# Article

GENES

### **IN SPACE** Genes in Space 2022 Finalists Contributing Ideas to Advance Space Biology

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#### INTRODUCTION

This fall, we will witness the beginning of a new chapter for NASA's space program: the launch of the Artemis 1 mission. Launches happen all the time, so what makes Artemis 1 special? It marks the kickoff of the Artemis program, which will bring humans back to the moon in 2025.

If Artemis and missions like it are to be successful, we will need to learn much more about how humans will fare under the conditions of space. Can we build sustainable systems to grow food in space? Can we protect our bodies from the dangers of cosmic radiation? How does microgravity affect our health and longevity? All of these questions - and many more - will need to be answered if we are to establish a permanent human presence in space.

Genes in Space is a competition for middle and high school students who want to get involved with the search for these answers. Each year, we invite students to design biology experiments that will help us understand how life is affected by the unique conditions of space. One winning experiment is selected to fly to the International Space Station (ISS), where it is carried out by astronauts.

The ISS is a testing ground where we develop systems and tools that support long-term spaceflight, and do experiments that help us understand how life is affected by the conditions of space. Since it was established as a permanently crewed, orbiting research outpost in the year 2000, more than 3,000 scientific investigations have been carried out aboard the ISS, in fields ranging from physical science to biology to Earth science.

Genes in Space was founded in 2015. In its seven years, the contest has inspired proposals from 8,600 students and launched nine winning experiments to the ISS. Each student-led investigation has pioneered the use of new technology in space, taught us something about how life is affected by cosmic conditions, and built a knowledge base that will inform the development of future safeguards for astronauts.

On the following pages, the 2022 Genes in Space finalists publish their award-winning proposals in hopes of seeding inspiration for future innovators. We hope you find their ideas as compelling as we did.



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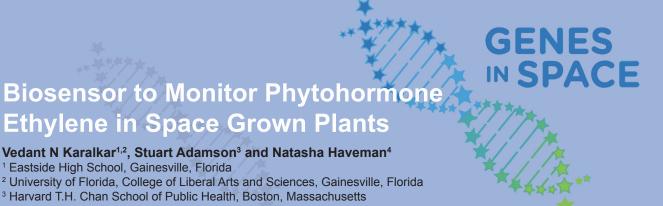


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Madhav Gulati and Vineeth Sendilrai Lambert High School, Suwanee, Georgia

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# Article



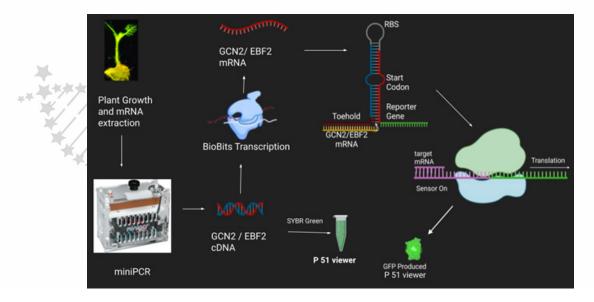
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#### ABSTRACT

In plants, the phytohormone ethylene plays a central role in stress response, increasing in response to changes in salinity, metals, hypoxia, air pollutants, flooding, and pathogen attack. In microgravity flooding of plant roots is a major concern, and great care has been taken to mitigate this with special water delivery systems. When these methods fail, early detection of ethylene spikes would allow for preventative actions that restore plant health, thereby reducing crop losses. As astronaut Mark Kelly observed, crop loss is an eminent threat aboard the ISS and having a precursor of ethylene to monitor it could be highly effective in crop loss prevention. Ethylene is typically detected using photoacoustic spectroscopy or gas chromatography. However, these analytical methods are impractical for real-time measurements on the International Space Station (ISS), due to their large footprint and time intensive protocols. We have proposed a novel method to use miniPCR and genetically encoded BioBits biosensors to rapidly and efficiently detect changes in the concentration and distribution of the ethylene response in plants.

Dormant, wild-type Arabidopsis thaliana seeds will be brought to the ISS. To simulate environmental stress, ethylene treatment will be performed by planting the seeds in media containing the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). Flooding stress will be examined by placing mature plants (5-week-old) into distilled water. After stress exposure, mRNA will be extracted and synthesized into cDNA for quantitative-PCR to measure changes in *GCN2* and *EBF2* expression levels. *GCN2* will be used as a biomarker for flooding stress, while *EBF2* will be used as a biomarker for environmental stress. Additionally, we will use BioBits in combination with a toehold system to generate a fluorescent signal for the rapid detection of the respective biomarkers.

Development of this tool will create a point-of-care sensor for the rapid detection of stress hormones in plants. This experimentation will lead to well-informed protocols aimed at simultaneously monitoring of multiple plant hormones critical to health. A rapid noninvasive sensor to measure plant health will lead to better crop productivity, stability, and safety of space cropping, an essential part of crew diets and biological life support systems.



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# Article



### Neuronal changes in space: A deeper look into the role of microtubules

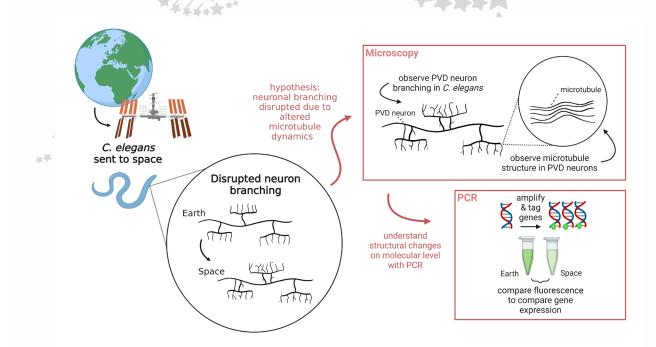
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#### ABSTRACT

To make long-term space travel possible, we need to have a deep understanding of neuronal health during long stretches in space and a confidence in our ability to protect it. Changes to the development of neurons have been previously observed in space. Microtubules, key components of the cytoskeleton that are centrally involved in neuronal development, become shorter and wavier in many cells in space, but these defects have not been characterized in neurons. We hypothesize that changes in the dynamics and structure of microtubules are causing neurons to develop differently in space.

To test this hypothesis, we will observe neuronal microtubules that have developed on earth and in space on both microscopic and molecular levels. Using C. elegans, which has a well-documented nervous system, we will first examine the development of fluorescently tagged PVD neurons by observing their branching organization, while simultaneously monitoring the structure of microtubules in each branch using Tubulin Tracker Deep Red. We will then use paclitaxel and colchicine as tools to cause microtubule polymerization and depolymerization, respectively. Such controlled manipulation of microtubules will allow us to stimulate and replicate potential changes we observe to microtubules in space. We will also monitor the expression of six microtubule-related genes using RT-PCR: *tba-1*, *tbb-2*, *cls-2*, *mei-1*, *unc-116*, and *bicd-1*, which will provide us insights into changes to the composition, growth, shrinkage, and transport performance of microtubules.

Comparing the results of PCR and microscopy in space to that on Earth will provide us a more complete picture of how microtubules affect neuronal development and will help us understand why neurons grow differently in space on the cellular and molecular level. This deep understanding will help protect the health of astronauts on long-term missions and can be applied in the development of drugs and treatments for cytoskeleton-related diseases on Earth.



# Article



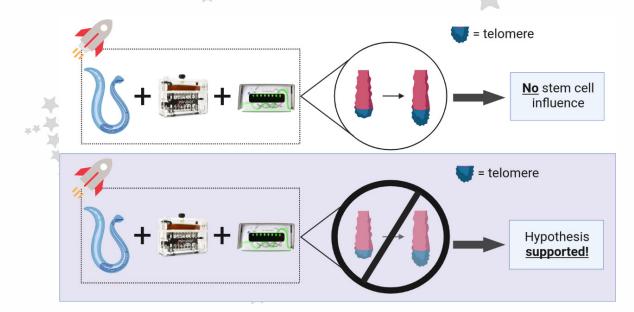
#### ABSTRACT

To ensure that we can safely explore the cosmos, we need to fully understand the challenges space travel poses to human health. Of particular concern is the fact that astronauts in space undergo physiological changes reminiscent of aging, such as muscle-wasting and increased arterial stiffness. However, their cellular processes suggest the opposite phenomenon.

While astronauts are in space, they develop longer telomeres. Numerous studies have supported the link between longer telomeres and attributes of youthfulness, such as decreased cell senescence, on Earth. In context, this finding suggests that spaceflight could paradoxically be the cellular "fountain of youth," despite the aging-like degeneration astronauts experience on a larger scale. A potential mechanistic explanation may be that the elongated telomeres reflect a healing response to the stressful conditions of outer space. Stem cells maintain long telomeres throughout their lives, and their elevated proliferation as a response to space-induced cell death could result in a higher population of them existing in tissue throughout the body, manifesting as observed telomere elongation.

To test this idea, the cause of spaceflight telomere elongation needs to be understood. We will conduct an experiment to investigate the potential impact stem cells may have on this phenomenon. To test the hypothesis that elongated telomeres are the result of stem cell dynamics, we propose sending *C. elegans*, a nematode without typical stem cells, to space for eleven consecutive days. After that period, telomere length of a specific chromosome would be analyzed using single-telomere length analysis (STELA).

If *C. elegans* do not develop longer telomeres it would suggest that stem cells may influence spaceflight telomere elongation. Alternatively, even if the opposite occurred, the evidence would suggest that astronaut-telomere elongation operates independently of stem cells. Through this experiment, we may advance closer to understanding the long-term health effects of spaceflight telomere elongation on astronauts as commercial spaceflight and long-term Mars missions dawn on the horizon.



### Article

# GENES IN SPACE

# Detecting Increased Risk of Developing Alzheimer's Disease During Long-Term Outer Space Travel

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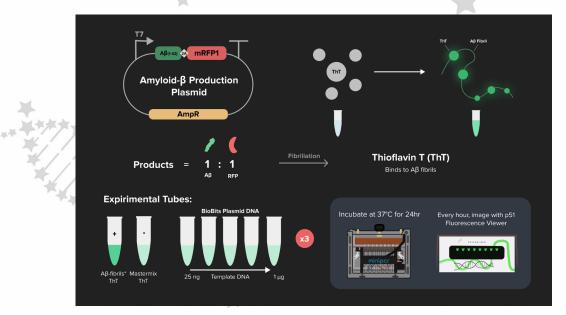
#### ABSTRACT

Long-duration space travel is on the rise in recent years, exposing astronauts to microgravity and 200 times more radiation than on earth for several months at a time. Studies show that long-term exposure to outer space conditions can alter brain structure and accelerate the progression of Alzheimer's disease. Associated symptoms of Alzheimer's disease include memory loss, impaired motor function, social withdrawal, and even premature death.

The amyloid hypothesis for Alzheimer's disease suggests that amyloid beta ( $A\beta$ ) protein fibrillates and forms plaque around neurons, causing neuronal degradation and disease progression. Our proposal aims to understand the effect of outer space conditions on the rate of protein fibrillation reactions. We hypothesize we will observe an increased rate of  $A\beta$  protein fibrillation, independent of protein concentration in space.

To test this hypothesis, we propose using BioBits cell-free technology to synthesize  $A\beta$ -42 proteins, a species of  $A\beta$  prone to fibrillation, tagged with mRFP1, to visualize protein production with red fluorescence. We will perform multiple parallel fibrillation assays on Earth and space with varying concentrations of BioBits-produced  $A\beta$ -42 across samples. Our samples will also contain ThT, a fluorescent probe that emits green fluorescence upon binding to  $A\beta$  fibrils in a concentration-dependent manner. If  $A\beta$ -42 is more prone to fibrillation in outer space, we hypothesize that we would see more red fluorescence in space than on Earth. These experiments, observed using the p51 Fluorescence Viewer, will allow us to evaluate protein dynamics in the microgravity environment of space.

The findings of this study would be the first time that proteins produced from BioBits will be functionally evaluated in space. These insights into protein kinetics will provide valuable data to advance our understanding of Aβ fibrillation in outer space conditions. Ultimately, our experiment would establish a system to study protein dynamics in a fast, easy, and replicable way in space.



# Article



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#### ABSTRACT

Sustaining long-duration space exploration relies on successful plant cultivation. However, the harsh conditions in space result in decreased plant immunity, increased drought stress, and inhibited root growth. Fortunately, novel plant growth-promoting rhizobacteria (PGPR) were recently isolated on the International Space Station, providing a promising solution to help plants overcome spaceflight conditions.

PGPRs interact with plant roots to fertilize the surrounding soil, strengthen plant immunity, and provide plants with increased resistance to abiotic stress. Since PGPRs change their gene expression based on the environment, understanding those changes in spaceflight can elucidate how PGPRs can be modified to better support plants in space. However, due to the lack of trained personnel on spacecraft, traditional methods to study gene expression such as RT-qPCR have technical limitations that require samples to be sent back to Earth. This inhibits experimental progress due to delayed results. An integrated microbial monitoring system that analyzes samples in-flight, providing on-demand results, is necessary for accelerating decision-making and independence on long-term missions.

This study proposes a rapid one-tube reaction that measures gene expression in crude soil samples in less than an hour. This assay, adapted from the SHERLOCK assay, utilizes CRISPR-Cas13 RNPs with a guide RNA that can bind to complementary RNA transcripts of interest. Once bound, the CRISPR-Cas13 RNP will become activated to nonspecifically cleave commercially available fluorophore-quencher RNA probes in solution, resulting in green fluorescence proportional to the quantity of RNA transcripts of interest.

Our experiment evaluates the efficacy of BioBits in the cell-free synthesis of CRISPR-Cas13 RNPs and the visualization of quantifiable green fluorescence through the p51 fluorescence viewer. Here, we propose to study two model PGPR genes: acdS which decreases plant stress, and iaaH which promotes root growth. We hypothesize that these genes may be underexpressed due to increased plant stress in space. This proof-of-concept CRISPR-based assay with the Genes in Space toolkit will enable on-demand environmental monitoring and disease detection in low-resource areas without access to professional training or expensive instrumentation.

