BACTERIAL TRANSFORMATION WITH pBAD

In this lab you will perform a procedure known as a genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (coding for) a protein, which gives an organism a particular trait. Genetic transformation literally means change caused by genes; it involves the insertion of genes into an organism in order to change the organism's traits. Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes can be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the gene involved in the disease.

You will use a procedure to transform bacteria with a gene that codes for a **Green Fluorescent Protein (GFP)**. The source of this gene is the bioluminescent jellyfish *Aequorea victoria*. The gene codes for a Green Fluorescent Protein that causes the jellyfish to fluoresce and glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein. Transformed bacteria glow a brilliant green color under ultraviolet light.

You will learn about the process of moving genes from one organism to another with the aid of a **plasmid**. In addition to one large chromosome, bacteria can contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for traits that may confer selective growth advantage under certain conditions. In nature, bacteria can transfer plasmids back and forth allowing them to share beneficial genes. This natural mechanism allows bacteria to readily adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due in part to the transmission of resistence plasmids.

The **pBAD plasmid** encodes the gene for the Green Fluorescent Protein (GFP) and a gene for resistance to ampicillin. pBAD also incorporates a special gene regulation system, the **arabinose operon**, which can be used to control expression of the fluorescent protein in transformed cells. The gene for the Green Fluorescent Protein can be switched *on* in transformed cells by adding L-arabinose to the growth

media. Selection for cells that have been transformed with pBAD is accomplished by growth on antibiotic plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose; they will appear fluorescent green when arabinose is included in the nutrient agar.

Bacterial transformation reactions require the use of recipient *E. coli* cells that have been made "leaky" while retaining viability. These cells are formally known as **competent cells**. Competent cells were prepared by taking cells during logarithmic growth phase and treating them with an ice-cold $CaCl_2$ solution. These *E. coli* cells more readily take-up and incorporate extracellular or "naked" DNA such as plasmids.

FIRST PERIOD

Materials:

1. Competent *E.coli* cells: 200 µl aliquots in microfuge tubes on ice.
2. pBAD plasmid: 10 ng, 25 ng, 50 ng, or 100 ng in MF tubes on ice.
3. Four plates: 2 LB with ampicillin, 1 LB with ampicillin plus arabinose, 1 LB.
4. Preheated (37°C) LB broth.
5. Automatic pipettors and tips.
6. Preheated (42°C) water bath.

Protocol: (Working in pairs)

1. Label two MF tubes, one **DNA** + and one **DNA -** .

2. Transfer pBAD to the **DNA** + tube and add 100 µl of competent cells to each of the two microfuge tubes. Incubate both tubes for 15-30 minutes on ice.

3. Transfer the microfuge tubes to a "float" in the 42°C water bath. Incubate for **exactly 90 seconds** and then transfer back to ice for 1-2 minutes.

4. Add 900 µl of preheated LB broth (37°C) and incubate both microfuge tubes at 37°C for 45-60 minutes.

5. At the end of the 37°C incubation period, spread 100 μl onto the agar plates as follows:

- ☞ **DNA** + tube: **LB+amp** & **LB+amp+arab** plates
- ☞ **DNA -** tube: **LB** & **LB+amp** plates.

6. Invert and store plates at 37°C for 18-24 hours. After 18-24 hours, move plates to the 4°C incubator until the next lab period.

SECOND PERIOD

1. Observe abd count all colonies on each of the four plates.
*****Wear goggles when using UV light source*****
Record and interpret your results.

2. Estimate transformation efficiency as follows:

$$\text{Transformation efficiency} = \frac{\text{total \# cells growing on LB+amp+arab plate}}{\text{Amount pBAD } (\mu g) \text{ spread on plate}}$$

BACTERICIDAL EFFECTS OF ULTRAVIOLET RADIATION

Radiation is one of the physical factors that have a tremendous effect on microorganisms. The electromagnetic spectrum includes microwaves, infrared rays, visible rays, ultraviolet rays, X-rays and cosmic rays. The radiation of most interest to the microbiologist is ultraviolet radiation.

Although ultraviolet light has very little power of penetration, it is strongly bactericidal, mutagenic and carcinogenic on direct contact. These effects are due to the dimerization of pyrimidines, particularly thymine, in the DNA chain. When thymine dimers are formed, the DNA is distorted and the thymines fail to pair with the adenines on the opposite strands, thus causing a mutation.

The bactericidal effects of ultraviolet rays are limited to a specific region of the ultraviolet spectrum. The radiation found to be both the most lethal and the most mutagenic to cells has a wavelength between 260 and 270 nm. This particular wavelength is strongly absorbed by the nucleic acids of the cell, causing them to be ionized. This wavelength is also absorbed by some proteins. In this exercise, bacteria that have been streaked on nutrient agar plates will be exposed to ultraviolet light for various lengths of time to determine the minimal exposure required to effect a 100 percent kill.

FIRST PERIOD

Material:

1. Broth culture of *Serratia marcescens*
2. Six nutrient agar plates
3. 1.0 and 0.1 ml pipets
4. Glass spreader
5. 95% ethyl alcohol in glass beaker
6. Ultraviolet lamp

Procedure: (work in groups of four)

1. Transfer **0.1 ml** of the *Serratia* broth culture to each of the 6 nutrient agar plates. Spread the inoculum with a flame-sterilized glass spreader and let dry.

2. Using four of the inoculated *S. marcescens* plates, place one plate at a time under the UV lamp. Remove the lid of the petri dish, cover half of each plate with a cardboard shield and then expose one plate for 10 seconds, one for 20 seconds, one for 30 seconds, and the other for 1 or 3 minutes. Remove the plates after the exposure period and replace their lids. Mark each plate with time of exposure.

3. Expose a fifth plate, with the cover on, to the UV light for 1 minute.

4. The sixth plate will be used as a control.

5. Incubate all plates at 25ºC for 48-72 hours.

SECOND PERIOD

Procedure:

1. Examine all plates and describe the effects of UV radiation on the growth of *Serratia marcescens*.

BACTERIAL MUTAGENICITY AND CARCINOGENESIS: THE AMES TEST

We have become increasingly aware of the number of chemicals to which we are regularly exposed. Pesticide residues are found in our food and water supplies; industrial wastes contribute noxious chemicals; and cigarette smoke contains suspected harmful chemicals. Many of the chemicals released into the environment are suspected **carcinogens** in that they many induce malignancies or cancers in humans. Many of the carcinogens are also known as **mutagens** because they cause changes in the nucleotide bases of the DNA molecule.

Bruce Ames and colleagues have developed an inexpensive and practical screening test for mutagenic and/or carcinogenic effects of compounds being considered for use as drugs or additives. The test is known as the **Ames test** and utilizes a histidine negative (his^-) **auxotrophic** strain of *Salmonella typhimurium* that will not grow on a medium lacking histidine. The test involves measuring the rate of back mutations (reversions) from the auxotrophic to the prototrophic or wild-type (his^+) state. The test organism is also deficient in DNA repair enzymes, which minimizes the potential repair of any mutations that may occur. Chemicals that are identified as mutagens by the Ames test can then be screened using other tests for carcinogenicity. **Not all mutagenic chemicals are necessarily carcinogenic.**

Many chemicals are not directly carcinogenic or mutagenic, but rather undergo chemical changes in the human body where they are converted to their carcinogenic states. These chemical changes take place primarily in the liver. The standardized Ames test incorporates the use of a rat liver enzyme extract that provides a source of activating enzymes to mimic the fate of the chemical in the human body. Rat liver extract will not be used in this experiment.

Soft "top" agar containing trace amounts of histidine (enough to allow the his^- cells to divide a few times), biotin (a vitamin), and 0.1 ml log-phase *Salmonella* cells is poured onto a glucose-minimal salts agar. The base agar lacks histidine. Sterile filter paper circles are treated with the chemicals being tested and are placed onto the "top" agar. A filter paper circle moistened with sterile water should be placed onto the "top" agar of one plate; this plate is an important control that allows you to determine the rate of spontaneous reversion to histidine

prototrophy. Mutagenic effects are observed by counting the number of revertant colonies growing on the plates. The strength of the mutagen is proportional to the number of revertant colonies (after correcting for the spontaneous reversions).

FIRST PERIOD

Material:

1. Broth culture of *Salmonella typhimurium* (his^-)
2. Three glucose-minimal salts agar plates
3. Three tubes of soft agar
4. Biotin-histidine solution
5. Sterile filter paper discs
6. Forceps
7. 1.0 ml pipets
8. Miscellaneous chemicals - potential mutagens/carcinogens
*****(Be careful and wear gloves!)*****

Procedure: (work in pairs)

1. Take a tube of soft agar from the 45ºC water bath and add 0.3 ml of the biotin-histidine solution. Mix well. Inoculate with 0.1 ml of *Salmonella typhimurium* (his^-) culture. Mix the tube contents quickly with swirling motion and pour onto the surface of the base agar plate. Tilt the plate gently to spread the soft agar evenly over the base layer.

2. In the laminar flow hood, moisten a sterile filter paper disc with the chemical to be tested. The discs should be moist, but not dripping. Use flame-sterilized forceps to place the saturated disc in the center of one of the previously prepared plates. You should make certain that the soft “top” agar has solidified.

3. Repeat the procedure using another chemical.

4. Repeat the procedure using sterile water as a control.

5. Incubate plates at 37ºC for 48-72 hours.

SECOND PERIOD

1. Observe the growth and appearance of colonies on all plates. Scattered colonies of revertant mutants will appear on the agar surface.

2. Observe the area around each disc for revertant colonies. A positive result is indicated by a relatively high concentration of revertant colonies surrounding the disc. Record your observations; drawings might be useful.

3. Count the number of colonies on the control plate and the test plates. Subtract the number of colonies on the control plate (spontaneous reversions) from the number of colonies on the test plates.