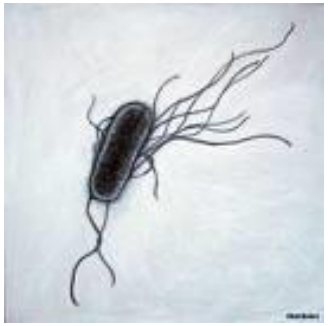


Team Biology

Katherine Cullis, Susanna Jacobs, Puja Kapoor, Kim Richard, Shellene Wright

Mission Overview

Objective:

To determine the effects various of near space conditions on a strain of non-pathogenic E. Coli bacteria

Expectation:

To observe genetic mutation resulting from the extreme conditions of near space (i.e.- radiation, extreme cold, low pressure and oxygen deprivation.)

Hypothesis:

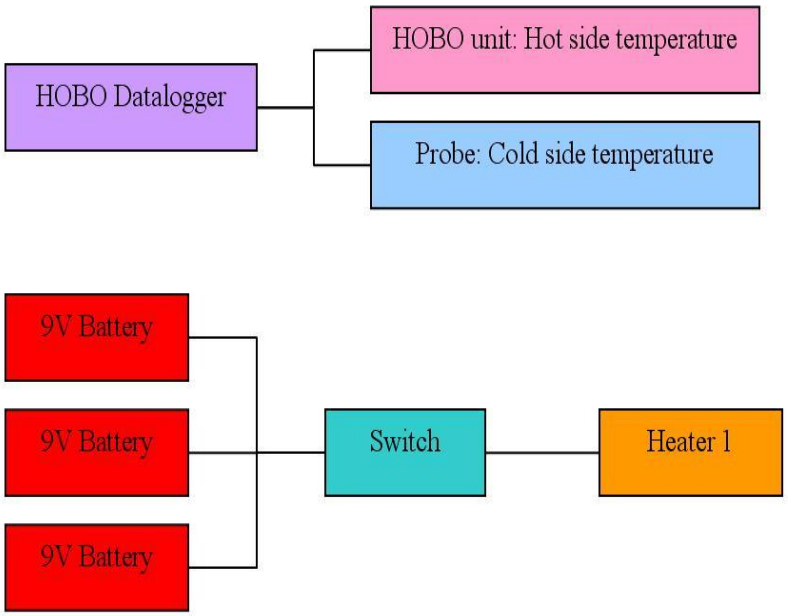
Evidence of genetic mutation will be observed in a heated bacterial culture plate which will be exposed to various types of radiation.

Why this mission?

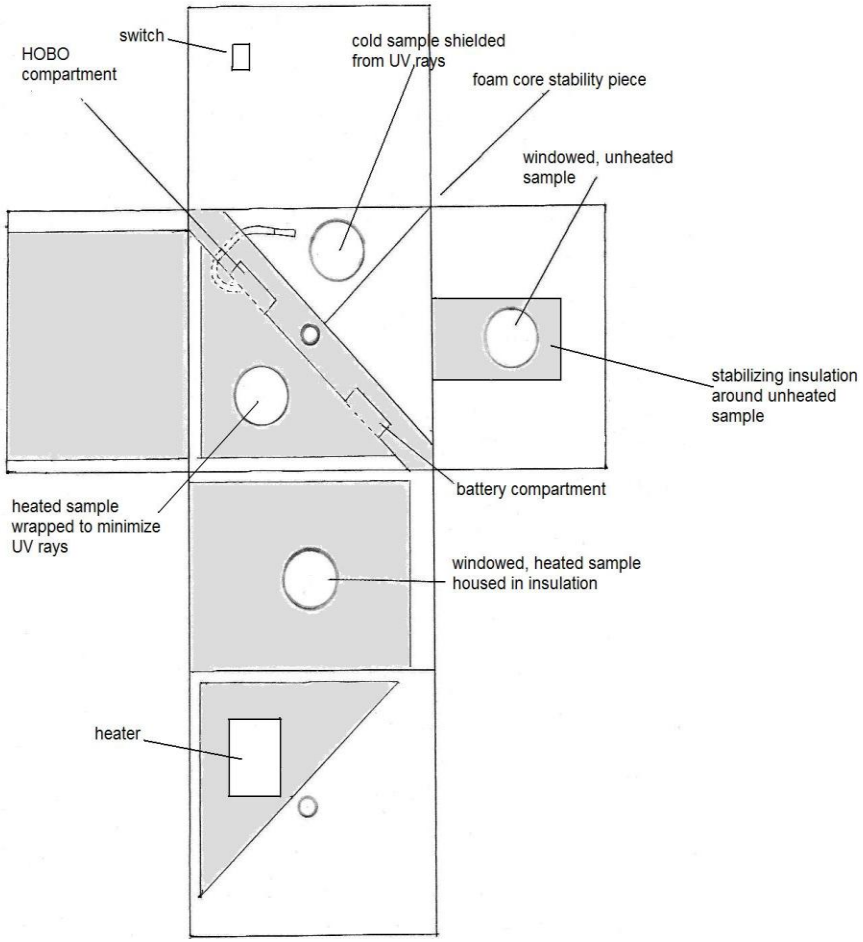
To take advantage of near space conditions to learn more about the conditions most conducive to genetic mutation and possible cellular differentiation.

Payload Diagram

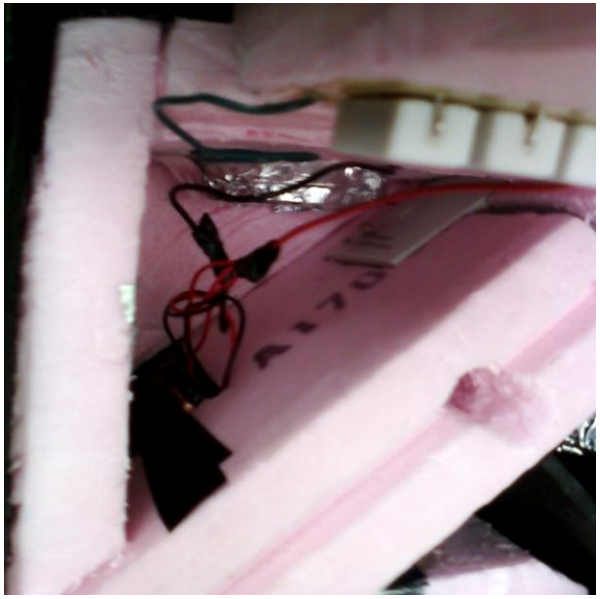
Functional Block Diagram



Sketch

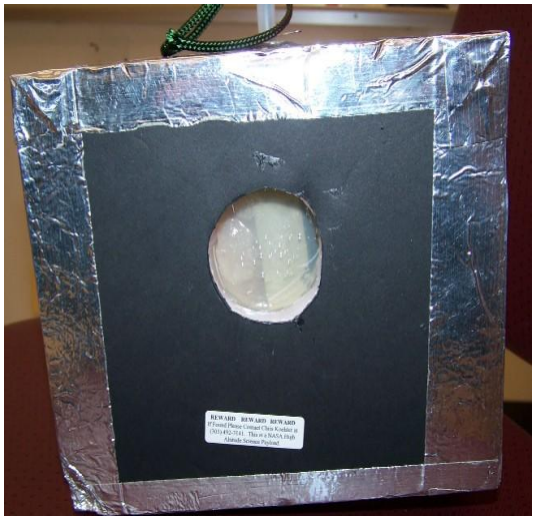


Final Design



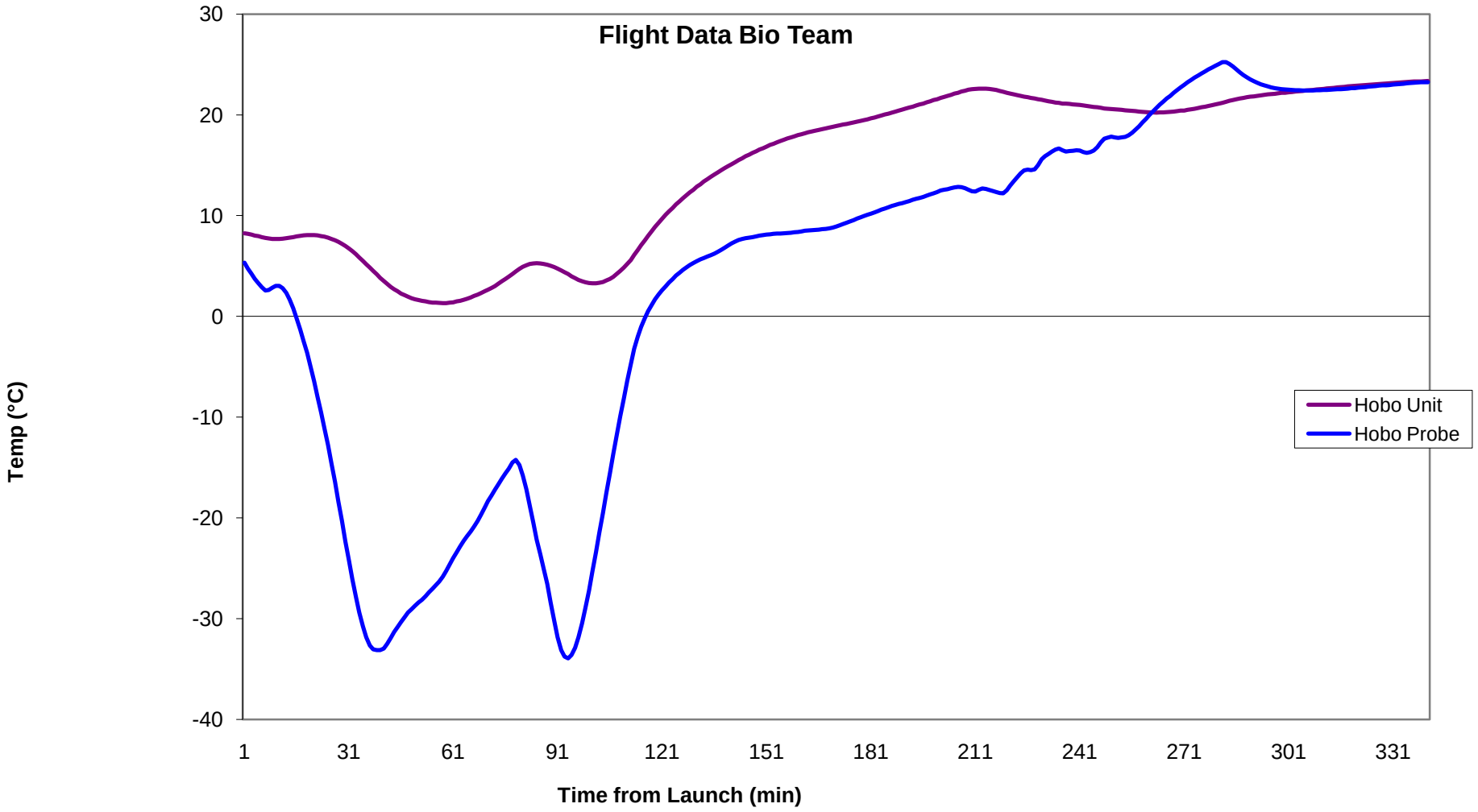
- After performing freezer tests at -80C, the polystyrene insulation performed the best in keeping our samples above zero degrees Celsius.
- The HOBO and batteries were seated within the insulation, increasing the security of the components in turbulent conditions.
- The insulation was twice as thick in the cross section in order to increase stability and insulating properties between the cold and warm sides respectively.
- Hot glue was sufficient in securing the biological plates in the windows and allowed us to keep our samples from becoming contaminated before launch.
- After performing tests for heater battery life and temperature, a 3 resistor, 3 battery heater performed optimally and was chosen for our satellite.
- We also performed tests on our media (LB agar) to ensure that the media would be stable in both the warm and cold sides of the satellite. The integrity of the media upon thawing proved to be the weakness of the standard concentration, and therefore the concentration of agarose (a solidifying agent) was doubled.

Post launch



•No external damage to the satellite was noted, all components remained in their proper places throughout flight and recovery.
•No internal damage, all components recovered successfully for analysis.

After analysis of the HOBO data, we see that the heater performed as expected and our samples were kept at desired temperatures for the duration of the flight.



Results

Predicted

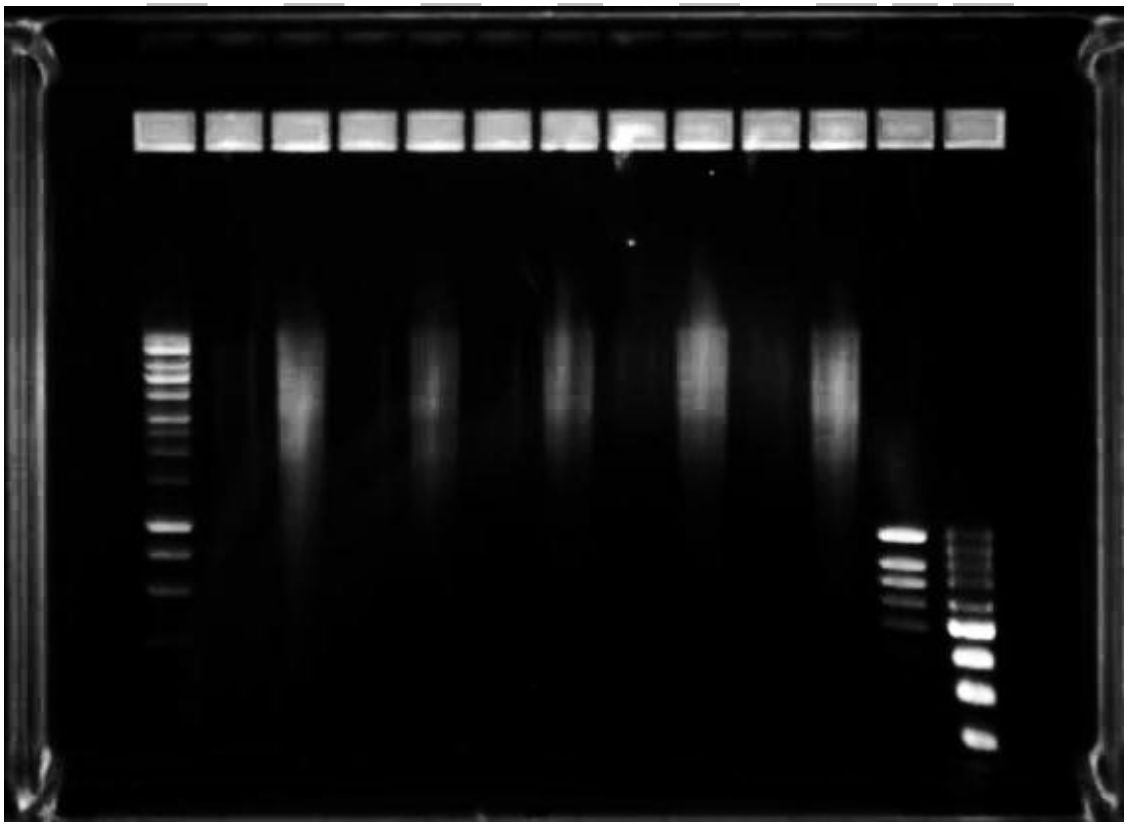
- We expect to observe at least some mutation of the bacterial genome. We believe mutation is most likely to occur in the bacterial plate which will be heated and mounted in a window for maximum radiation exposure.
- If this is accurate, we will observe differences in the genetic sequence of the bacteria after we have recovered it as compared to the control sequence obtained before launch. We may also be able to visualize these mutations using restriction fragment length polymorphisms (RFLP) on a gel electrophoresis plate.
- We do, however, acknowledge that the duration of flight may be too brief to effect any mutations, in which case the genetic sequence of the post-flight bacteria will be identical to the pre-flight control sequence.

Actual

- We did observe mutations in all samples that we were able to analyze. One sample failed to sequence (heated foil sample) but all others showed deviation from the control sample. Upon further genetic isolation and sequencing, we believe we will find mutations in the heated foil sample. The highest deviation occurred in the heated, windowed sample, as predicted.
- The gel electrophoresis test did not yield definitive results for identification of mutations. Although some bands in the genetic sequence can be seen, gel electrophoresis plates are best used for smaller pieces of DNA, not genomic sequences.
- The duration of the flight proved to be long enough for results. A longer flight would be beneficial in finding more precise results.

E. coli JM109 bacteria DNA Gel Electrophoresis

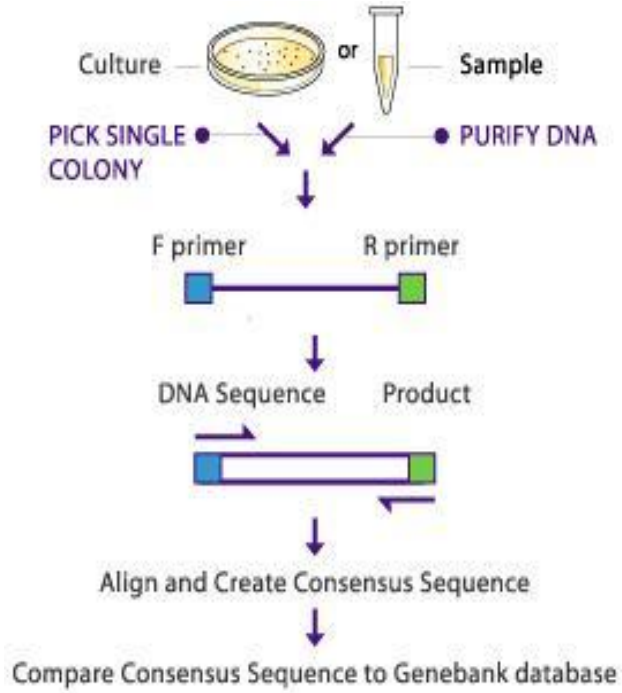
Experimental Treatments



Environmental conditions

Control Heated Cold

The results seen here are an indication that the pieces of DNA we were analyzing were too large. The samples on lanes 1, 12, and 13 are “ladder” samples that are manufactured and pre-cut to known lengths. This allows us to compare our sample sizes to a ladder in order to identify the size of our piece of DNA. Typically, a sample would be cut by restriction enzymes and the bands that illuminate would be distinguishable to the eye. However, in our test, the DNA in question was not a small sequence, but a whole genome of E. coli and the quantity of DNA was too large, causing the smearing effect seen here. Small separations can be seen here but they are not clear enough to identify differences in the samples.



Genetic Sequencing

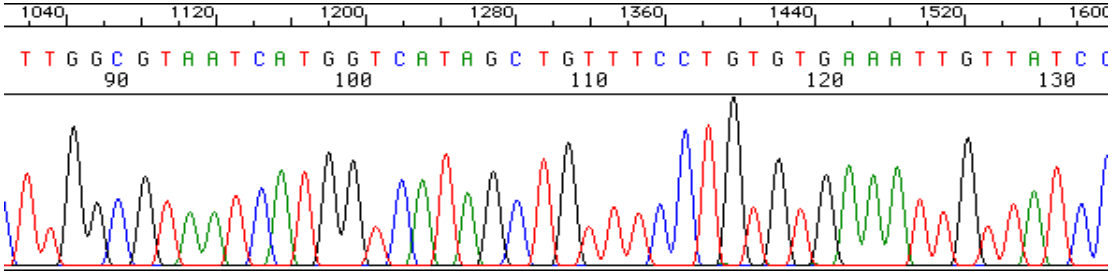
- Cultures were regrown in liquid broth, restreaked on sterile plates, and an individual colony was selected for DNA extraction.
- DNA extraction was performed via the Qiagen DNeasy Blood and Tissue Kit.
- Upon completion of DNA extraction, we used a biophotometer to quantify the amount of DNA present in our samples. (below)
- The extracted DNA was then used in the Applied Biosystems BigDye Terminator Cycle Sequencing Kit to purify it and prepare it to be run through the genetic sequencer.
- The following day we began running the DNA through the genetic sequencer. Our results were successful for all samples except the sample obtained from the heated foil covered plate. For reasons unknown, these samples produced indecipherable results when sequenced.

Sample Identity	Quantity of DNA in ng/µl
Heated Windowed Plate	129.8
Heated Foil Plate	73.9
Cold Windowed Plate	116.0
Cold Foil Plate*	129.8
Control Plate	129.8

*50% dilution

•Our specific strain of ***E. coli***, JM109, is not sequenced on Genbank (a large public database that allows users to compare their experimentally acquired genetic sequences to known archived genetic sequences), so all data is based on a percentage match (base pairs in common per 100) with a very well studied strain of ***E. coli***, DH1.

•These results are excellent support for our original hypothesis that the most mutation would occur in the heated windowed culture plate. The two cold plates were likely somewhat protected by the fact that they were deep frozen and not actively trying to respirate in the highest elevations during flight. As such, very little deviation from the original sequence was observed. The heated windowed plate, however, was trying to respirate during flight and was exposed to the maximum number of potentially mutagenic factors. As a result, it appears to have undergone multiple mutations during flight.



Sample Identity	% Match to DH1
Control	99%
Cold Windowed Plate	98.5%
Cold Foil Plate	97%
Heated Windowed Plate	93%
Heated Foil Plate	99%*

*second analysis

Follow-up analysis and results

- The inconclusive findings of the gel electrophoresis warrants additional testing better suited to genomic DNA. Performing a Southern Blot test will allow us to look for mutations with more accurate results.
- Specific mutation sites are being studied to better understand why unexpected mutations occurred, and what implication that may have to coding regions of the DNA in question.
- Repeated sequencing of the DNA from the original samples yielded results for the sample that failed to sequence at a 99% match with some evidence of DNA breakage.
- Sequencing of samples allowed to replicate showed rapid rates of cellular repair.

Benefits to NASA or the Scientific Community

Currently, one of NASA’s objectives is to study the effects of extreme conditions, like those present in space and near space on the life and functionality of cells. NASA Astrobiology Roadmap Goal 5, Objective 5.1 states: "Conduct environmental perturbation experiments on single microbial species to observe and quantify adaptive evolution to astrobiologically relevant environments."

Our experiment was designed to determine the effects on the genetic material of bacterial cells caused by various combinations of extreme conditions.

With this information, we will gain better understanding of the survivability of space and near space conditions to one of life’s simplest forms-bacteria.

This is significant, as we do send the normal flora indigenous to our bodies into space with every astronaut.

In conclusion

- The samples flown produced results similar to what was predicted, the evidence of mutations is promising in the heated, windowed sample and warrants further investigation.
- There are additional methods of analysis that we can use to produce more distinct results, such as the Southern Blot.
- While the mission was successful, there is more analysis that can be done. We would like to look more closely at the sites of mutation and their function, as well as possible back mutations, and repair mechanisms.
- Ideally, we would love the opportunity repeat the Balloon Sat flight as well as a longer duration flight.