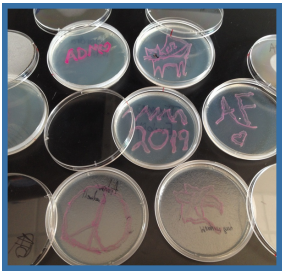
RFP Art

Honors/AP Biology
Time: 45 min

How are fluorescent proteins used?

Learning Objectives

Students streak a plate, a protocol commonly used by synthetic biologists to grow up cells with modified DNA. Students should understand that cell division, and therefore bacterial growth, is exponential. Students should also understand that a colony grows from a single cell and that all cells in a colony have identical DNA unless the DNA of a cell mutated.



Materials

Bacteria with an RFP (red fluorescent protein) gene
http://www.enasco.com/product/LM00724M
Agar plates (one per pair)
https://www.enasco.com/product/Z13903M
Toothpicks (one per student)
Permanent marker

Approximate Cost: $25 for 20 students

Procedure

Explain the idea of plasmids to students. Ask them what type of protein they would design a plasmid to code for if they were trying to see whether or not there was a plasmid in the cell.
Introduce them to the idea of using a colored protein as an "indicator" that signals to the researcher whether or not inserting a plasmid into a cell was successful. Ask students how cells grow: if a cell has an RFP plasmid in it, will it grow into a red colony? At what rate do cells grow? If one cell becomes two cells in twenty minutes, then how many cells will you have in forty minutes if you have eight cells now?
Give each pair of students an agar plate and have them write their names on the lid of the plate. Turn the plate upside down and draw a line across the bottom, dividing the plate in half. Give each student a toothpick. Each student should take turns dipping the toothpick into the bacterial culture and then streaking half of the plate in any pattern they want. Don't press too hard with the toothpicks—they shouldn't break the surface of the agar. Store plates at 37 degrees Celsius overnight or at room temperature for 48 hours. Students can take pictures of their plates and see if their designs grew.

Background

A plasmid is a circular molecule of DNA that a bacterium can take up and express. For molecular biology, a plasmid consists of a vector backbone, which carries a particular antibiotic resistance gene, and the insert, which is some genetic device or component of a device. RFP is red fluorescent protein, which fluoresces red under certain wavelengths of light.

In epifluorescent microscopy, a laser is used to illuminate an organism expressing a fluorescent protein such as RFP. Computer software that works with the microscope can detect the level of fluorescence, which tells us how strongly the fluorescent protein is expressed.

Fluorescent proteins like RFP are used as reporters in synthetic biology, sometimes just to indicate that a device works, and sometimes to signal some event, where activation of RFP is the last of a series of steps in a genetic device's function.

Example: A biosensor is an organism programmed to detect a particular substance in the environment. Here is an example of a device designed to detect cobalt, a metal that can be toxic to humans: http://parts.igem.org/Part:BBa_M45102 The promoter, which controls gene transcription, is sensitive to cobalt. Cobalt in the environment of a bacteria expressing this device will have the gene activated by cobalt and express RFP, which serves to report the presence of cobalt.

Critical Thinking Questions

Looking back at the structure of plasmids, notice the backbone contains antibiotic resistance. Why is this necessary?

In a biosensor like that described above, what does the level of intensity of the fluorescence indicate?