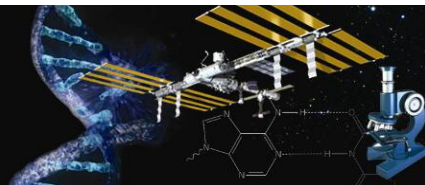




MATH AND SCIENCE @ WORK

AP* BIOLOGY Educator Edition



Space Bugs

TI-Nspire™ Lab Activity

This lab may replace or reinforce AP Biology Lab 6: Molecular Biology. The Math and Science @ Work Free-Response problem, *Microgravity Effects on Human Physiology: Immune System*, may be used in conjunction with this lab.

Instructional Objectives

Students will

- analyze the nucleotide composition of DNA;
- evaluate restriction maps of bacterial plasmids;
- analyze models of DNA gel electrophoresis; and
- predict the sizes of unknown DNA fragments by comparing them to known fragment sizes.

Teacher Preparation

Load the TI-Nspire file, *Space_Bugs.tns*, to the students' handhelds.

Class Time Required

This lab requires 60-80 minutes.

- Introduction: 10 minutes
 - Read and discuss the background section with the class before students work on the problem.
- Student Work Time: 40-60 minutes
- Post Discussion: 10 minutes

AP Biology Framework Alignment

Big Idea 3: Living systems store, retrieve, transmit, and respond to information essential to life processes.

- **Enduring understanding 3.A:** Heritable information provides for continuity of life.
 - 3.A.1: DNA, and in some cases RNA, is the primary source of heritable information.
 - 3.A.2: In eukaryotes, heritable information is passed to the next generation via processes that include the cell cycle and mitosis or meiosis plus fertilization.

Grade Level
10-12

Key Topic
Molecular Biology-DNA
Gel Electrophoresis

Teacher Prep Time
10 minutes

Lab Time
60-80 minutes

Materials/Equipment
- TI-Nspire Learning
Handhelds
- TI-Nspire file,
Space_Bugs.tns

**AP Biology
Framework Alignment**
Essential Knowledge:
3.A.1, 3.A.2, 3.A.4, 3.B.1,
3.C.1, 3.C.2

**NSES
Science Standards**
- Science and Technology
- Life Science

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- 3.A.4: The inheritance pattern of many traits cannot be explained by simple Mendelian genetics.
- **Enduring understanding 3.B:** Expression of genetic information involves cellular and molecular mechanisms.
 - 3.B.1: Gene regulation results in differential gene expression, leading to cell specialization.
- **Enduring understanding 3.C:** The processing of genetic information is imperfect and is a source of genetic variation.
 - 3.C.1: Changes in genotype can result in changes in phenotype.
 - 3.C.2: Biological systems have multiple processes that increase genetic variation.

NSES Science Standards

Science and Technology

- Understandings about science and technology

Life Science

- Molecular basis of heredity

Background

This lab activity is part of a series of activities that apply Math and Science @ Work in NASA's scientific laboratories.

From the beginning of the human spaceflight era, NASA has been mindful of the need to monitor the health and well-being of its astronauts. As such, specialists from the Immunology Laboratory at the NASA Johnson Space Center are investigating the effects of spaceflight on various aspects of human physiology, primarily the responses of the human immune system. The goal of this laboratory is to investigate the mechanisms responsible for physiological changes of the immune system, so that appropriate countermeasures or interventions can be developed for future human space exploration missions.

Another important aspect of human immunology is the study of infection by different pathogens. As part of this investigation, lab scientists from the Microbiology Laboratory at NASA Johnson Space Center routinely analyze infectious agents (bacteria, viruses, and fungi) which co-exist with astronauts on the space shuttle and on the International Space Station (ISS).

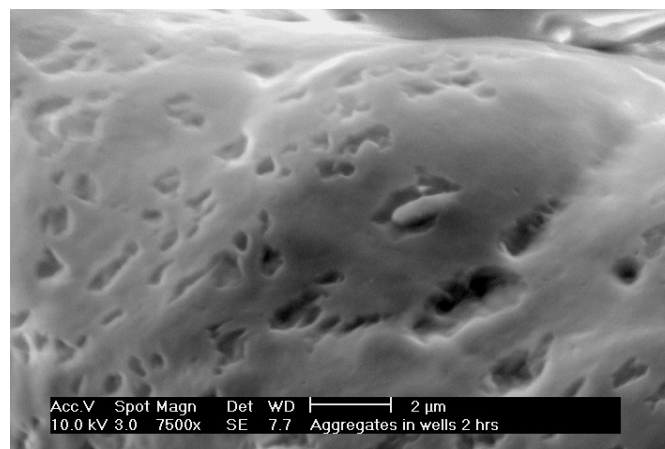


Figure 1: Environmental Scanning Electron Microscopy (ESEM) image of *Salmonella enterica* Typhimurium invading intestinal cell aggregates grown in vitro



Multiple spaceflight experiments have demonstrated that microorganisms change the way they grow and respond when cultured in the spaceflight environment. One change which concerns NASA scientists is evidence that microorganisms may change their virulence (disease-causing potential) during spaceflight. If microbes do cause disease at a higher rate in space, the astronauts are at greater risk, and it may be necessary for more protective measures to be put in place during a mission.

To better understand the potential risk, samples of microorganisms are collected from surfaces, air, and water located in the spacecraft. When these samples are returned to Earth, the DNA of these "space bugs" is evaluated to determine the presence of infectious agents which might infect the crew. While these "space bugs" are similar to microorganisms found on Earth, the microgravity environment may cause these microbes to behave differently than on Earth. Therefore, scientists continue to investigate how these microorganisms may change in space in order to better protect the crew.

Some microbial changes during spaceflight are the results of the way microbes respond to their environment. These are temporary changes. Other changes can occur that are permanent. These are DNA mutations, which can occur due to radiation. Changes can also occur when pieces of DNA (often in the form of circular DNA called plasmids) are shared with one another. Mutations caused by both radiation and exchange of plasmids can cause a number of changes in microorganisms, including changes in the virulence of organisms.

A variety of techniques are used to measure changes in DNA. A common way is through a process called DNA gel electrophoresis. This technique separates DNA based on its length and ability to move through a gel.

Lab Procedure

On your TI-Nspire handheld open the file, *Space_Bugs*. Work through the entire activity and answer the questions embedded throughout the document.

Solution Key

Throughout this activity, students are provided information and questions within the TI-Nspire document, *Space_Bugs.tns*. A solution key, *Space_Bugs_Solutions.tns*, is also provided for the instructor to review with students using TI-Nspire software.

Mission (TI-Nspire page 1.2)

In this activity, you will be asked to analyze the basic composition of DNA. You will then look at how DNA is "cut up" with special molecular scissors (called *restriction enzymes*), so it can be analyzed. Finally, you will observe and evaluate the results of some simulated *DNA gel electrophoresis* trials.

Lab Activity and Questions (TI-Nspire pages 1.3-4.11)

DNA from all organisms has the same basic structure. There are only four building blocks of DNA molecules, called *nucleotides*: **A**denine, **T**hymine, **G**uanine, and **C**ytosine. The rungs of the DNA ladder are made of two of the four nucleotides called *base pairs*, and they are either T-A (or A-T) or G-C (or C-G). Therefore, in any sample of DNA, the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine. This is known as *Chargaff's Rule*; and in 1953, it helped Watson and Crick determine the *double-helix* structure of the DNA molecule, for which they won a Nobel Prize in 1962.

Staphylococcus aureus TW20 (*S. aureus*), generally referred to as staph, is commonly isolated from the nasal area of all humans, including astronauts. *S. aureus* is very common, and can cause a wide variety of diseases under the right circumstances, including boils, food poisoning, bone infections, and



severe skin infections. Studies aboard spacecraft have shown that, despite all precautionary measures, *S. aureus* is still commonly passed between crew members.

- 1.6 The DNA of *S. aureus* consists of 32.78% G-C base pairs. What percentage of the *S. aureus* DNA consists of A-T base pairs?

67.22%

- 1.7 What percentage of the *S. aureus* DNA nucleotides is guanine?

16.39%

- 1.8 What percentage of the *S. aureus* DNA nucleotides is cytosine?

16.39%

- 1.9 What percentage of the *S. aureus* DNA nucleotides is adenine?

33.61%

- 1.10 What percentage of the *S. aureus* DNA nucleotides is thymine?

33.61%

- 1.11 The entire, circular chromosome of *S. aureus* consists of 29,585 base pairs. How many nucleotides comprise this chromosome?

59,170

- 1.12 How many of the *S. aureus* base pairs are A-T?

19,887

- 1.13 How many of the *S. aureus* base pairs are C-G?

9,698

- 1.14 How many thymine nucleotides are there in the *S. aureus* genome?

19,887

- 1.15 How many cytosine nucleotides are there in the *S. aureus* genome?

9,698

- 1.16 The human genome consists of about 3.15×10^9 base pairs, and 61.2% of these base pairs are

A-T. How many of each of the four DNA nucleotides are in the human genome?

Adenine = 9.64×10^8

Thymine = 9.64×10^8

Cytosine = 6.11×10^8

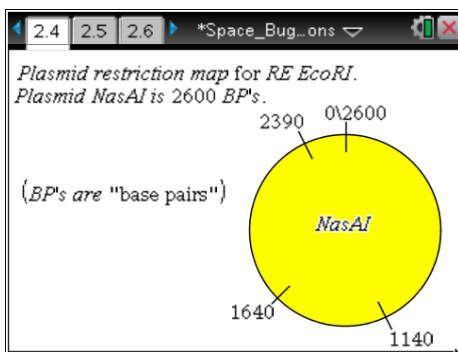
Guanine = 6.11×10^8



Many bacteria produce special chemicals called *restriction enzymes* (REs), which are produced as a bacterial defense system to cut up foreign DNA from, for instance, a virus. REs cut DNA at specific locations called *restriction sites*. If DNA is exposed to a restriction enzyme, the DNA will be cut at a restriction site regardless of the organism (humans included).

In the laboratory, molecular biologists can use restriction enzymes to cut up DNA from two different organisms and then splice these pieces of DNA to one another, thus creating *recombinant DNA*. Once new DNA is placed into a host organism, the organism's genome will be changed, thus allowing it to make new proteins.

Page 2.4 (on the TI-Nspire handheld) shows a model of a *bacterial plasmid*, which is a small piece of DNA, separate from the main chromosome that some bacteria possess. Plasmids have become very useful to genetic researchers. Bacterial plasmids, which are proteins such as *insulin* and *human growth hormone*, are now made for human use.



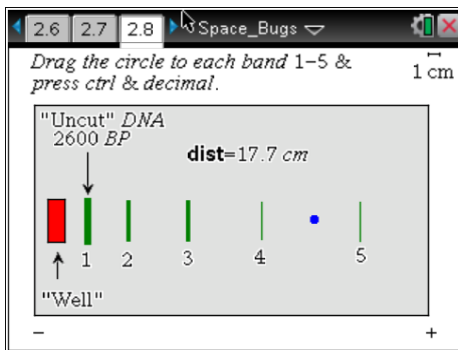
2.5 If the NasAI plasmid is digested with the restriction enzyme EcoRI, how many DNA fragments will be produced per plasmid?

4

2.6 If the NasAI plasmid is exposed to EcoRI, what will be the sizes of the fragments produced?

1140, 750, 500, 210

Page 2.8 shows a gel that was produced using EcoRI as the restriction enzyme. Find the distance of the DNA migrations by dragging the circle to each band numbered 1-5. *Do not drag it to the well.* When the circle is on each band, press **ctrl** and **decimal**. This will record the band distance and automatically record it in the spreadsheet and graph on the pages following.



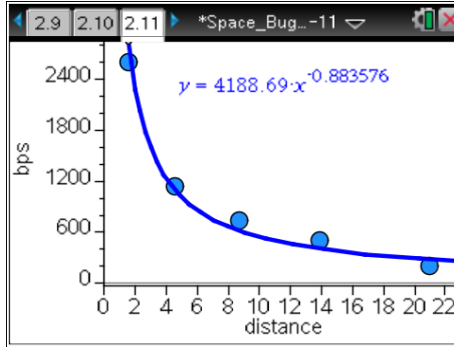
2.9 Why is the well into which the DNA is placed near the negative electrode?



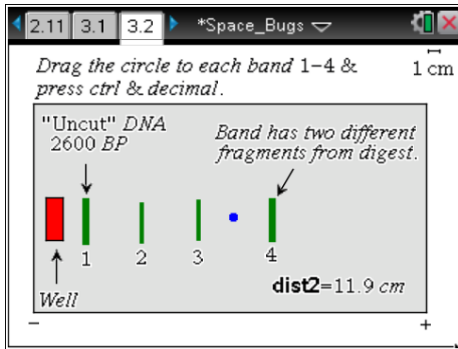
DNA is negatively charged and will move toward the positive electrode.

Teacher Note: The screenshots below show the spreadsheet and graph that were generated by the data collection on 2.8. Students will be using the equation of the regression line shown later in the activity to analyze two more gels.

distance	ecor1_bp
1.50943	2600.
4.5283	1140.
8.65409	750.
13.8868	500.
20.9308	210.



The following page shows a gel that was produced using a different restriction enzyme, XbaI, on the NasAI plasmid. Follow the instructions to measure the distance of the DNA migrations. *Do not drag the circle to the well—only to the four bands.*

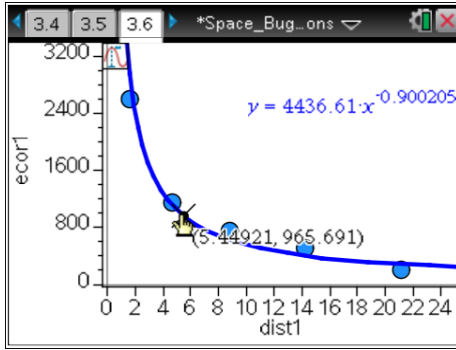


distance2	xba1_bp
1.40881	
5.43396	
9.35849	
14.6918	

Use the distances that were recorded in the spreadsheet to predict the sizes of the DNA fragments in the XbaI gel on page 3.2. You will do this by using the regression model that you made for the EcoRI gel.

Go to the next page, click on the regression model, press **ctrl, menu**, and select **Graph Trace**. Move along the regression model by gently sliding your finger on the touchpad until you find the approximate distance (the x-value). Record the corresponding base pairs (the y-value) in the spreadsheet on page 3.3.

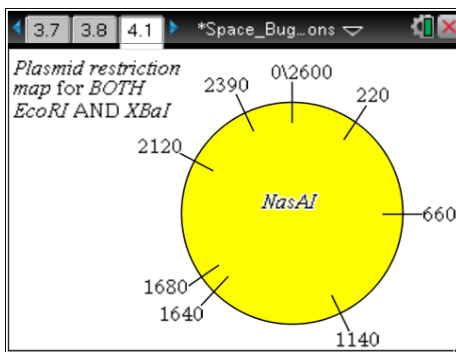
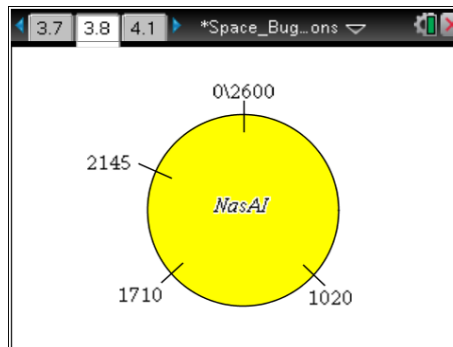
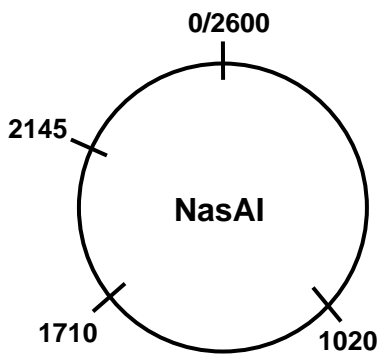
Alternatively, insert a calculator page and use the regression equation from the EcoRI graph to make your predictions.



A	B	C	D
distance2	xbal_bp		
1.40881	3259.		
5.43396	967.		
9.35849	593.		
14.6918	395.		

3.7 Using the information from the XBaI gel on page 3.2, draw a possible plasmid restriction map on your student handout for the NasAI plasmid when it is exposed to the restriction enzyme, XBaI, or insert a geometry page and draw it there.

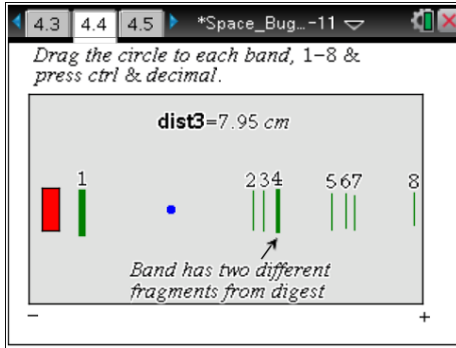
To insert a geometry page, press **ctrl** and **doc**, then select **Add Geometry**. To create a circle, press **menu** and select **Shapes > Circle**. To create segments, press **menu** and select **Points & Lines > Segment**. To add text, press **menu** and select **Actions > Text**.



4.2 If the plasmid is digested with both EcoRI and XBaI, how many DNA fragments will be produced per plasmid?

8

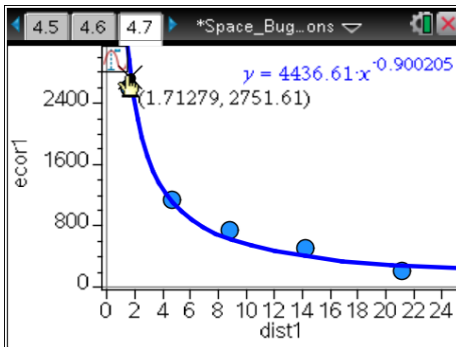
The following page shows a gel that was produced using both restriction enzymes, EcoRI and XBaI, on the NasAI plasmid. Measure the distance of the DNA migrations by dragging the circle to the bands numbered 1-8. Do not drag the circle to the well—only to the eight bands.



A	B	C	D
distance3	both_enz		
=capture('dis			
1	1.70943		
2	13.7849		
3	14.5899		
4	15.4956		
5	19.4201		
B6			

Use the distances that were recorded in the spreadsheet on page 4.5 to predict the sizes of the DNA fragments in the gel on page 4.4. Go to the graph on the next page, select Graph Trace, and move along the regression model until you find the approximate distance (the x-value). Record the corresponding base pairs in the spreadsheet.

Alternatively, insert a calculator page and use the regression equation from the *EcoRI* graph to make your predictions.



A	B	C	D
distance3	both_enz		
=capture('dis			
1	1.70943	2738.	
2	13.7849	419.	
3	14.5899	397.	
4	15.4956	376.	
5	19.4201	307.	
B7	286		

- 4.8 If the plasmid is exposed to both restriction enzymes, what will be the sizes of the fragments produced?
220, 440, 480, 500, 40, 440, 270, 210
- 4.9 Not counting the uncut DNA, the gel only has seven bands of DNA instead of eight. Explain.
Two of the fragment sizes are identical, so they show up as one band in the same location.
- 4.10 Explain why many of the DNA fragments in this gel are much smaller (migrated further) than in the other two gels.
Two restriction enzymes were used, so the DNA fragments were cut into smaller pieces.
- 4.11 Explain why the smaller DNA fragments end up furthest from the well.
Smaller fragments have an easier time getting pulled through the gel.

Contributors

This problem was developed by the Human Research Program Education and Outreach (HRPEO) team with the help of NASA subject matter experts and high school AP Biology instructors.

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