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Project ALIEN

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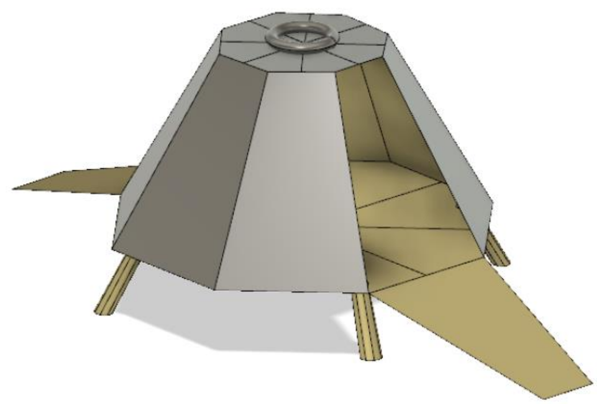

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I. Overview

Project ALIEN (Alternative Lifeform Identification and Exploration Navigator) is a comprehensive and robust plan to send humans to Mars to look for life on the Martian surface. ALIEN will use a ballistic capture trajectory to get to Mars and stay in areostationary orbit for the 30-day surface mission, during which two surface crewmembers will perform a variety of experiments to check for existing life in Gale Crater as well as explore the viability of terrestrial microbes in a Martian environment. Two landers will arrive before the crew to provide a rover and a research hub. After the surface mission, the two surface crewmembers will reunite with their mission control counterparts in orbit around Mars and return to Earth via the ballistic capture trajectory. Project ALIEN was designed for the 2020 NASA RASC-AL Competition which involved a broad mission design for a rocket and launch, a rover and landers, and formation of novel experimentation.

II. Project Background

Project ALIEN fits within the scope of a large strategic (flagship) mission. For the most recent of the National Academy of Science's Decadal Surveys on Planetary Science for the decade 2013-2022, the highest-priority mission recommended as a flagship mission was the Mars 2020 rover, designed for sample return and rooted in the Mars Science Laboratory/Curiosity mission (National Academies Press, 2018) (National Academies Press, 2011). A logical next step is to move beyond robotic-based sample return and utilize human scientific capabilities by creating a surface mission to Mars. ALIEN looks to leverage capabilities highlighted in previous Decadal Surveys.

The recent Decadal Survey also included an endorsement for 1% of NASA's mission budgets to go towards education and outreach activities, with an emphasis on direct involvement by the subject matter experts in developing these plans and engaging the public (National Academies Press, 2018) (National Academies Press, 2011). Project ALIEN includes an educational outreach plan designed in conjunction with those developing the science and engineering portions of this project, in order to meet this objective. Because these plans are already developed, this is at zero cost and leaves the rest of the 1% budget open for future development.

III. Crew Concept of Operations (ConOps)

The orbiting crew will serve as mission control for the surface crew, as the communication delay between Earth and Mars requires another source of more immediate mission control than can be provided by an Earth-based system. Sample schedules for the crew can be seen in Figure 1. ConOps encompasses a three-phase endeavor to discover microbial life within the Gale Crater, and a secondary focus to determine terrestrial microbe adaptability to the Martian environment; of this three-phase approach, Phase I is Respiration Analysis, Phase II is Media Proliferation, and Phase III is Sequencing. Contamination from terrestrial microbes is addressed through experimental controls shown in Figure 5 which partners with the terrestrial microbe adaptability focus. Therefore, to establish controls prior to the Martian life discovery study, the terrestrial adaptability studies must be performed first. All experiments will be done under controlled environments following international standards to prevent terraforming. Rules and regulations of the ISS regarding decontamination will be upheld (Cobb, 2016).

Figure 1 Sample schedules for the surface crew and orbiting crew, respectively.

Time	Activity
6:00 AM	Wake up call
6:00 AM - 8:00 AM	Exercise, breakfast
8:00 AM - 9:30 AM	EVA prep
9:30 AM - 2:00 PM	**EVA for regolith sampling**
2:00 PM - 2:30 PM	EVA wrap-up
2:30 PM - 3:00 PM	Lunch
3:00 PM - 5:00 PM	Remaining exercise, group meeting
5:00 PM - 6:00 PM	Dinner
6:00 PM - 8:00 PM	Equipment maintenance
8:00 PM - 9:30 PM	Relaxation time
9:30 PM	Lights out

Time	Activity
6:00 AM	Wake up call
6:00 AM - 8:00 AM	Exercise, breakfast
8:00 AM - 2:30 PM	**Communication, mission control tasks**
2:30 PM - 3:00 PM	Lunch
3:00 PM - 5:00 PM	Remaining exercise, group meeting
5:00 PM - 6:00 PM	Dinner
6:00 PM - 8:00 PM	Equipment maintenance
8:00 PM - 9:30 PM	Relaxation time
9:30 PM	Lights out

IV. Experimental Background

The Curiosity rover's Sample Analysis at Mars (SAM) instrument discovered fundamental elements to known life on Earth within the Gale Crater of Mars using evolved gas analysis (EGA) mass spectrometry (Mhaffy, P. R) (McAdam, A. C. et. al, 2020) (NASA, n.d.). EGA uses thermal analysis to identify elements within gas samples (Extrel, 2020). Atmospheric elements and compounds such as H_2O, CO_2, O_2, H_2, SO_2, H_2S, HCl, NO, and CO were discovered with this instrument despite evidence of atmospheric loss (Mhaffy, P. R) (McAdam, A. C. et. al, 2020) (NASA, n.d.). SAM also led to the discovery of other compounds such as iron sulfides, magnesium sulfides, iron sulfates, oxychlorine, and nitrate salts (McAdam, A. C. et. al, 2020). The presence of these elements in the Martian environment, which are key contributors to organismal survivability on Earth, leads to the conclusion that microbial life either exists or once existed on Mars. The presence of these similar elements in the Martian and terrestrial environments, particularly arsenic and oxidized nitrogen-bearing compounds indicative of the presence of a biogeochemical cycle of arsenic and nitrogen cycle, respectively, warrant an investigation of the adaptability of terrestrial microbes to the martian environment (Oremland et. al. 2002) (Stern, J. C, et. al, 2015).

In this proposed terrestrial microbe adaptability study, the distinction between the possible discovery of novel life on Mars and contaminants from Earth will be based on analysis of three microbes that live in comparable terrestrial extremophile environments to those found on Mars (Plum, et. al., 1993). These bacteria, *B. Arsenicoselenatis* (MLS10), *B. Selenitireducens* (E1H) and *Alkalilimnicola Ehrlichii* (MLHE-1), which utilize nitrogen and arsenic as electron acceptors in metabolism, were chosen for comparative analysis due to their ability to survive in alkaline and hypersaline environments similar to those found on Mars and their ability to employ arsenic in metabolism.

Table 1 shows some of the chemical properties of the bacteria. Respiratory initiators for the adaptability study were chosen based on the electron donors from this table. It is expected that these bacteria will survive and adapt gradually to the Martian climate, offering possible insight into the type of life that may exist or once have existed on Mars using the known products of terrestrial metabolism as possible indicators of respiring life. From here, the proposed microbial discovery study will then involve astronauts on the Martian surface utilizing these results as an experimental basis to search for evidence of current extraterrestrial life.

Table 1. Properties of the three terrestrial microbes involved in the microbe adaptability study[37]

Organism	Taxonomy	Electron donors	Electron acceptors	pH	Salinity
Bacillus selenitireducens	**Gram+low G+C**	**Lactate, pyruvate, fructose, glucose starch**	**Se(IV), Se(0), S(0), As(V), fumarate, nitrate, nitrite, low O_2**	**9.8 range: 7.3–11.2**	**60 range: 20–220**
Bacillus arsenicoselenatis	**Gram+low G+C**	**Lactate, malate, citrate, starch**	**Se(VI), As(V), nitrate, Fe(III), fumarate**	**9.5 range: 7.3–10.1**	**60 range: 10–140**
Alkalilimnicola Erhlichii	**γ-Proteobacteria. Gram Negative**	**As(III), H_2S2-, acetate, H_2**	**Nitrate, air**	**9.3 range: 7.3-10**	**30 range: 15-190**

V. Mission Design

A. Launch Plan

1. Launch Vehicle

Table 2 highlights key specifications of New Glenn as compared to NASA's Space Launch System (SLS) and the Falcon Heavy. New Glenn was chosen as the option to optimize budget considerations as well as provide a sufficient trans-Mars Payload capability.

	New Glenn [3]	SLS [4]	Falcon Heavy [5]
Trans-Mars Payload	Est. 20 tons (3-Stg)	Block 1B: 37 tons Block 2: 45 tons	13.6 tons
Cost per Launch	$323 million [2]	$876 million	$150 million
Fuel/Oxidizer	First Stage: LNG/LOX Upper Stage: LH2/LOX	LH2/LOX	RP1/LOX
Precedent	Test launches Estimated 2021 launch	Significant launch delays Estimated 2021 launch	LEO, GTO launches First flight: 2018

Table 2. Comparison of key specifications of potential launch vehicles. For this mission, New Glenn has been selected. Specifications that are major factors against a given vehicle are in red.

2. Selected Trajectory

The trajectory chosen for this mission is a ballistic capture trajectory, also known as a weak stability boundary trajectory or WSB. This trajectory is based on weak stability boundary theory, and entails an interplanetary transfer to get the spacecraft from Earth to a point in the Martian orbit around the Sun.

Once the spacecraft enters the Martian orbit, a burn is done to slow the spacecraft's orbit as Mars continues at its same orbital velocity, allowing Mars to catch up to the spacecraft. When Mars gets close enough, the spacecraft enters Mars's sphere of influence (SOI) where Martian gravity is the dominant factor in the spacecraft's motion, shown in Figure 2 (Topputo, F., Belbruno, E., 2015). At this point, Landers 1 and 2 will maneuver to the target landing site. The crewed module containing Lander 3 will enter into an areostationary orbit at 13,000 km above the target landing site, and will proceed as described in the Mission Timeline section. The crew module's return to Earth will also utilize a WSB.

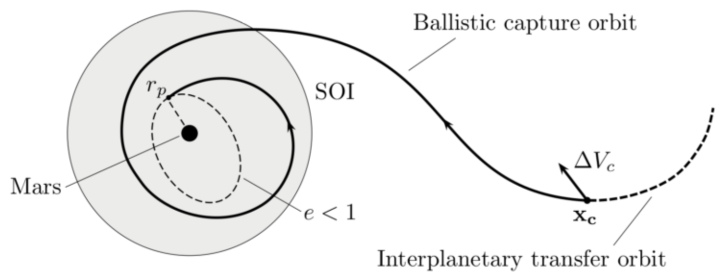


Figure 2 WSB trajectory (Topputo, F., Belbruno, E., 2015). Once the modules enter into the SOI, they will be maneuvered to the landing site or the areostationary orbit. Specific maneuvers and corresponding delta Vs are being determined.

An areostationary orbit was chosen to eliminate communication blackouts between the orbiting crew and the surface crew. Because the areostationary orbit rotates with the same period as Mars, it remains above the same point on the Martian surface. This means that the surface crew will have a direct line of communication to the orbiting crew, and will also not need to wait for launch windows to return to the orbiting module at the close of the mission. This is a safety feature as well, as it means that the mission can be ended at any point. Additionally, areostationary orbits are stable for 126 days around the equatorial plane without station-keeping maneuvers, so for ALIEN's 30-day surface duration, no additional maneuvers are necessary (Silva, J.J, Romero, P., 2013).

Trajectory selection was made in consideration of two main factors: crew safety and cost, in terms of fuel cost, mission duration, and launch window availability. WSB was chosen over the more traditional Hohmann transfer due to these factors, shown in Table 3. The burn used in WSB to slow the spacecraft is not time-critical, which is important in a Martian mission because of the communication delay from Earth. Removing the time-dependent nature of the burn allows mission control on Earth to be in control of the trajectory. The delta V and transit duration are variable in WSB depending on the position of Mars relative to Earth at time of launch. The main reason for selecting WSB is due to the launch windows. WSB can be launched at virtually any time; there are no set launch windows. Hohmann transfers have a narrow launch window that recurs every 26 months, meaning if a launch were to be delayed by weather or any other factor, it would significantly alter the mission timeline.

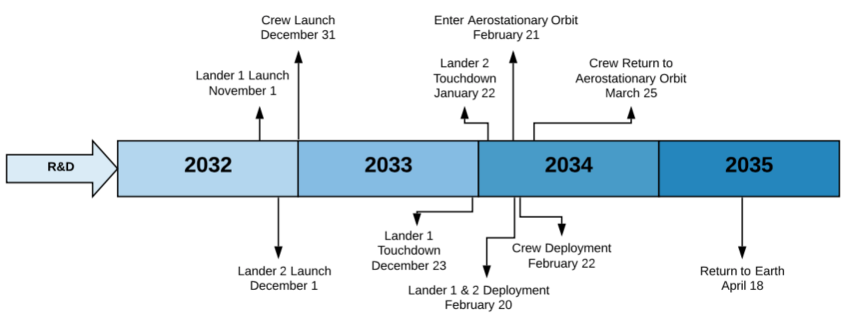
	Ballistic Capture [6]	Hohmann Transfer [8]
Safety	No time-critical insertion burn	Requires a time-critical insertion burn
ΔV	Approximately 13.4 km/s	Approximately 14.4 km/s
Duration	234-599 days (one way)	259 days (one way)
Launch	Flexible launch windows	Narrow launch window - 26 months
Precedent	Uncrewed lunar missions	Uncrewed Mars missions
Landing	Maneuverable to landing site	Maneuverable to landing site

Table 3 Comparison of WSB with Hohmann transfer. Items in red are major factors against selection.

B. Mission Timeline

The timeline is shown in Figure 3. All launches will occur with a 30-day separation. Landers 1 and 2 will not enter into areostationary orbit and instead will go directly to the landing site, where they will remain in their closed configuration until one day before the crew module's arrival in areostationary orbit. Landers 1 and 2 will deploy two days before the surface crew lands, to allow the rover to charge without unnecessary exposure to weather conditions. Transit time between initial launch and entry into areostationary orbit for the crew module will be approximately 448 days.

Figure 3 Mission timeline.



Launch dates are tentative pending weather, and specific transit times will be dependent on the exact launch dates using a WSB. There is some flexibility in the timeline. Because WSB does not depend on a narrow launch window, the specific launch dates can easily be adjusted to accommodate launch conditions. Since areostationary orbits are stable well beyond the surface mission duration, the surface crew can be held in orbit to wait out inclement weather on the surface of Mars that would not be conducive to a surface mission. A 10% margin of food and water supplies will be brought on the orbiting module to ensure this capability.

C. Landers

1. Lander Design and Payload

Landers 1 and 2 share a design based on Pathfinder, because it has been shown to work on Mars (The Lander Structure). Both will use the Skycrane to get to the Martian surface in place of the airbags used in Pathfinder; this eliminates any bouncing and allows precise landing in the correct orientation (United States, NASA, 2012). Lander 1 will contain GROVER, and Lander 2 will be a habitable research hub. The lander petals will open autonomously to begin charging before crew arrival. Lander 3 is based on the

Apollo lander, modified for Mars and with an added fifth leg for increased stability with design being finalized (Apollo News Reference). Its payload will be the crew and a secondary transport vehicle that will allow the crew to travel from Lander 3 to Lander 1, where GROVER is located. The secondary transport vehicle will have basic functionality for the crew to reach the primary rover. The crew will need EVA suits on for the trip to GROVER, as the secondary transport vehicle is not habitable. Lander specifications are shown in Table 4, with design shown in Figure 3.

	Lander 1	Lander 2	Lander 3
Design Basis	Pathfinder	Pathfinder	Apollo
Dimensions (m)	8x8	8x8	6.985x9.4488
Wet Mass (kg)	28165	27165	TBD
Dry Mass (kg)	21627	20627	16375
Power (W)	300	300	2300
Cost (millions)	$1,714	$1,714	$4,893

Table 4 Lander specifications. The wet mass of Lander 3 is being finalized as the exact amount of fuel needed is determined.

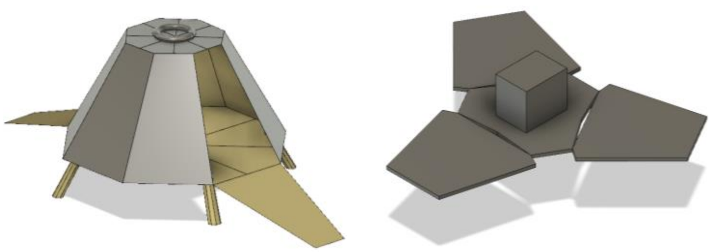


Figure 3 Design of Landers 1 and 2. The true design is on the left with petals down to charge, and a simplified design is on the right. The right is being used in ongoing computational fluid dynamics analysis to verify minimal risk in case of a dust storm.

Solar panels on the inside of the petals on Landers 1 and 2 are responsible for charging GROVER and the research hub. Final solar panel selection will be determined, with several in consideration for their sizing and charging capabilities on Mars. Specifically, the team is deciding between Spectrolab solar arrays, MMA designs that have been used on cubesats previously, and AAC-Clydes with spring-loaded mechanisms for solar tracking (Chapter 3: Power). The determination that the landers will deploy two days before crew arrival on the surface of Mars was made based on the charging time for batteries on the surface of Mars; previous missions show that these batteries will be fully charged within a two-day timeline, pending good weather (Electrical Power). As explained above, the surface mission can be held until appropriate weather conditions are reached, giving also flexibility to fully charge the payloads.

2. Landing Site

The selected landing site is Gale Crater, with considerations shown in Table 5. Gale Crater has a diameter of 96 miles, is located near the northwestern part of the aeolis quadrangle at latitude 5.4°S and longitude 137.8°E. Because of the established presence of water in the crater, byproducts of water such as clays and sulfates could also be found that may preserve signs of past life. Curiosity Rover also found an alluvial fan, thought to be formed by the deposition of materials as water velocity decreases. The base of Mount Sharp in the crater contains clays and sulfates, and warrants further investigation for signs of life (Gale Crater, 2019). Additionally, in comparison to other crater landing sites, the soil analysis results obtained by Curiosity showed a similar composition (Yen, A.S., et. al, 2013). The specific part of Gale Crater that each lander will target in its landing is being determined.

	Jezero Crater [15]	Gale Crater [14]	Victoria Crater [16]
Precedent	Planned mission, 2021	Curiosity Rover, 2012	Opportunity Rover, 2012
Evidence of Water	Concluded to have once been a lake	Evidence of multiple sites with water and an Alluvial fan	No evidence of water found
PROS	Calculations suggest that there are valuable sediment layers	Considered a dry lake Investigation of bottom layer of Mount Sharp No significant weather-related issues for Curiosity Rover Landing site variety	Access to bottom (older) layers of the surface
CONS	NASA already has a planned search-for-life mission to this crater Weather predictions would be based on assumptions	Inclined surface could increase exploration difficulty	High probability of dust storms Opportunity's life was threatened in multiple occasions

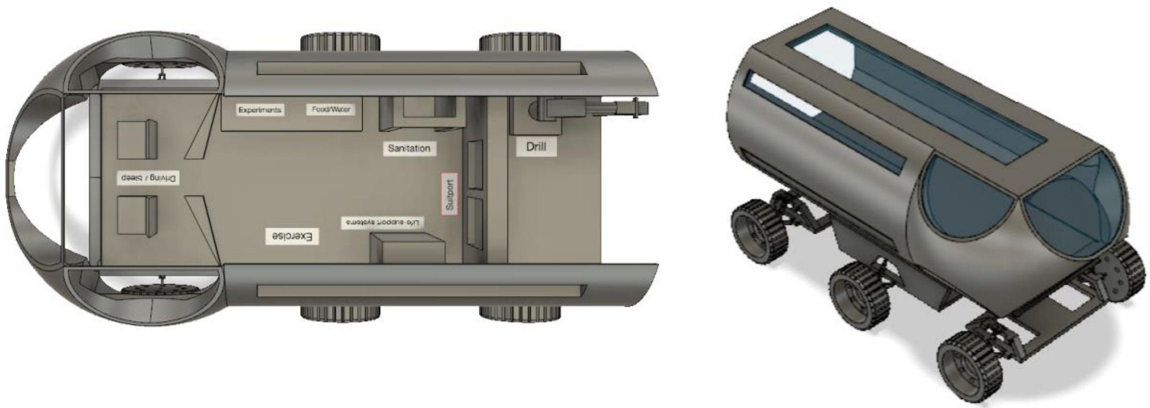
Table 5. Landing site selection. Items in red are major factors against selection.

D. GROVER

The Ground Reconnaissance Operations Vehicle for Extraterrestrial Research is the proposed habitable rover based on NASA's SEV (Space Exploration Vehicle, 2020). GROVER has a total mass of 4000 kg and an estimated total power draw of 1324 W, with an average expected power draw of 600 W at a given time; of this, driving power is 100 W on average, with ECLSS and science equipment making up the remainder. ECLSS and radiation shielding appropriate for the Martian surface will be used.

The design is based on the SEV, with minor modifications made to account for ALIEN's specific experimental considerations as shown in Figure 4. GROVER allows two crewmembers to live inside and carry out daily activities. There is a sanitation area for washing and waste management aboard the vehicle that is shielded to minimize bacteria spread. The seats are dual-function, as seats for driving the vehicle that fold down into beds. An exercise area with basic exercise equipment is included to mitigate bone density and muscle loss in the Martian environment. There is also a space for experiments and tests below the food prep area, with removable shelving and storage options to eliminate risk of cross contamination between the food and experiments. Two suit ports along the back wall are for crew to enter and exit the vehicle for EVAs. The drill is stored in the back of the rover; simple clamps will hold it in place during travel, allowing for portability as needed.

Figure 4 GROVER design. Left shows layout inside the rover, while right is the general rover design.



1. Payload and Equipment Descriptions

The following equipment will be included onboard GROVER, shown in Table 6: a portable miniature mass spectrometer to analyze the chemical composition of samples; radiometer and Geiger counter for determination of the radiation environment; compact shaking incubator; agar powder to culture any specimens found on the Martian surface; compact centrifuge; freezer to match the Martian surface temperature for specimen storage; DAN to look for water on Mars by measuring the energy of neutrons escaping from the surface, as lower energy indicates the presence of Hydrogen; ROPEC (ROtary PErcussive Coring) drill, detailed below; MinION with the necessary adapter (Flongle) for real-time DNA and RNA sequencing, with a controller and data acquisition tool (MinIT); VolTRAX, an automation system for sample preparation; a laptop to connect to automated equipment and for analysis; UV light to disinfect surfaces to prevent forward contamination on the surface of Mars; and a K-band pyramidal horn antenna for communications. Food and water will be included as well. Food will be stored on Lander 2 and GROVER, as the crew will return to Lander 2 at least every two weeks. GROVER will house enough food to last two weeks, plus food for an additional margin of one week, and will restock with each visit to the lander.

Device	Power (W)	Mass (kg)	TRL
Portability Transportable Mass Spectrometer [18]	4.6	11	9
Radiometer	0	0.037	9
Geiger Counter [19]	5	1	9
Benchmark Incu-Shaker Mini Incubator [20]	300	10	9
Fisher BioReagents LB Agar, Miller powder [21]	0	1.5	9
Eppendorf 5415D Centrifuge [22]	180	8.4	8
Freezer-MDF-C8V1-PA [23]	300	67	7
DAN (Dynamic Albedo of Neutrons) [24]	0.1	2.6	9
ROPEC (ROtary PErcussive Coring) Drill [25]	100	4	9
MinION with Flongle and MinIT [26]	35	0.136	8
VolTRAX [27]	15	0.35	5
ThinkPad 755 Laptop [28]	135	1.67	9
UV Light	230	20	9
Communication-SAR-2507-42-S2 [29]	15	0.127573	9
Food (2 people for 2 weeks, plus margin)	N/A	562.5	N/A
Water (2 people for 2 weeks, plus margin)	N/A	60	N/A

Table 6 GROVER Master Equipment List with specifications.

The ROPEC drill is a NASA design built specifically for extracting samples of Martian regolith. The drill can be fitted with five different drill bits to extract rock, powder, or other configurations of the regolith. ROPEC is capable of autonomous sample acquisition and delivery from a depth of 5 cm, and can deposit the entire drill bit with a captured rock core into the desired place (Chu, et. al., 2014). This autonomy and versatility makes ROPEC compatible with ALIEN's mission.

2. Power Production and Consumption

A 90-kg lithium-ion battery with a capacity of 11250 W-hr will be used that can be charged by an array of solar panels and regenerative braking. The LG 375Q1C-V5 NEON R BLK/WHT Module solar array will be used along with regenerative braking to generate the power necessary to run GROVER. The solar array will be made up of 375 W solar panels that are 1.702 x 1.016 m (LG 375Q1C-V5 NEON R BLK/WHT Module Specifications). These panels could be stored under GROVER and pulled out as needed. With an array of 6, maximum operating efficiency would generate 2250 W; however, on the surface of Mars, we estimate around 1200 W due to the 492.14 $\frac{W}{m^2}$irradiance at the surface at Gale Crater, and estimating 20% efficiency (Mission Technology: Power). This is double our average expected power draw of 600 W. Additionally, regenerative braking like that used in certain automobiles will be used to stop the vehicle while simultaneously charging the battery. When the electric motors within the wheel assembly are commanded to slow the vehicle, the mechanical energy from the moving vehicle is translated into electrical energy that can be harvested back into the battery for later use (Solberg, 2010). This will add to GROVER's power production, even in a dust storm where solar power generation may be lowered.

VI. Experimental Methodology

Terrestrial bacterium *B. Arsenicoselenatis* (MLS10), *B. Selenitireducens* (E1H) and *Alkalilimnicola Ehrlichii* (MLHE-1) will be tested for adaptability to the environment and to analyze changes in them throughout the mission. These bacteria are alkaliphiles that live in high salinity environments, halophiles who prefer salty environments, and respire anaerobically in the presence of arsenic and nitrogen compounds. The samples of the microbes will be extracted from Mono Lake, California about 35m deep, where these bacteria coexist (Oremland, et. al., 2002) (Oremland, et. al., 2004). A control sample from Mono Lake will be kept to note any changes to the microbes. The samples will be kept in media that chemically recreates the conditions of the lake and will be frozen right before take off, then defrosted while the crew is en route to Mars. Microbes will be sequenced before launch to determine a control sequence. Samples will also be sequenced a week after they have left the atmosphere to detect any changes, and then throughout experimentation.

Since halophiles will be used, they are required to stay within extremely precise ranges of pH, temperature, oxygen concentration, and salinity. Microbes will be kept in an airtight container as they cannot survive in the presence of oxygen and ideal solutions will be created for the microbes before launch. The most effective way of preserving the samples will be to freeze them when they are not needed as they can be transported much more easily. When the microbes are needed, they can be thawed once they are in the proper environment and experiments can begin. The terrestrial microbe testing will remain in the rover as the sudden temperature change can kill the microbes along with radiation exposure. This extends to any martian microbes that can be possibly found which is why all samples are kept on the outside of the rover and "shielded" by regolith. Having a research lander allows Martian samples to remain outside, reducing the risk of possible microbes dying.

Adaptability of the bacterium will be studied in two phases; in Phase I, the pH, salinity and temperature of the microbes will be changed gradually while en route to Mars until conditions match Mars; in Phase II, lactate, pyruvate and malate will also be introduced in the samples to encourage respiration and study the adaptability and functions of the microbes in regolith. Phase II will occur in the refrigerator located in the back of GROVER. All science objectives can be seen in Table 9.

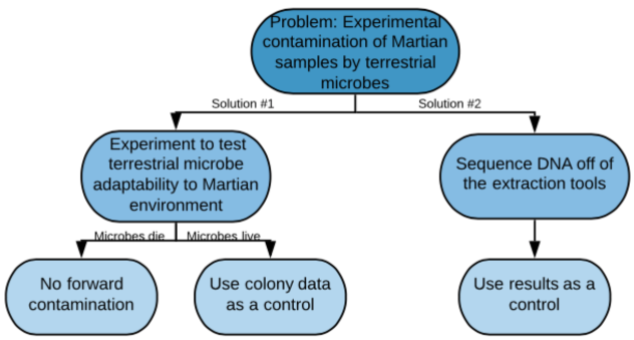


Figure 5 Forward contamination prevention flowchart. Details methods for identifying and mitigating sample contamination with terrestrial microbes.

ROPEC will be used to take core samples near suspected hot spring locations identified by the Curiosity rover, along with other points of interest that contain sediments usually carried by water. It is important to protect core samples, as there is more radiation exposure on the surface than underground. In the first EVA, the crew will fill the walls of a box with regolith; samples will be brought back to the lander in this regolith-layered, radiation-resistant transport box on the back of GROVER. The regolith will shield against the bulk of the radiation, protecting the sample inside. The sample box will be stored outside the rover to maintain the temperature and reduce risk of overheating any live specimens in the samples.

Phase I of Martian microbial discovery involves analyzing potential respiratory responses initiated by an array of chemical reactions from Martian samples to indicate the presence of respiring life, relying on the assumption that Martian microbes behave like terrestrial microbes (Merino, et. al., 2019). DAN will initially be used to find locations where there is a lot of hydrogen present. Samples of Martian brine will be obtained; raw samples will be sequenced to remove possible sources of DNA contamination or degradation. Then, three experiments will be carried on the brines to determine if there is bacterial respiration by exposing samples to chemical compounds that are predicted to interact with arsenic or selenium compounds within regolith samples. Brine samples will be treated with deionized water. One sample will be exposed to Arsenic and the other to Selenium (Stolz, et. al., 1999). The samples will be observed for any changes and will be tested for any presence of H_2 using DAN; those with H_2 detected will be further analyzed.

In Phase II, samples that reacted with one of the respiration-inducing chemicals will be proliferated in media corresponding with the respiration-inducing chemical. The chemical(s) that encourage reactions will be used to create a nutrient-based agar to further experiment with regolith, and to investigate any growth and potentially enumerate microbes if any are present.

In Phase III, samples with signs of respiration will be genomically sequenced using VolTRAX and MinION within Lander 2. Genomic DNA (gDNA) will be isolated from samples before the sequencing process through basic pipetting techniques. Solid samples, such as hardened sediment samples, will be ground into a powder form to make reactions with solutions easier. VolTRAX will be used to prepare samples; this removes human error within the experiment and allots the crew time to perform other duties while samples are being prepared (VolTRAX, 2019). Should a situation occur where VolTRAX cannot be utilized, the traditional, manual method of sample preparation will be used. Next, the solution will be run through MinION to produce high throughput reads. MinION will be used in tandem with the MinIT and Flongle devices (MinION, 2019). MinIT replaces the need for a laptop and can connect to any bluetooth device for quick and easy access to results, in addition to processing information much faster than a laptop. Flongle is an adaptor for the MinION that allows faster sequencing, utilization of smaller samples, and performance at a lower cost. Sequencing results will be run through BLAST (Basic Local Alignment Search Tool) by the orbiting crew to compare the obtained sequence to other recorded sequences. Sequenced samples will be cultured and cryopreserved for transport back to Earth so that further analysis can be done.

VII. Expected Results

Based on the known arsenic and selenium metabolic reactions of the three terrestrial microbes in Table 7 and the assumption that life on Mars is metabolically similar to the terrestrial microbes, we expect to find novel life using the products of the reactions found below as identifying markers of life.

Table 7. Lists expected metabolic outcomes based on microbial characteristics in Table 1 (Oremland, et. al., 2004).

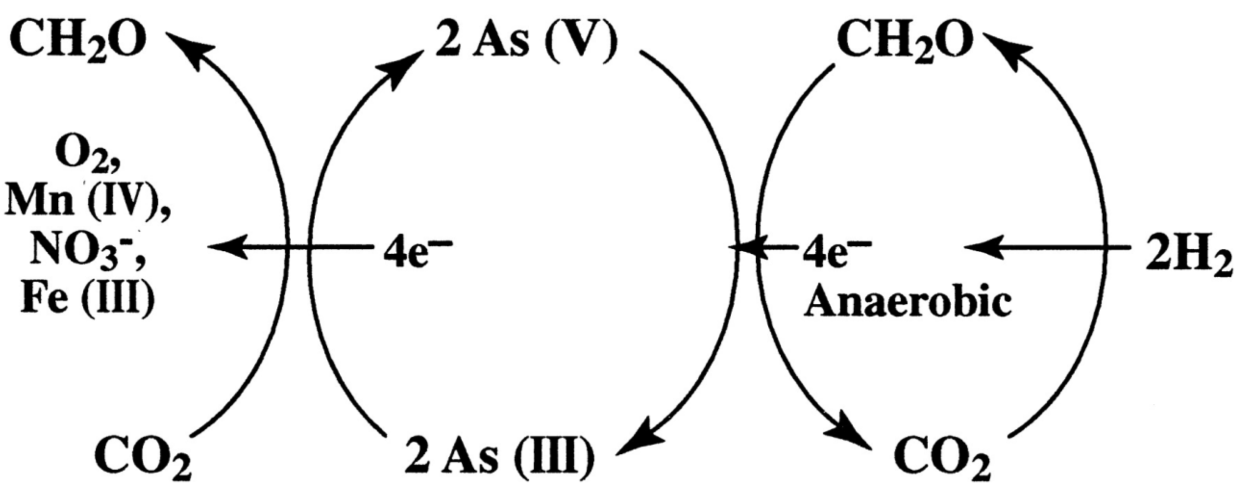
Organisms	Reaction
B. selenitireducens, B. arsenicoselenatis	$Lactate^{-} + 2\ HAsO_4^{2-} + 2\ H^{+} \rightarrow 2\ H_2AsO_3^{-} + acetate^{-} + HCO_3^{-}$
B. arsenicoselenatis	$Lactate^{-} + 2\ SeO_4^{2-} \rightarrow acetate^{-} + 2\ SeO_3^{2-} + HCO_3^{-} + H^{+}$
B. selenitireducens	$Lactate^{-} + SeO_3^{2-} + H^{+} \rightarrow Se(0) + acetate^{-} + HCO_3^{-} + H_2O$
B. arsenicoselenatis + B. selenitireducens	$3\ Lactate^{-} + 2\ SeO_4^{2-} + H^{+} \rightarrow 2\ Se(0) + 3\ acetate^{-} + 3\ HCO_3^{-} + 2\ H_2O$
MLHE-1	$H_2AsO_3^{-} + NO_3^{-} \rightarrow H_2AsO_4 + NO_2$

For Martian samples exposed to water, we believe that possible microbial life will cause the products seen in Table 3 (Stolz, Oremland. 1999). Hydrogen acts as the electron donor in the reactions allowing arsenic and selenium to accept these electrons and go through the arsenic cycle seen in Figure 6.

Table 8. The reduction and oxidation reactions of arsenic and selenium in the presence of water (Stolz, Oremland. 1999).

1/4 Se + 3/4 H_2O → 1/4 $[HSeO_3]^-$ + 2 H^+ + 1/2 H_2
1/3 As + 1/2 H_2O → 1/2 $[H_2AsO_4]^-$ + 1/2 H_2
1/3 As + H_2O → 1/3 H_3AsO_3+1/2 H_2

Figure 6. Diagram depicting the possible biogeochemical arsenic cycle that is mediated by microorganisms and strong oxidizers (Oremland et. al. 2002).



Finally, out of the three terrestrial microbes, we hypothesize that MLHE-1 would be the best adapted bacterium to the Martian environment because it's an autotroph that oxidizes arsenic, hydrogen, or sulfide and reduces nitrate (JGI, n.d.). MLHE-1 also has a flexible metabolism allowing it to grow and thrive in barren environments with varying inorganic electron donors and a lack of organic material (Oremland et. al 2002).

VIII. Risk Analysis and Mitigation

A complete risk analysis has been done for each component of ALIEN. Mitigation strategies have informed all finalized design components, and have been explained in the appropriate sections. The complete risk analysis plan is beyond the scope of this proposal, but is available upon request.

Science Objective	Measurement Objectives	Measurement Requirements	Instrumentation	Mission Requirements
Respiration Analysis	Analysis of potential respiratory responses initiated by an array of chemical reactions from Martian samples to indicate the presence of respiring life within samples	Martian brine collection, core samples from beneath regolith layer, Arsenic and Selenium to interact with samples, Enteropluri test tubes	Regolith tubes, DAN	Samples must immediately be put into sample tubes; solid samples must be put into liquid form for media preparation and sequencing
Media Proliferation	Samples that reacted with one of the respiration inducing chemicals will be proliferated in media corresponding with the respiration-inducing chemical	Utilization of the chemical compound that caused a result	Qubit 4 fluorometer	Positive results from respiration analysis
Sequencing	Sequencing of proliferated media and raw samples obtained from the surface	Should be performed within confines of rover or lander due to environmental factors affecting instrumentation	Qubit, VolTRAX, MinION, laptop	Must be performed in the rover or lander
Terrestrial Adaptability	Exposure to environmental factors, determination of survival/adaptability to Martian atmospheric conditions	pH, temperature, and salinity of sample must be gradually changed; samples introduced to Martian soil	Microfuge tubes, freezer, compound microscope, DAN, regolith boxes, MinION	First part must be performed during trajectory to Mars; second performed sequentially to prevent instant microbial death, and must be an anoxic environment

Table 9. Science traceability matrix.

IX. Financial Analysis

Project ALIEN is less than $9.3 billion, which is approximately 40.7% of NASA's FY2020 budget (A Budget for America's Future: Budget of the U.S. Government, 2020). A preliminary budget is shown in Table 10.

Table10. Itemized budget for Project ALIEN.

Item	Quantity	Cost ($)	Item	Quantity	Cost ($)
GROVER (based on SEV)	1	180189147	VolTRAX ($8150 per unit) 27	2	16300
Food ($40 per meal, 10% margin)	3353	134120	ThinkPad 755 Laptop ($4049 per unit) 28	2	8098
Mini Mass Spectrometer 18	1	5000	UV Light ($5 per unit)	2	10
Radiometer	1	15	Flongle ($1860 per unit) 26	2	3720
Geiger Counter 19	1	400	MinIT ($2400 per unit) 26	2	4800
Incubator 20	1	2550	Solar Array (LG 375Q1C-V5 NEON R BLK/WHT) 30	6	450
Agar (500 g) 21	1	190	ROPEC Drill 25	1	5000000
Centrifuge 22	1	1095	Landers 1 & 2 ($1713 million per unit) 9	2	3427764000
Freezer 23	1	7000	Lander 3 11	1	4892554000
Communications 29	1	500	Launch (mission analogs: $323 million per launch) 2	3	696000000
DAN 24	1	500	R&D		10000000
MinION ($12400 per unit) 26	2	24800	**Total**		**$ 9,211,716,695.00**

X. Educational Outreach Plan

Lesson plans have been developed based off of ALIEN's mission in alignment with Pennsylvania Common Core standards and will be taught to students in the Pittsburgh community in the coming months. Middle school and high school students will do a research project on the factors that come into play with Martian terraforming. Elementary school students will do a carving channel lab to simulate the Martian terrain and demonstrate that it could have been shaped by water in the past. Lesson plans are available on request to any interested parties.

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