of GFP, thus, causing the cell or organism to turn green when exposed to UV light. In other words, GFP will only be expressed under the conditions that would express your gene of interest.

GFP reports on the myc oncogene:

Let’s create an experiment that would demonstrate the use of GFP as a reporter gene. You are a graduate student at U.C. Berkeley studying breast cancer. You would like to learn more about the oncogene myc, which, when found in high amounts, can cause breast, lung, or cervical cancers. When the myc gene is turned on, cell growth and proliferation occurs non-stop and tumors form in animals. Under normal conditions, the myc gene is not turned on continuously and tumors are not formed. You have been asked to test some drugs that have been designed to slow the process of breast cancer tumor growth. You would like to know if these drugs are turning off the myc gene. If they do turn off the myc gene, these drugs may aide in stopping tumor formation.

You decide to try out the new hot reporter GFP system. When you put the myc regulatory region in front of the GFP gene, GFP should be continually expressed and the cells will turn green when exposed to UV light. But if something blocks the myc regulatory region, GFP will not be expressed, and the cells will remain colorless.

Designing your experiment using the GFP gene:

First you insert the myc regulatory regions (Figure 1) upstream from the GFP gene as shown in the vGLO plasmid (Figure 2).

Figure 1: The myc gene under control of the Pmyc promoter.

Below is your new construct of the vector, vMYCGLO alongside the original plasmid without the myc regulatory sequences, vGLO.

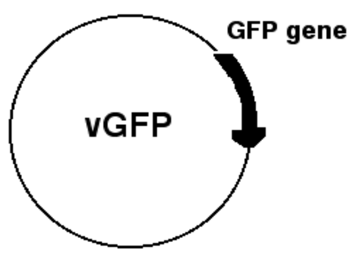

Figure 2: The vGLO vector containing the gene for GFP.

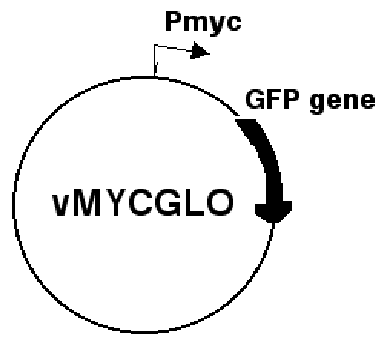

Figure 3: The new vMYCGLO vector

In your new vector, vMYCGLO, GFP is now under the regulation of the myc regulatory genes. You grow human tissue culture cells with either the vMYCGLO or the vGLO vectors for 2 days in normal growth medium. Next, you add Drug A to the growth medium of only one culture with vMYCGLO (Figure 4). The other culture containing vMYCGLO will be maintained on normal growth medium as a control. You do the same with cultures containing vGLO. After 24 hours, you expose these cultures to UV light (see Figure 4 for results).

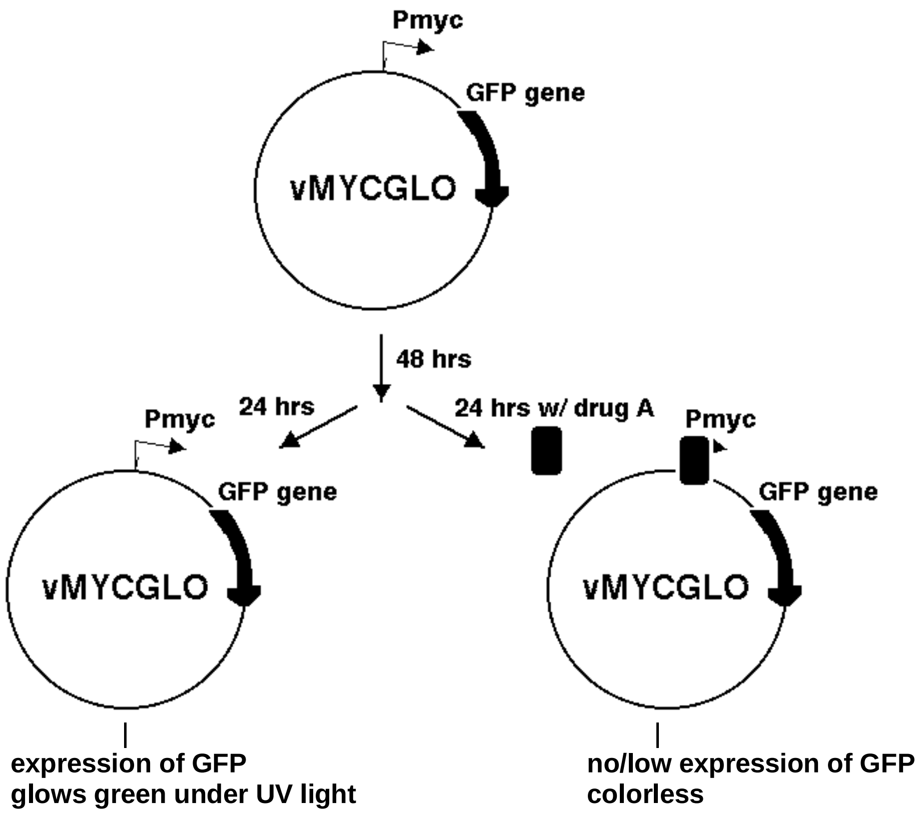

Figure 4: The new vector with GFP gene under the control of Pmyc is exposed to no drugs (Control) on the left and drug A on the right. The Control sample shows the expression of GFP. While the sample treated with drug A blocked the expression of GFP.

The controls with vGLO remain colorless since GFP cannot be expressed without any regulatory regions to promote transcription. The culture with vMYCGLO that was **NOT EXPOSED** to Drug A **glowed bright green** when exposed to UV light. Whereas the culture **EXPOSED** to Drug A **remained colorless**. This suggests that Drug A inhibits the expression of GFP. Since GFP expression is controlled by the regulatory region of myc, this indicates that Drug A can turn off the expression of the myc genes. You have just found a drug that may be useful in breast cancer therapy!

This scenario is just one way a scientist can use GFP to report on the expression of another gene. I bet you can now come up with your own set of experiments using GFP as a reporter gene.

References:

http://www.clontech.com/archive/APR96UPD/cmycGFP.shtml
Using GFP to study the oncogene c-myc. Nuclear localization of a c-myc-GFP fusion protein.

Glossary for Using GFP as a Molecular Tool

cancer
A type of disease caused by cells that divide and grow uncontrollably, invading and disrupting other tissues and spreading to other areas of the body (metastasis).

cloning
The process of asexually producing a group of cells (clones), all genetically identical, from a single ancestor. In recombinant DNA technology, the use of DNA manipulation procedures to produce multiple copies of a single gene or segment of DNA is referred to as cloning DNA.

DNA
The material inside the nucleus of the cells that carries genetic information. The scientific name for DNA is deoxyribonucleic acid.

fluorescence
A phenomenon shown by certain substances when they are hit by ultraviolet radiation. The substance absorbs high frequency wavelengths and emits it at a lower frequency light. This emission stops as soon as the high frequency radiation is removed. For example, GFP absorbs the higher frequency blue light emitted by aequorin, undergoes a chemical reaction, and emits the lower wavelength green light.

gene
A unit of inheritance; a small section of the DNA strand. Genes contain the code for a specific product, typically a protein such as an enzyme.

gene cloning (DNA cloning)
A lab technique which uses DNA manipulation procedures to produce a recombinant DNA molecule and then to make multiple copies of it by inserting it into the genome of a host microorganism which is then grown in culture.

gene expression
The process by which a gene's coded information is converted into the structures present and operating in the cell. Expressed genes include those that are transcribed into mRNA and then translated into protein and those that are transcribed into RNA but not translated into protein (e.g., transfer and ribosomal RNAs).

gene regulation
The process by which genes are turned on and off. This process can occur by a variety of mechanisms.

genetics
The study of the patterns of inheritance of specific traits.

genetic engineering (gene manipulation, genetic manipulation)
The manipulation of an organism's genetic endowment by introducing or eliminating specific genes through modern molecular biology techniques. A broad definition of genetic engineering also includes selective breeding and other means of artificial selection. See recombinant DNA technologies.

green fluorescent protein (GFP)
A protein found in jellyfish that fluoresces, or emits a green visible light when excited by UV light with a wavelength of 395 nanometers. It can function as a biological marker when co-expressed with other proteins. The structure of the protein is cylindrical with the glowing component, an amino acid complex called a *fluorophore*, in the middle of it.

malignant tumor
An abnormal uncontrolled growth of tissue that has potential to spread to distant sites of the body. Cancer exerts its deleterious effect on the body by:

- destroying the surrounding adjacent tissues: e.g. compressing nerves, eroding blood vessels, or causing perforation of organs.
- replacing normal functioning cells in distant sites: e.g. replacing blood forming cells in the bone marrow, replacing bones leading to increased calcium levels in the blood, or in the heart muscles so that the heart fails.

molecular biology
The study of the biochemical and molecular processes within cells, with emphasis on the processes of replication, transcription, and translation.

molecular genetics
The study of the flow and regulation of genetic information between DNA, RNA, and protein molecules.

oncogene
A gene, one or more forms of which is associated with cancer. Many oncogenes are involved, directly or indirectly, in controlling the rate of cell growth.

proto-oncogene
A gene, usually one that codes for a regulatory protein, that can become an oncogene if it mutates.

MYC oncogene
The presence of this proto-oncogene results in tumor formation (carcinomas and sarcomas) and is found in the avian myelocytomatosis virus (a virus found in birds). If translocated, it can cause Burkitt's lymphoma; if amplified, it can cause lung, breast, and cervical carcinomas. It is found in chicken and has an analog in humans which is located on the long arm of chromosome 8.

operon system

operon
A controllable unit of transcription consisting of a number of structural genes transcribed together. Contains at least two distinct regions: the **operator** and the **promoter**. For an example, see the lac operon page.

i gene (lac I operon)
A gene that is a part of the **lac operon.** It codes for a repressor protein, which prevents the transcription of genes that code for the enzymes that bring lactose into the bacterial cell and use it as a carbon source. The repressor protein prevents the unnecessary production of these enzymes when no lactose is present.

promoter
A site on DNA to which RNA polymerase will bind and initiate transcription.

operator
Site of repressor binding on a DNA molecule; part of an operon.

recombinant DNA
Recombinant DNA is a fragment of DNA incorporated artificially into the DNA molecule of a suitable vector so that it can express itself many times. This way a large quantity of the DNA in question can be obtained. The DNA is usually one that contains genes of interest, such as interferon, insulin, or an oncogene. The DNA may also be intended to fix mutated genes causing diseases, such as hemophilia or sickle cell anemia. The vector could be plasmids, bacteriophages, and cosmids (packaged plasmid DNA into a phage particle).

recombinant DNA technologies
Procedures used to join together DNA segments in a cell-free system (an environment outside a cell or organism). Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into a cellular chromosome.

reporter gene
A gene that is used to locate or identify another gene**.** It is often used to examine the factors that regulate the gene of interest, in effect, it “reports” on the gene that is being examined.

translocation
The movement or reciprocal exchange of large-chromosomal segments, typically between two different chromosomes.

ultraviolet radiation (UV)
Electromagnetic radiation produced by the sun and or produced when an electrical current passes through ionized gas between two electrodes. It consists of wavelengths between 200 and 400 nanometers. Exposure to excessive amounts of UV radiation damages DNA and can cause health problems such as skin cancer and cataracts in the eyes.

Vectors

cloning vector
A DNA molecule originating from a virus, a plasmid, or the cell of a higher organism into which another DNA fragment of appropriate size can be integrated without loss of the vectors capacity for self-replication. Vectors introduce foreign DNA into host cells, where it can be reproduced in large quantities. Examples are plasmids, cosmids, and yeast artificial chromosomes. Vectors are often recombinant molecules containing DNA sequences from several sources.

expression vector
A cloning vector that contains the necessary regulatory sequences to allow transcription and translation of a cloned gene or genes. A vector which is able to replicate and transcribe cloned DNA.

host-vector system
A combination of a bacterial host cell (i.e. a specific strain) and a virus vector (i.e. a particular bacteriophage strain) which work well together for DNA cloning.