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Author: Ms. Yana Charoenboonvivat

School of Aerospace Engineering, Georgia Institute of Technology, United States, yanac@gatech.edu

Mr. Milad Mozayyani

Georgia Institute of Technology, United States, mmozayyani3@gatech.edu

Mr. Mirza Samnani

Georgia Institute of Technology, Atlanta, United States, msamnani6@gatech.edu

Mr. Alexander Chipps

Georgia Institute of Technology, Atlanta, United States, achipps3@gatech.edu

Mr. Uttoreo Saha

Georgia Institute of Technology, Atlanta, United States, usaha3@gatech.edu

Ms. Martina Tehubijuluw

Georgia Institute of Technology, Atlanta, United States, mtehubijuluw3@gatech.edu

Dr. Rachel Moore

Georgia Institute of Technology, Atlanta, United States, rmoore305@gatech.edu

Prof. Christopher Carr

Georgia Institute of Technology, Atlanta, United States, cecarr@gatech.edu

CUBESAT LUNAR CYCLER PLATFORM TO MEASURE DARWINIAN EVOLUTION BEYOND  
LOW EARTH ORBIT**Abstract**

Life can be defined as a “self-sustaining chemical system capable of Darwinian evolution.” Thus, evolution represents a high-confidence biosignature in the context of astrobiology. However, detecting evolution in space has been deemed to be impractical under mission constraints such as mass, volume, and power. The Biological Exploration Payload 2 (BioX2) is targeted for launch to the International Space Station (ISS) in May 2022 as a technological demonstration to detect Darwinian evolution in space. BioX2 will grow and evolve *Bacillus subtilis* under selective pressure provided by ultraviolet (UV) radiation, which simulates representative radiation of an early planetary surface environment in the absence of a protective ozone layer. BioX2 will then extract and sequence DNA from surviving/mutated cell population in an automated process. However, BioX2 will not be able to characterize the effects of deep-space radiation on microbial evolution due to radiation shielding provided by Earth’s magnetosphere in low Earth orbit. Understanding the effects of deep-space radiation on microbial evolution is essential, especially concerning human biology as we plan prolonged crewed missions to the Moon and Mars as part of NASA’s Artemis program. Therefore, a subsequent BioX3 cislunar CubeSat mission that integrates BioX2’s core astrobiology experiment is being proposed. BioX3 will distinguish itself from current cislunar CubeSats by flying a lunar cycler trajectory, an orbit that passes the Moon and Earth repeatedly. BioX3 will leverage lunar cyclers by taking advantage of scalable mission times from months to years in deep-space environments, consistent science data downlink capabilities during periodic Earth flybys, and relatively less propellant for the allotted mission duration. Thus, the goals of the study are to (i) assess current technological capabilities for a biological CubeSat to fly lunar cyclers (ii) develop a technology road map for BioX3. A science traceability matrix was developed to define BioX3’s astrobiology goals and elucidate the mission and science instrument requirements that will enable BioX3 to achieve those goals. A concept

of operations and system requirements for BioX3 were generated. A trade study was then conducted to identify candidate components for BioX3; the process involved literature review and testing BioX2's prelaunch capabilities. The technology gaps and a technology roadmap for the development of BioX3 were then reported. This manuscript describes BioX3's mission design, the development of current spacecraft technology necessary to operate BioX3, and how BioX3 may provide low-cost and routine cislunar access that could enable validation of technologies to enhance human lunar exploration.