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| ***GENES R US BRIEFING AND ASSIGNMENT***Some genes and their protein products have enormous commercial value. Examples include: insulin used by diabetics for lowering blood sugar and enzymes added to laundry detergent for removing stains. Scientists in the Research and Development Division of **Genes-R-Us** **Inc.** have developed a process in which proteins that produce a color and fluoresce are produced by genetically transformed bacteria. |  |

You have been hired to work on an extension of this **RAINBOW** project. The members of your team will be trained to carry out the steps used to create color-producing bacteria using our Standard Operating Procedure (SOP).

**Background information**:

Scientists can insert genes into bacteria. The genes inserted in the **RAINBOW** process are on a circular piece of DNA called a ***plasmid*** (the plasmid we use is called **pFLO®**). The bacteria with the inserted genes are used as factories that produce large quantities of the gene and its product. The process of giving bacteria (or another organism) a new gene is called ***transformation***.

Our transformation process uses the bacterium *Escherichia coli* as the “factory” to produce the fluorescent proteins. We insert the **pFLO** ® plasmid into a highly specialized, harmless strain of *E. coli*.

Transformation is a rare event, so to make it easier to find transformants (transformed bacteria), a gene for ampicillin resistance was included on the **pFLO** ® plasmid. Expression of this gene, which produces the enzyme β-lactamase, allows transformed *E. coli* to grow in the presence of **ampicillin**, an antibiotic that usually prevents the growth of bacteria. The β-lactamase enzyme dismantles ampicillin, rendering it non-functional. Hence, transformed bacteria will live in the presence of ampicillin, while untransformed bacteria will not live.

Proper controls are essential in any experiment. In this experiment we will grow *E. coli* on agar plates which contain nutritional requirements for the bacteria. Our control is another sample of bacteria which is treated **exactly** the same as the bacteria we’re transforming, except the control does not receive the **pFLO** ® plasmid.

Controls help you understand what happened if things go wrong and to know things work out as expected. For example, if there are no bacteria on your **pFLO** ®-transformed plate, is this because none of them were transformed (a definite possibility) or because all the bacteria were flat-out dead already? A control plate helps answer the question. If the bacteria on the control plate are alive, you can rule out that the procedure killed the bacteria.

Controls also validate that the protocol was performed correctly when the experiment appears to have been successful. Experiments can utilize positive and negative controls.

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| **Overview of the Transformation Process:**1. Grow the bacteria on a stock plate.2. Make the bacteria “competent.” This makes them able to accept new DNA. The method we use involves coating the bacteria and the DNA with positively charged molecules, to minimize charge repulsion of the DNA by the bacteria.3. “Shock” the bacteria with heat, then chill to push the DNA into pores in the bacteria.4. Grow the bacteria on LB agar. Bacteria on an agar plate containing the antibiotic ampicillin will only grow if they have received the plasmid. ***E. coli* colonies are usually white. Those producing our fluorescent protein will be colored****and fluoresce under a UV light source.** |

The bacteria cannot move, so they will grow and divide in the same spot on the plate. Small numbers of bacteria cannot be seen without a microscope, but colonies (millions of daughter cells from one bacterium) can be seen. This allows you to identify, count, and isolate bacterial colonies, each of which arose from a single bacterial cell.

As part of your job assignment at Genes-R-Us, your team has been given the task of increasing the efficiency of transformation. The higher the efficiency of transformation, the more protein-producing bacteria colonies will grow.

A prominent wildlife protection organization recently requested our collaboration on a project to preserve the genomes (genetic information) of endangered species. The project uses the transformation process to create “libraries” of bacteria. Each transformed bacterium carries a different small piece of the genetic material being preserved. The challenge for Genes R Us is to increase the efficiency of the transformation process so that these libraries are complete. Our scientific advisory board is keen to help with this project. Achieving higher transformation efficiency will reduce the costs and increase the success to the project.

**Your first assignment is to familiarize yourself with our standard operating procedure (SOP #T-1) by reviewing a step-by-step guide of the transformation process, then practicing the techniques using a virtual lab program. You will then outline the protocol in the form of a lab flow chart that your team will use as you conduct the procedure.**

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**pFLO Plasmid Map**

**Materials Needed Per Team**

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| ***Equipment***microcentrifuge tubesrack for microcentrifuge tubespermanent marker pensterile toothpickssterile graduated transfer pipets2-20 μl micropipet + tipsfloating tube rack for waterbath100-1000 μl micropipet + tips (shared) 2 sterile plate spreaders (not disposable) | ***Reagents***disinfectant (10% bleach or 70% alcohol)sterile distilled watersterile Luria Broth (LB)2 LB agar plates2 LB agar/amp/X-gal platespBLU® plasmid (0.01 μg/μl)50mM CaCl2 (cold) 250 l per samplecontainer for crushed ice |

**Materials Needed Per Class**

42°C waterbath

37°C incubator

container for waste

*E. coli* stock plate

crushed ice