INS 099 Data Analysis **Dr. Onyenwoke, Kenyon College**
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Day 2. The Virtual Ames Test: Linking Chemicals and Mutations

based on the Wisconsin Program for Scientific Teaching Virtual Ames Test, supported by HHMI: http://scientificteaching.wisc.edu/

Background: mutations and selection

How do we determine if a chemical/compound is "dangerous," or harmful to life?

- Many diseases and ailments have been linked to long-term exposure to certain chemicals used in medicine, industry, agriculture, etc. **Can you think of any examples of such diseases?** This assertion has led many people to view all synthetic chemicals as dangerous.

- The extensive use of synthetic pesticides in the 1940s and 1950s included a variety of chemicals used in large quantities that would ultimately have negative consequences for organisms and the environment. **What are other examples of harmful chemicals?**

An area of debate about chemical safety is the regulation of chemicals based on their potential for causing cancer.

The most famous example of a test that predicts the mutagenicity/ carcinogenicity of chemicals is the **Ames Test**, developed by Bruce Ames. The test has been used extensively for years to predict the safety of chemicals.

The Ames test is based on several theories and assumptions:

- DNA is the universal genetic material in plants, animals, and microorganisms. Therefore, the ability of a chemical to cause mutations in the DNA of a bacterium may be used to predict its ability to cause mutations in all DNA, which, in turn, may be correlated with its potential to induce cancer.
- Genetic changes in cells can be induced by chemicals that alter DNA, causing mutations.
- Cancer is defined as the uncontrolled proliferation of cells in animals. The change to a cancerous condition is often caused by a genetic change, i.e., mutation, in the cell.

Definitions

Streptomycin: an antibiotic that is used as the means of selection; it differentiates strains of bacteria that are sensitive (do not grow on streptomycin) from those that are resistant (grow on streptomycin).

Mutagen: a chemical (or a physical phenomenon, such as X-rays) that causes changes in the sequences of bases in DNA; exposure to a mutagen will increase the frequency of mutation in a population.

Mutagenicity: the potential of a chemical for causing mutations or changes in DNA.

Mutant: an organism that differs genetically from its parent.

Spontaneous mutant: a mutant that arises from random changes in the DNA; changes can be caused by mistakes during DNA replication.

Frequency of mutants: the proportion of the population accounted for by mutants; for spontaneous bacterial mutants, the frequency is typically one mutant for a given trait in every million (10^6) to 10 million (10^7) cells.

Carcinogenicity: the ability to cause cancer in animals.

Key Concept

The Ames test is based on the assumption that **mutagenicity is associated with carcinogenicity,** and that **mutagenic activity in bacteria is predictive of mutagenic activity** in humans.

Lab Background and Materials

Mutations in DNA appear at random in any given population of bacteria. A chemical mutagen **increases the frequency** at which mutations appear. The Ames test measures the ability of a chemical to increase the mutation rate in bacteria. The mutations are detected by measuring a characteristic that results from changes at the DNA level, such as the ability to grow under certain conditions.

In this lab simulation, you will conduct a modified Ames test. You will be provided with:

- A virtual strain of *Escherichia coli* (*E. coli*) that is sensitive to (killed by) the antibiotic streptomycin. Natural cultures contain mutants that will grow in the presence of the antibiotic. You can alter the number of bacterial cells that you wish to incubate. You may choose from 10^0 (**What does this equal?**) to 10^{10} (**What does this equal?**) cells by selecting the number in the scroll-down menu.
- Several virtual chemicals ("test substances") to test for mutagenicity are available. Chemicals that cause mutations should increase the frequency of streptomycin-resistant mutants in a population of cells.
- Two types of media are available: Nutrient Agar and Nutrient Agar + Streptomycin. Nutrient Agar is a general medium that allows many culturable bacteria, including *E. coli*, to grow well.
- 70 hours of incubation time at 28 °C is performed. Each dot that you see on the petri plate represents an isolated colony of *E. coli*.

Assignment:

The Virtual Ames test is located at:

http://plantpath.wisc.edu/~PP123/virtual_ames/experiment/spudswin.html

You will be writing a lab report based on hypotheses you will develop concerning the mutagenic/carcinogenic properties of some common, and not so common, chemicals. This lab report will be in the scientific paper format and include:

1) **A title**
2) **An introduction where you introduce the problem along with some background information that you will find on the problem. This is also the part of the report where you shall propose your hypotheses about the types of chemicals (from the pull down list) that are likely to be mutagens. Specifically are these chemicals likely to be mutagens or not? Explain why? Present your hypotheses in a useful format within the lab report. Gather information from any sources you can find and include this information in you report. Cite where you found this information.**
3) **Materials and Methods. Design an experiment to test your hypotheses. How many replicates will you be doing? Record what you did to test your hypotheses, i.e., all parameters used and report those methods here in enough detail so that someone could read your lab report and do exactly the same things you have done.**
4) **Results and Discussion. What did you find out through the use of your methods? Report that data. What is the standard deviation of your replicates? Calculate the background mutation rate (spontaneous mutation rate). Compare this rate to the mutation rate with the test chemicals (induced mutation**

rate). Explain what this data means. How will you report this data? Tables? Graphs?

Additional questions to address in your lab report:

1. **Are there any apparent flaws in using the Ames test to predict carcinogenicity?**
2. **Should we base regulation of food additives on Ames test?**
3. **Should we use the Ames test to determine which foods to eat or products to use?**
4. **If a major pesticide was extremely mutagenic as determined by Ames test, would you advocate that farmers not use it?**
5. **If your favorite food was very mutagenic in the Ames test, would you stop eating it?**
6. **If you expose a population of genetically altered bacteria to streptomycin, what are the apparent risks/dangers?**
7. **What factors/variables does the virtual Ames test ignore?**

Based upon material found at: http://plantpath.wisc.edu/~PP123/virtual_ames/virtualames.html