STREAM

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Student Lab Report

Introduction

EBPI's Muta-Lab™ kit is a convenient approach for detecting mutagenic activity or mutagenic materials in environmental water, sediment and air samples as well as food products, cosmetics and biological fluids. Mutagenic materials may be hazardous due to their ability to alter the structure and function of DNA. Mutations are often the initiating factor inducing cancerous transformations of normal cells. EBPI designed a kit that allows students to test samples in their own environment for mutagenic effects.

Purpose

With this simple lab activity, students gain practical knowledge by conducting a real-world procedure by testing different samples and watch as the bacteria in their samples perform a reverse mutation. Students will also learn how different chemicals and compounds can cause various types of mutations, the importance of metabolic activation, as well as how bioengineered bacteria are utilized in a battery of biological testing techniques. These bacteria are specifically engineered to mutate to their natural phenotype when exposed to a mutagen and thereby produce histidine or tryptophan. The consequence of this reverse mutation is bacterial growth, and this growth qualified by a shift in the colour of the colour indicator. This activity is designed for any classroom environment and requires no specialized equipment or reagents. Students will acquire samples (optional), perform a perfunctory preparation of their samples, conduct dilutions, and analyze as well as interpret their results.

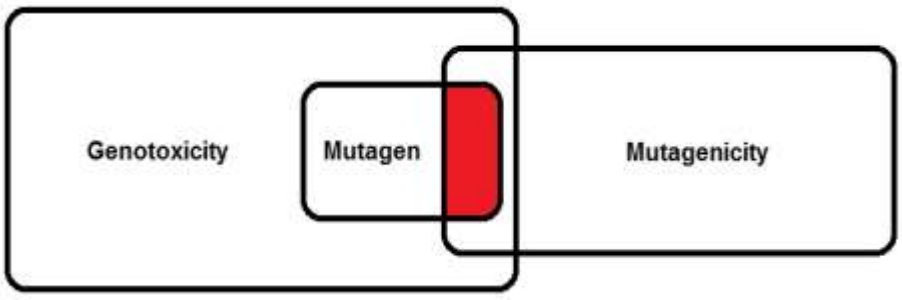
WARRANTY

EBPI warrants that, at the time of shipment, the Muta-Lab™ kit is free of defects in material and workmanship, and complies with the company specifications. Since actual experimental conditions prevailing at user's laboratory are beyond the control of EBPI or its representatives, EBPI makes no other warranty, express or implied, with respect to the product. Notification of any breach of warranty must be made within 120 days of delivery. The sole and exclusive remedy of the customer for any liability of EBPI of any kind, including liability based upon warranty (express or implied, whether contained herein or elsewhere) is limited to the replacement of the product or the refund of the invoice price of the product.

Concept: Genotoxicity and Mutagenicity

Genotoxicity and Mutagenicity

Genotoxicity and mutagenicity are closely related terms. A genotoxic agent interacts with DNA and/or cellular apparatus that regulate the fidelity of the genome. Any such interaction can result in mutations and possibly lead to the development of cancer. While the concepts of genotoxicity and mutagenicity are related, it is important to note that all mutagens are genotoxic, however, not all genotoxins are mutagenic. Simply put, mutagenicity is a particular case of genotoxicity.



Mutagen and Mutagenicity

Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material of cells or organisms. Changes may involve a single gene or a block of genes. A mutagen is an agent, such as a chemical, radiation, or ultraviolet light that is capable of inducing a mutation or increasing the rate of mutations in an organism. Mutagenicity is strongly correlated with carcinogenicity. Many mutagens are known carcinogens.

Mutagenicity Testing

During the 1950s, bacteria strains with specifically engineered mutations were developed which allowed for relatively simple testing of the mutagenic nature of a substance. Testing for mutagenicity has since become a standard process in the risk assessment of cosmetics, pesticides, pharmaceuticals, and medical use products. The tester strains of bacteria have been mutated from their natural phenotype to be unable to produce histidine, in the case of *Salmonella*, or tryptophan, in the case of *E. coli*. When grown in a medium that contains little or no histidine (or tryptophan) the bacteria are unable to grow. However, in the presence of a mutagen, the bacteria are mutated back to their natural phenotype and survive. The growth of the surviving bacteria, called revertants as these bacteria have undergone a reverse mutation back to the natural phenotype, can be measured and correlated with mutagenicity. The strains have also been developed to be sensitive to a range of types of mutagenic agents.

The Muta-Lab™ kit is based on the most popular and validated bacterial reverse-mutation test, known as the `Ames Test'. Traditionally, reverse-mutation tests have used agar plates, known as `pour plate', `plate-incorporation' or `agar-overlay' assays. An alternate method performed entirely in liquid culture is the `Fluctuation test', originally devised by Luria and Delbruck (1943) and modified by Hubbard et al. (1984). The Muta-Lab™ kit employs the Fluctuation test method and includes all the reagents and disposable items to perform the test in a non-specialized laboratory.

The Reaction

Bacterial strains used in the ‘Ames test’ carry a specific mutation in a biosynthesis or metabolism pathway. As a result, Ames strains are dependent on the supplementation of the nutrient normally processed by this pathway and will not survive in normal growth media. However, specific mutations caused by toxicant interactions at sites in the bacterial DNA can produce reversions back to the wild type genome, a state in which the bacteria regain the ability to synthesize histidine.

The Escherichia coli strains used in these assays contain mutations at selected sites in the lactose metabolism pathway which are reverted by a specific type of interaction, like a frameshift or base-pair mutation, and permits the mechanism of DNA damage to be identified. The use of reversion mutations to detect genetic alterations makes the modified E. coli strains ideal screening tools to assess mutagenic potencies of chemical mixtures in low concentrations that may exert synergistic effects when present together. Exposure and incubation of the bacterial strain with a test sample, followed by selection of the revertant mutant colonies in a lactose deficient growth media provides rapid and facile identification and quantification of mutagenesis.

Expectations

The Muta-Lab™ is a bacterial based assay that tests for mutagenicity. The kit is based on the Ames test which is the gold standard to examine the potential of mutations in human genetics. Once your class completes the experiment and the colour change occurs, the results can be recorded to determine mutagenicity levels and each group will complete a lab report for evaluation. The Muta-Lab™ is designed for students to work in six groups of three to four students.

Contents of the Muta-Lab™ Kit

Each kit contains sufficient components for 12x 96-well microplates. The bottles and vials in the Muta-Lab™ are labelled with clear, bold letters.

Each kit should contain the following:

- 48x sterile test tubes
- 1 120ml reagent mixture Bottle
- 12 96-Well Sterile Microplates

Reagents:

A: Buffer (concentrate) 22 mL (1 unit)	H: Thiamine
B: Supplemental Media 5 mL (1 unit)	V: Reagent V (Strain Dependent)
C: pH Indicator 2.5 mL (1 unit)	W: Reagent W (Strain Dependent)
D: Vitamin 1.5 mL (1 unit)	X: Reagent X (Strain Dependent)
E: Sugar 40% 5 mL (1 unit)	Y: Reagent Y (Strain Dependent)
F: Sterile Distilled Water 250 mL (1 unit)	Z: Reagent Z (Strain Dependent)
G: Growth Media 5 mL (1 unit)	

Test Strains (ONE of the following is provided with each Muta-Lab™ kit)	Standard Mutagens (ONE of the following is provided with each Muta-Lab™ kit)
• *E. coli lacZ **GST-** (Base-Pair mutation) or **GST+** mutant base-pair detection with human GST T1-1 expression.* • *E. coli lacZ **DJ701** mutant frameshift detection or **DJ702** with human CYP P450 1A2 recombinant expression.*	• 4-Nitroquinoline 1-oxide (4-NQO, 150 µL) – for use with **DJ BP** • 2-Nitrofluorene (2-NF, 150 µL) – for use with **DJ FS** • Ethylene dibromide (EDB, 150 µL) – for use with **DJ GST+** **(High volatility!)** • 2-aminoanthracene (2-AA, 150 µL) – for use with **DJ 702**

Collecting the Samples

You have the option of either having your students acquire samples from pre-determined or assigned sources that you and/or your class discussed or you can acquire the samples. One sample are required from each source. Ensure your students understand and follow safety measures when acquiring the samples. The samples you or the students acquire must be liquid or able to be made into a liquid or dissolved in a solvent such as water or dimethyl sulfoxide (DMSO). The class should have a total of 12 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 2	- Sample 3	- Sample 4	- Sample 5	- Sample 6

Preparation of the Samples

Day Prior to the Experiment

Using aseptic technique, open one bottle of the (G) Growth Medium and add the necessary reagents (see below). Open one vial of lyophilized bacteria. Transfer all the Growth Media containing added reagents (G) to the vial of bacteria and mix. Cover with the rubber stopper and incubate the bottle at 37°C overnight (16 to 18 hours).

Strain	Standard Mutagen	Growth Conditions
DJ 701 FS	2-NF (2 ug/mL)	Using aseptic techniques, open one bottle (G) Growth Media, add 20 µL of (W) and 12.5 µL of (Y). Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate the bottle in a 37°C.
DJ 702 FS CYP 1A2 Expression	2-AA (100 ug/mL)	Using aseptic techniques, open one bottle (G) Growth Media, add 20 µL of (W), 10 µL of (V), 25 µL of (X), 12.5 µL of (Y) and 50 µL of (Z). Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate the bottle in a 37°C.
DJ GST - BP	4-NQO (5 ug/mL)	Using aseptic techniques, open one bottle (G) Growth Media, then add 50 µL of (Z). Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate the bottle in a 37°C.
DJ GST+ BP GST T1-1 Expression	EDB (1 ug/mL)	Using aseptic techniques, open one bottle (G) Growth Media, then add 10 µL of (V) and 50 µL of (Z). Open one vial of lyophilized bacteria and add the above nutrient mixture. Cover with rubber stopper, swirl and incubate the bottle in a 37°C.

Day of the Experiment

Sample preparation and dilution should be carried out on the day of the assay. Before commencing with the experiment, examine the bacteria grown overnight for any signs of turbidity. Continue only if turbidity exists.

Ensure that the samples collected are pH neutral and sterile. Start the preparation of your sample material and dilution procedures as early as possible on the day of the assay. Once the samples are properly filter-sterilized and diluted (if necessary), they have to be dispensed into the sterile tubes provided in the kit.

Whether the students provide you the samples or you collect the samples, please note the following:	If the sample is **water-based**, make sure that the samples are filter-sterilized to ensure no foreign bacteria contaminate your test, which may compromise the results. Aqueous samples may need to be filtered for sterility using a 0.22 µm filter.	If the sample is **solid-based**, the samples needs to be sterilized and in liquid form. It is recommended that 0.1 g of sample be dissolved in 0.5 mL of dimethyl sulfoxide (DMSO). This volume should be diluted with water to a total volume of 17.5 mL.

Preparing the Reaction Mixture:
Aseptically measure and mix these components in one of the 120 mL sterile Media bottles supplied with the kit.

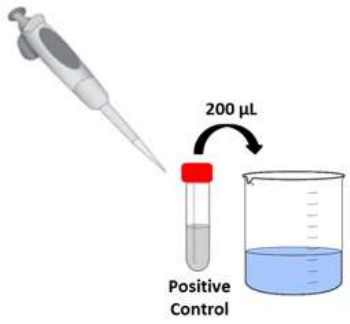
See the right columns for the volume amounts needed to put it together.

Components	Volume
A: Buffer (concentrate)	10.81 mL
B: Supplemental Media	0.06 mL
C: pH indicator	2.38 mL
D: Vitamin	1.19 mL
E: Sugar	4.75 mL
F: Sterile distilled water	10.81 mL
H: Thiamine	2.81 mL

Preparing the Controls:

For **positive control** treatments, add 200 µL of appropriate positive control (see the "Preparation of the Samples – Day Prior to the Experiment" to see which strain you are using) and 29.80 mL of sterile filtered water to a labelled 50 mL sterile tube or sterile beaker.

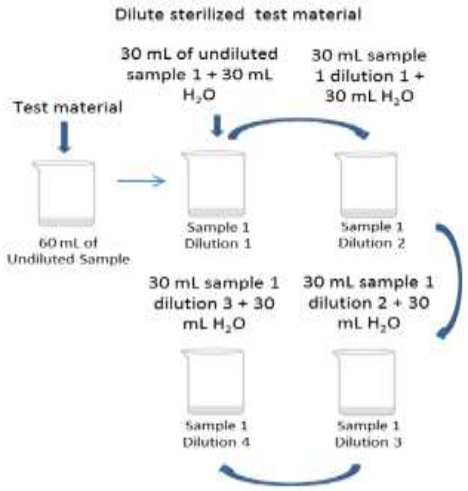
For **negative control and blank** treatments, sterile water can be dispensed directly from the provided container or from a labelled sterile beaker. Appropriate controls are essential to monitor the function of the bacteria.



Preparation of Sample Dilutions:

Prepare an undiluted sample by pouring 60 mL into a labelled sterile beaker or tube. Prepare a set of 2-fold dilutions by pipetting 30 mL of sterile water into three (3) other sterile containers. Transfer 30 mL of the undiluted sample into one of the other sterile tubes that contains sterile water. Mix this solution thoroughly and transfer 30 mL from this tube into the third tube.

Repeat these steps twice more and discard 30 mL from the final sterile container. You have now prepared a set of four dilutions for your sample. Each sterile tube should contain 30 mL of total volume.



Student Station Preparation

Below is the suggested distribution of supplies for the preparation of the lab stations for the day of the experiment:

STUDENT LAB STATIONS

Each group should have the following:

- 2x 96-well microplates
- 1x beaker for the Sample (sterile)
- 8x sterile 15 mL test tubes
- 5 mL vial of the Reaction Mixture (see previous page for preparation)
- Test sample

FRONT OF THE CLASSROOM

- 1x vial of bacteria
- 1 bottle of positive control (2 controls used when express strains are incorporated)
- Parafilm for

REQUIRED INSTRUMENTATION

- Micropipettes using disposable tips in the range of 5-200 uL
- 37°C incubator
- Sterile beakers or containers to prepare sample dilutions

Lab Safety Protocols – Review this with your class before beginning the experiment

Storage:

- For reference, the mutagenic standards are sodium azide, 2-nitrofluorene, ethylene dibromide and 2-aminoanthracene
- These mutagenic standards should be stored frozen (-4°C to -20°C) along with the lyophilized bacteria
- Protect from high temperatures and temperature changes
- If you plan to store the kit for a long period of time, keep the bacteria frozen
- Mutagenic standards, bacteria, and some of the reagents should be kept in total darkness

Handling:

- Bacterial strains are non-pathogenic laboratory strains; it is advised that good laboratory practice be used
- Sterilization of the bacteria either in an autoclave or a bleach bath is recommended after use
- The LacZ E.coli strains are considered Bio-Safety Level 1 handling
- Use the biohazard bag in the kit to collect and properly dispose of all used components after the completion of the Muta-Lab™

Procedure – Bacterial Muta-Lab™

1. Preparing test tubes with controls or samples

Part A: Aseptically add 0.625 mL of the reaction mixture to all tubes containing a sample or control using the provided single channel pipette and fresh tip (8 tubes). Ensure you are careful to avoid any type of bacterial contamination by holding caps, not touching dispensing tools to container lips and resealing test tubes immediately after additions are made.

Preparing the Controls:

For **positive control** treatments, add 20 µL of appropriate positive control to a 15ml tube and label it "Positive Control".

For **negative control and blank** treatments, sterile water can be dispensed directly from the provided container or from a labelled sterile beaker. Appropriate controls are essential to monitor the function of the bacteria.

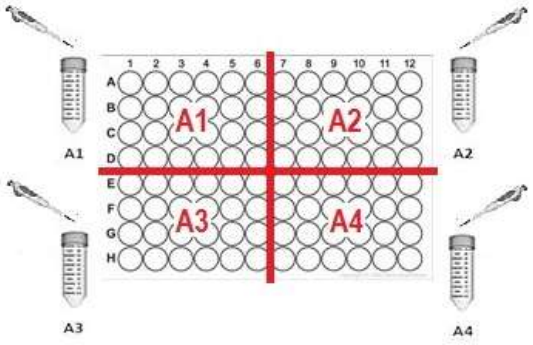
Part B: Dispense the amounts seen below to each corresponding labelled 15 mL sterile test tube. Each test tube must contain a total volume of 5 mL after the respective components are added. See the chart below to determine what needs to be added.

Microplate	Test Tube	Quadrant	Treatment	Bacteria Added (µL)	Standard (mL)	Sample (mL)	Water (mL)	Reaction Mixture (mL)
Microplate 1: Background & Controls Blank Sample (A1) / Background (A2) Background (A3) / Positive Control (A4)	A1	A1	Sample Blank	-	-	4.375	-	0.625
	A2	A2	Negative Control	5	-	-	4.375	0.625
	A3	A3	Negative Control	5	-	-	4.375	0.625
	A4	A4	Positive Control	5	0.02ml	0	3.355	0.625
Microplate 2: Sample Sample 1 Dilution 1 (A5) / Sample 1 Dilution 2 (A6) Sample 1 Dilution 3 (A7) / Sample 1 Dilution 4 (A8)	A5	A5	Sample 1 Dilution 1	5	-	4.375	0.004	0.625
	A6	A6	Sample 1 Dilution 2	5	-	0.437	0.043	0.625
	A7	A7	Sample 1 Dilution 3	5	-	0.043	0.437	0.625
	A8	A8	Sample 1 Dilution 4	5	-	0.004	4.375	0.625

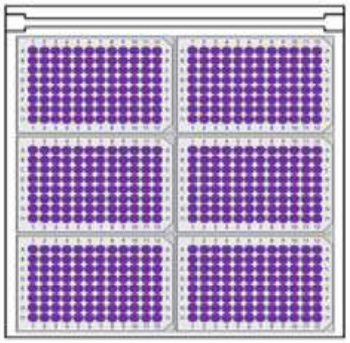
2. To each treatment tube containing the material to be tested, add 10 uL of the bacterial test strain broth culture, which has grown overnight, and mix thoroughly. (Ensure that the bacteria are fully suspended in the vial before withdrawing the micropipette).

3. Mix all 4 Centrifuge tubes well.

4. Divide each 96 well microplate into 24-well quadrants. Ensure each quadrant is clearly labelled. Use pipette to transfer 200 uL of solution A1 to each well in the quadrant. Ensure that a fresh sterile tip is used for each sample to avoid cross contamination. Repeat this step for A2, A3, A4, etc. until both microplates are done.



5. Cover the plate with a lid and seal in sterile airtight plastic bag(s) to prevent evaporation. If evaporation is allowed to occur during incubation, the solutes will concentrate in the wells and the test may not work properly.



6. Incubate the plates in a sterile airtight plastic bag at 37 ^{o}C for three to seven days. Monitor the progress of colour development and record daily revertants after day 3.

CLEAN UP – When you complete the experiment, place all used material in the autoclave bag for sterilization and disposal. Remember that most of the material has come in contact with either bacteria or potentially the positive control, which is a suspected mutagen. Proper disposal is essential.

To Educators: Please review the information as a visual guideline of the experiment results, recording the results, further analysis, and the answer sheet for the questions.

Recording the Results

Guidelines – Counting the Microplates

All yellow, partially yellow or turbid wells are positives. All purple wells are negative.

The `Blank' (i.e. no test material added) plates will show the level of spontaneous or background mutation of the assay organism. Students will record the number of positive wells for each plate using the provided table of the results significance handout.

Assessing Accuracy of the Results

Check the following to ensure the experiment worked properly:

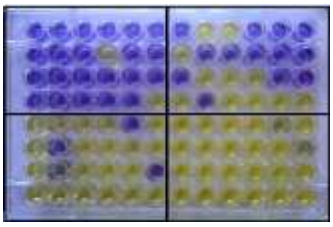
"Blank" quadrant: If yellow or turbid wells are observed, then the results are invalid.

Positive control: Nearly or all yellow, since it is a known mutagen for your strain.

Background rates of mutation should contain only a few yellow wells. If the background rate is too high, it would be impossible to tell whether your samples were mutagenic or not. If a treatment plate contains all purple wells, acute toxicity of the sample to the tester strain occurred.

Average score for negative or background control is ≥ 0 and ≤ 6 revertant wells per 24-well section on day 6	Average score for positive (standard mutagen) controls is ≥ 22 revertant wells per 24-well section on day 6

Figure: Muta-Lab™ control microplate after completing the experiment.



If the treatment microplate contains all purple wells, or rates of reversion that are significantly below the background, acute toxicity of the bacteria from the sample occurred. Try the test again using more dilute concentrations to look for mutagenicity.

If one or all the above criteria are not met, then the entire test is **invalid**. If all the controls and background quadrants are **acceptable**, then the students can compare their sample mutation rates to the background mutation rate for the statistical significance of the difference using the Results Significance Table (see the previous page).

Results Significance: Once the student groups complete their experiment, they can record the results from their microplates. If a sample results in a number of positive wells greater than the background, then the 'Significance Values Table' (the Table below) is used in order to determine the level of certainty that the sample is mutagenic. During this time, you can also assess and evaluate their results to note accuracy and any possible errors. Students should be encouraged to record their errors in their lab reports.

Number of positive wells in background quadrant	Number of positive wells in treatment to achieve associated significance %			Number of positive wells in background quadrant	Number of positive wells in treatment to achieve associated significance %		
	95%	99%	99.9%		95%	99%	99.9%
0	3	5	9	13	19	21	23
1	5	8	11	14	20	22	24
2	7	9	12	15	21	22	24
3	8	11	14	16	21	23	-
4	10	12	15	17	22	24	-
5	11	13	16	18	23	24	-
6	12	15	17	19	23	24	-
7	13	16	18	20	24	-	-
8	14	17	19	21	24	-	-
9	15	18	20	22	-	-	-
10	16	19	21	23	-	-	-
11	17	19	22	24	-	-	-
12	18	20	22				

Interpreting the Results: Once the students record their results, the students can answer the following questions:

1. Did you see mutagenicity or toxicity?
2. Were the results what you expected?
3. Group the class results according to the response and comment on reasons for the responses seen.

Further Analysis: You can pick further analysis questions based on your preference and the project stream's overall objectives.

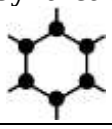
Questions and Answers

1. Explain the purpose of running the Muta-Lab™? In relation to reverse mutation?	The purpose of the Muta-Lab™ is to test whether different compounds are mutagenic at certain concentrations. A compound is classified as mutagenic if it has a significantly higher mutation rate as compared to the negative control. It may also indirectly give information about toxicity if a sample has a much lower mutation rate than the background, implying bacteria died before they could spontaneously mutate. The idea of a reverse mutation is used in order to track rates of mutation. Using the Muta-Lab™, the bacterial strain was purposely mutated so it does not produce histidine (an amino acid). Once that supply of histidine is exhausted, bacterial survival and colony formation is dependent on the mutant bacteria undergoing mutations and reverting to a phenotype that is able to produce histidine.
2. In your own words, describe the difference between mutagenicity and genotoxicity.	Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material of cells or organisms. A genotoxic agent regulates the fidelity of the genome. All mutagens are genotoxic, but, not all genotoxins are mutagenic. Accordingly, mutagenicity is a particular case of genotoxicity.
3. Why are "standard mutagens" included in the test?	Standard mutagens are included to ensure the test is working properly. The standard mutagen (or positive control) provides a demonstration of mutagenicity to which the results of the unknown samples can be compared.
4. Why is a "blank" quadrant included in the test?	A blank quadrant is included to ensure that the reagents used in the test are not producing a colour change. Comparing the results of the positive control to the blank demonstrates that the observed colour change is the result of a mutation in the bacteria and not from the reagents used. A colour change in the blank quadrant could also indicate contamination from bacteria which would grow and reproduce in the reaction mixture and induce a colour change.
5. Explain all components of the reaction mixture for the negative and positive control. Why are these controls needed?	a. Negative Control shows the spontaneous mutation rate of the bacteria, if it is not exposed to any possible mutagens. You could also include the solvent used to dissolve your samples in the negative control in order to make sure the solvent is not mutagenic or toxic to the bacteria. b. Positive Control shows what the response of the bacteria will be if it is, in fact, exposed to a mutagen. It is expected that the response in this preparation will be very high (with 24/24 wells (or close) turning yellow after the incubation period).
6. If your sample were to have a mutation rate less than that of the background, what would that mean? How would you adjust the protocol in order to determine whether your sample were in fact mutagenic or not?	If your sample has a lower mutation rate than the spontaneous background, it could mean that your sample is toxic to the bacteria, limiting growth and potential for mutation. If this is the case, you should perform dilutions of your sample in order to determine if there is a limit of toxicity, and if it becomes non-toxic, does the response rise above the background to prove mutagenic, or not.
7. You perform a series of experiments in which you test proteins purified from soybeans added to your reaction mixture. You find that the bacteria grow surprisingly well, with all 24 wells turning yellow. Does this necessarily mean that soybean proteins are highly mutagenic?	These results could mean one of two things: a) your sample is highly mutagenic or b) your protein sample included amino acids, with extra histidine being introduced with your sample. This would allow all his- bacteria to continue growing and reproducing after the minimal amount of histidine supplied by the reaction mixture was used, creating a population of his- mutants. This creates enough acidic compounds and metabolic byproducts to lower the pH, resulting in colour change. Instead of showing mutagenicity, the colour change reflects the initial his- bacteria was able to grow to a level large enough to create pH change without reverting to his+ in order to survive.

Student Lab Report

Group #	Group Student Names	Role

Checkmark the appropriate Stream below:

Water 	Chemical 	Soil 	Synthetic 

Note: At the beginning of class, your teacher will have the lab stations ready. Remember to check your lab station, ensure you have the appropriate supplies, and read over the safety protocols.

Pre-Lab Notes	

Instructions: Read over the preparation and the procedure for the specific biotechnology kit that you will be working on. Complete the lab and record the results.

Preparation
Bacterial Muta-Lab™

To Students: Please follow the instructions and information below to help prepare for the lab experiment.

Expectations

The Muta-Lab™ is a bacterial based assay that tests for mutagenicity. The kit is based on the Ames test which is the gold standard to examine the potential of mutations in human genetics. Once your group completes the experiment and the colour change occurs, record the results to determine mutagenicity levels and each group will complete a lab report for evaluation.

Collecting the Samples

Your educator will discuss whether your group will need to acquire samples from a pre-determined or assigned sources or if your educator will provide the samples. If you will be doing field work, ensure you acquire the samples safely. If you are collecting the samples, ensure it is in liquid form or able to be made into a liquid or dissolved in a solvent such as water or dimethyl sulfoxide (DMSO). There will be a total of 6 samples for the class organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 2	- Sample 3	- Sample 4	- Sample 5	- Sample 6

If you are completing the site sample study: Once you acquired your sample, submit your sample to the Educator.

Lab Station Preparation

At the beginning of the lab, you should have the following available in the front of the classroom and lab station:

FRONT OF THE CLASSROOM

- 1x vial of bacteria
- 2x bottles of positive control (2 controls used when express strains are incorporated)
- 2x Ziplock bags

GROUP LAB STATION

- 2x 96-well microplates
- 1x beaker for the Sample
- 8x sterile 15 mL test tubes
- 5 mL vial of the Reaction Mixture
- Test sample

REQUIRED INSTRUMENTATION

- Micropipettes using disposable tips in the range of 5-200 uL
- 37°C incubator
- Sterile beakers or containers to prepare sample dilutions

Before starting an experiment it is important to ensure all of the materials, supplies and equipment needed are available and ready. Be sure to speak to your educator if there is anything missing.

Ensure that you review the Lab Safety Protocols before beginning the experiment.

Storage:

- For reference, the mutagenic standards are sodium azide, 2-nitrofluorene, ethylene dibromide and 2-aminoanthracene
- These mutagenic standards should be stored frozen (-4°C to -20°C) along with the lyophilized bacteria
- Protect from high temperatures and temperature changes
- If you plan to store the kit for a long period of time, keep the bacteria frozen
- Mutagenic standards, bacteria, and some of the reagents should be kept in total darkness

Handling:

- Bacterial strains are non-pathogenic laboratory strains; it is advised that good laboratory practice be used
- Sterilization of the bacteria either in an autoclave or a bleach bath is recommended after use
- The LacZ E.coli strains are considered Bio-Safety Level 1 handling
- Use the biohazard bag in the kit to collect and properly dispose of all used components after the completion of the Muta-Lab™

Results & Analysis
Bacterial Muta-Lab™

Instructions: Once you obtained the results, read over the guidelines below before recording your results then complete the lab report to submit to your educator.

If you have any errors from the experiment, please describe them here and the reasons why this occurred:

Recording the Results:

Visual Guidelines – Counting the Microplates	Assessing Accuracy of the Results
All yellow, partially yellow or turbid wells are positives. All purple wells are negative. The `Blank' (i.e. no test material added) plates will show the level of spontaneous or background mutation of the assay organism. Record the number of positive wells for each plate using the table of the results significance (below).	Check the following to ensure the experiment worked properly: ▪ "Blank" quadrant: If yellow or turbid wells are observed, then the results are invalid. ▪ Positive control: Nearly or all yellow, since it is a known mutagen for your strain. ▪ Background rates of mutation should contain only a few yellow wells. If the background rate is too high, it would be impossible to tell whether your samples were mutagenic or not. If a treatment plate contains all purple wells, acute toxicity of the sample to the tester strain occurred.

Use the "**Significance Values Table**" below to help determine the level of certainty that the sample is mutagenic:

Number of positive wells in background quadrant	Number of positive wells in treatment to achieve associated significance %			Number of positive wells in background quadrant	Number of positive wells in treatment to achieve associated significance %		
	95%	99%	99.9%		95%	99%	99.9%
0	3	5	9	13	19	21	23
1	5	8	11	14	20	22	24
2	7	9	12	15	21	22	24
3	8	11	14	16	16	21	23
4	10	12	15	17	22	24	-
5	11	13	16	18	23	24	-
6	12	15	17	19	23	24	-
7	13	16	18	20	24	-	-
8	14	17	19	21	24	-	-
9	15	18	20	22	24	-	-
10	16	19	21	23	-	-	-
11	17	19	22	24	-	-	-
12	18	20	22				

Average score for negative or background control is ≥ 0 and ≤ 6 revertant wells per 24-well section on day 6	Average score for positive (standard mutagen) controls is ≥ 22 revertant wells per 24-well section on day 6

If one or all the above criteria are not met, then the entire test is invalid. If a treatment plate contains all purple wells, or rates of reversion that are significantly below the background, acute toxicity of the bacteria from the sample may have resulted. Try the test again using more dilute concentrations to look for mutagenicity. If all the controls and background quadrants are acceptable, then you can compare their sample mutation rates to the background mutation rate for the statistical significance of the difference using the "Results Significance Table" (see above).

Once the microplate experiment is done and the results from the controls are verified, your group can evaluate the mutagenicity of the samples by comparing sample quadrants to respective background quadrants. Use the 'Significance Values Table' to determine the level of significance of the difference between the background and the sample response to classify compounds are either mutagenic or non-mutagenic. You need to record these results in days 5, 6, and 7 after your microplates are sealed in Ziploc plastic bags. You can place the significance of the difference between the sample and the background (e.g. 95%, 99%, or 99.9%) by using the 'Significance Values Table' (see the previous page).

GROUP _____	**QUADRANT**	**PLATE DESCRIPTION**	**NUMBER OF POSITIVE WELLS**			**SIGNIFICANCE**
			Day 5	**Day 6**	**Day 7**	**Yes or No based on 95% certainty**
PLATE 1	1	Sample Blank				
	2	Negative Control				
	3	Negative Control				
	4	Positive Control				
PLATE 2	1	Sample 1 Dilution 1				
	2	Sample 1 Dilution 2				
	3	Sample 1 Dilution 3				
	4	Sample 1 Dilution 4				

Notes:

Interpreting the Results:

Did you see mutagenic changes or acute toxicity?

Were the results what you expected?

Further Analysis: Complete the following questions.

Explain the purpose of running the Muta-Lab™? In relation to reverse mutation?

In your own words, describe the difference between mutagenicity and genotoxicity.

Why are "standard mutagens" included in the test?

Why is a "blank" quadrant included in the test?

Explain all components of the reaction mixture for the negative and positive control. Why are these controls needed?

If your sample were to have a mutation rate less than that of the background, what would that mean? How would you adjust the protocol in order to determine whether your sample were in fact mutagenic or not?