A Green Light for Biology: Making the Invisible Visible

Secrets of the Sequence Video Series on the Life Sciences • Grades 9 – 12

Teaching materials developed by VCU Life Sciences.

Virginia Commonwealth University

Classroom Tested Lesson

Video Description

"Secrets of the Sequence," Show 124, Episode 2
"A Green Light for Biology" – approximately 10 minutes viewing time

This discovery known as Green Fluorescent Protein has revolutionized molecular biology. Protein molecules inside the body move invisibly, but the advent of GFP has made the invisible…visible. The protein, found in jellyfish, helps researchers track molecules of all kinds, in real time, and shows how they build and maintain cells, and function in concert with other cells.

Ward Television
Producer: Paul Gasek
Associate Producer: Trish Golden
Featuring: Osamu Shimomura; Vincent Pieribone, Cellular and Molecular Physiology, Yale University School of Medicine; Jennifer Waters Shuler, Nikon Imaging Center, Harvard Medical School
Lesson Author; Reviewers: Ryan Templeton and Kristin Householder; Catherine Dahl, Dick Rezba, Kieron Torres
Trial Testing Teachers: Valerie May, Pam Sparks

National and State Science Standards of Learning

National Science Education Standards Connection

Content Standard C: Life Science

As a result of their activities in grades 9-12, all students should develop an understanding of:

- the cell,
- matter, energy, and organization in living systems, and
- molecular basis of heredity.

Content Standard E: Science and Technology

As a result of activities in grades 9-12, all students should develop:

- abilities of technological design and
- understandings about science and technology

Selected State Science Standards Connection

Use http://www.eduhound.com (click on "Standards by State") or a search engine to access additional state science standards.

West Virginia

A. The Cell
    4. Cell functions are regulated. Regulation occurs through changes in the activity of the functions performed by proteins and by the selective expression of certain genes. This regulation allows cells to respond to their environment and to control and coordinate cell growth and division.
        a. Examine the importance of DNA and proteins in cell regulation.
        b. Discuss mishaps in cell regulation (e. g., tumors).

B. The Molecular Basis of Heredity
    1. In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, T, G, and C). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular "letters") and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome.
        d. Evaluate the impact of DNA technology on society (e.g., bioengineering, forensics, genome project, DNA fingerprinting).

Virginia

BIO.3 The student will investigate and understand the chemical and biochemical principles essential for life. Key concepts include:

b) the structure and function of macromolecules.

BIO.6 The student will investigate and understand common mechanisms of inheritance and protein synthesis. Key concepts include:

g) events involved in the construction of proteins;
h) use, limitations, and misuse of genetic information; and
i) exploration of the impact of DNA technologies.

Testing: Sample related multiple choice item from State Standardized Exams

DNA that is derived from the DNA of two or more different species is called –

a) recombinant DNA *
b) mitochondrial DNA
c) chloroplast DNA
d) plasmid

(Source: Virginia 2002 Biology test)

Overview

In this video segment students see how a simple discovery led to the application of a powerful tool that has revolutionized the field of cellular biology – Green Fluorescent Protein (GFP). This particular protein has the unique property of biofluorescence. In biofluorescence, available light is absorbed and converted into light of another wavelength, producing a different colored light. In the case of GFP, photons of light in the blue wavelength are converted into photons in the green wavelength.

By attaching the gene for GFP to other genes that code for proteins, scientists are able to view cellular proteins in real time under a microscope. This technique uses recombinant technology to "attach" the gene for GFP onto another gene that scientists wish to study. Whenever the gene is expressed, GFP is also expressed and bound to the protein of interest. Exposing cells that carry these proteins to blue light results

in a glowing green color. Scientists can then trace the pathways a particular protein takes inside a cell, or observe where the protein stays inside of a cell.

This segment also explains the difference between bioluminescence and biofluorescence. The segment does not review how DNA can be spliced and recombined, so a brief discussion should precede this segment. The segment illustrates many applications of GFP technology among the examples provided. Students will see glowing jellyfish, glowing cells and the special microscopy needed to visualize these structures. One stunning part of the segment shows the export of proteins from a Golgi apparatus using time-lapse photography. Only the protein of interest is illuminated, but students can clearly see how the Golgi exports the proteins to other parts of the cell using vesicles.

Video Preparation

Preview the video and make note of the locations at which you will later pause the video for discussion.

Before Viewing

1) Ask: "Can you name an organism that gives off light?"
   Fireflies

2) Explain: Special enzymatic reactions (luciferase) create light in fireflies. Certain chemicals glow because they emit photons of light when excited.

3) Ask: "Can you think of some uses for chemicals that can emit light?"
   Paint used in watch dials, light sticks

4) Explain: Luminescence is the ability of something to produce its own light, Fluorescence, on the other hand, is the ability of something to emit light when exposed to other light. Some minerals, for example, fluoresce when exposed to UV light.

5) Briefly review the concept of proteins and protein expression. Students will need a basic understanding of protein expression to benefit most from this segment.
   Ask: "Can we see proteins?"
   No

6) Show students pictures of stained cells.
   Ask: "What makes it possible for us to see cell structures more clearly?"
   Stains and dyes used to highlight structures

During Viewing

1) START the video.

2) PAUSE the video after the computer explanation that describes the difference between bioluminescence and biofluorescence (2.54 minutes into the video). With the students' help, create a chart on the board summarizing differences between bioluminescence and biofluorescence:

Example:

Biofluorescence	Bioluminescence
Use incoming light to emit fluorescent light	Produces own fluorescent light
Use GFP (converts blue incoming light to green fluorescent light)	Use lucerferase + $O_2 \rightarrow$ luminescent light

3. RESUME the video and play to the end.

After Viewing

1) Ask students the following questions.
   - How did scientists use GFP to change the way they view cells?
   - Why would a scientist want to use GFP instead of stains and dyes that fluoresce?

2) What are some useful applications of GFP in cellular biology?

3) Have students list other examples of genes that have been transferred to other organisms. (This could be an assignment where students research the topic.)

4) Conduct the following demonstration to illustrate how motions can be clarified using fluorescence.

Demonstration:
Materials:
- green glow sticks (at least 2 per class)
- a very darkened room

Procedure:
a) Divide students into two groups. Each group should choose a short word that they will later 'write in the air' in the dark. The word is unimportant but you could limit them to a biology topic area.
b) Turn the lights off and blacken windows if possible. Immediately have one person in a group "write" the word in the air using his or her hand. The other group should try to guess the letters and the word that is being spelled. The darker the room, the better the effect.
c) Then have the same group member hold a glowing glow stick in his/her hand and spell the same word. Hopefully, students will see how following the hand with a visible label greatly improves their perception.

5) Conduct the student activity: Observation of GFP Expression

Teacher Notes for a Student Activity: Observation of GFP Expression

1. Choose one of the following commercially available activities:

A) In this activity, students observe colored fluorescence in E. coli bacteria transformed with genes from a marine cnidarian -- specifically utilizing two genes isolated and cloned from the bioluminescent jellyfish *Aequorea victoria*. The success of the genetic exchange will be indicated by a color glow given off by the bacteria when placed under long-wave UV (black) light. The genes, already placed in a bacterial plasmid, have been mutated for enhanced fluorescence. The kit, which is available from Ward's

Biological Supply (#88 W8233), contains enough materials for ten transformations, a teacher's guide, and student copy master. Micropipettes and a long-wave UV light are required and available separately.
Ordering Information: Kit number. 88W8233,
Note: Includes perishable materials. Please specify delivery date.
http://www.wardsci.com

B) In this activity, students use the Biotechnology Explorer program to observe GFP expressed by a unique jellyfish gene. The protein that glows brilliant green when viewed with the light of an inexpensive hand-held ultraviolet lamp. The gene for the fluorescent green protein was originally isolated from the bioluminescent jellyfish *Aequorea victoria*. The gene has been modified by Maxygen, Inc. to introduce specific mutations into the DNA sequence that greatly enhance fluorescence in the protein. The modified form of the jellyfish gene has been cloned into Bio-Rad's patented pGLO plasmid and is available in Biotechnology Explorer kits. Using the jellyfish gene to transform bacteria, students directly observe the results of gene transfer, gene regulation, gene expression, and protein purification.
Ordering Information:
Kit Number: 166-0003EDU pGLO Bacterial Transformation Kit, provides materials for 32 students or 8 workstations.
Kit Number: 166-0005EDU Green Fluorescent Protein (GFP) Chromatography Kit, provides materials for 32 students or 8 workstations.
http://www.bio-rad.com or 1-800-424-6723

2) Distribute Student Handout.

3) Have students make an attempt to answer the question below (on their Student Handout) without using any references. Then tell them to research the question on the Internet to see how closely their ideas matched up.

Ask: "What useful purposes do you think GFP-linked proteins would have for humans?"

Cancer research and treatment

Name:_____

Student Handout:

Make an attempt to answer the following question. Once you have answered it, do some research on the Internet to determine exactly how these GFP proteins are being used and see how closely your answer matched up.

What useful purposes do you think GFP-linked proteins would have for humans?

Additional Resources

Because Web sites frequently change, some of these resources may no longer be available. Use a search engine and related key words to generate new Web sites.

Fluorescent labeling
http://www-biology.ucsd.edu/~davek/intro.html
http://www.evrogen.com/p1.shtml

GFP
http://www.rcsb.org/pdb/molecules/pdb42_1.html
http://pantheon.cis.yale.edu/~wfm5/gfp_gateway.html
http://www.ebi.ac.uk/interpro/potm/2003_1/Page_1.htm
http://abcnews.go.com/sections/science/DailyNews/rabbit000918.html

Teacher Resources on GFP
http://www.ascb.org/teachers/green.html

Bioluminescence
http://www.lifesci.ucsb.edu/~biolum/
http://www.sio.ucsd.edu/explorations/biolum/

Recombinant DNA Technology
http://www.amgen.com/rnd/RecombinantDNA.html

Books and Articles

Mary Batten (2000). The Winking, Blinking Sea: All about Bioluminescence. Millbrook Press.
Roger Y. Tsien (1998): The Green Fluorescent Protein. Annual Review of Biochemistry 67, pp. 509-544.

Genomic Revolution
http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml
The Web site to the government-funded Human Genome Project with links about genomics, the history of the project, and more.

Secrets of the Sequence Videos and Lessons
This video and 49 others with their accompanying lessons are available *at no charge* from www.vcu.edu/lifesci/sosq