ASTRO Electrostatic Sampler Performance

HASP Final Report

Massachusetts Institute of Technology
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Payload Class: Small ***Flight No:*** 2013 ***Payload ID: 2013-06***

Table of Contents

Project Introduction and Motivations

Motivations

Initially, we were driven by a biological query: Given that organisms evolve in order to adapt to changing conditions, how would the extreme environment of high altitudes affect genetic variation? This project has allowed us the opportunity to begin to address this question through the development of a biotic aerosol sampling device. In addition to the academic pursuit of knowledge, this project also provided students with the opportunity to enhance their technical skills in the areas of design, prototyping, and electronics.

Introduction

The MIT Air Spora Trapping and Recovery Operation (ASTRO) team's first payload flew to 120,000 feet in September 2013 on the High Altitude Student Platform (HASP) in collaboration with NASA's Columbia Scientific Balloon Facility. The overall goal of the project was to fabricate a functional bioaerosol sampler effective for low sample concentrations, with the overall objective of documenting any biological particles found at high altitude. These particles are becoming of increasingly greater interest due to their newly hypothesized roles in ice crystal formation and oxidizing activity in clouds. The collection technology used for the ASTRO payload also has potential applications in air quality monitoring.

ASTRO tested the effectiveness of bioaerosol sampling at high altitudes using a custom-designed electrostatic collector. The concept behind the electrostatic sampler is that particles found at a float altitude of 120,000 feet are likely to be positively charged due to photoelectric ionization by cosmic rays. Charge differences were the mechanisms for sample collection.

Upon payload recovery and sample collection, the genetic material from the collected canisters were subjected to a variety of biological processing protocols, including Polymerase Chain Reaction (PCR), that determined relative abundance of genetic material found on various surfaces. The ratio of genetic material abundance for various surfaces indicated effectiveness of canister sealing, canister sterilization, and function of the electrostatic sampler as a whole. This was the primary area of focus for this flight, because it provided a proof of instrument function. After biological processing, the electrostatic sampler was found to be successful, as it was capable of collecting bioaerosols upon the electrode.

Our efforts were supported by our research advisor, Dr. Christopher Carr (MGH). We received our initial funds from the generosity of Dr. Carr, Dr. Maria Zuber, and Dr. Gary Ruvkun. We would also like to thank the Earth, Atmospheric, and Planetary Sciences (EAPS), Aeronautics and Astronautics, Biological Engineering, and Electrical Engineering and Computer Science (EECS) departments for their support. Experiments were performed using the facilities at our research station at Massachusetts General Hospital Simches Lab and at student workshops on campus.

Mechanical Subsystem

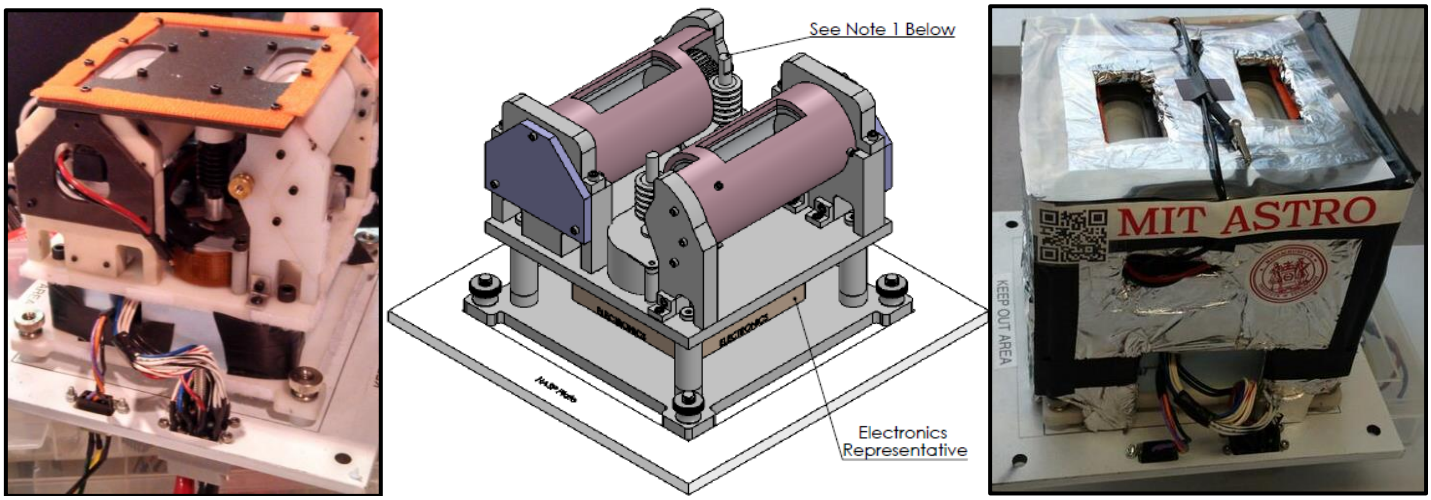


Fig 1) From left to right – 1) Assembled payload without thermal insulation ; 2) SolidWorks rendering of system ; 3) Completed payload with mylar & foam thermal insulation and static dissipative film

Overview

The mechanical design went through many changes before payload construction. Initially, we had envisioned flying an electrostatic sampler with a microfluidic component. After encountering issues with vapor pressure of collection fluid, we settled on a two chamber electrostatic collection payload. Figure 1 shows the design and fabrication of this two chamber payload. Future modifications could incorporate a microfluidics process into the design in order to facilitate sample concentration and prevent sample loss from downstream concentration processes.

Basic Mechanical Design

There are two collection canisters:

- ✓ **Canister 1**- collected integral atmospheric sample from 28,000 ft altitude to float altitude
- ✓ **Canister 2** – collected samples at float altitude

Each canister was composed of two nested cylinders with a cut running the length of the collector. Once aligned, the cuts allowed for airflow through the device. An electrode made of copper impregnated graphite spanned the center of the inner canister. A nominal voltage of -500V was then applied to the electrode in order to facilitate electrostatic collection.

These nested cylinders were lubricated with Krytox, a lubricant rated for space-related technologies due to its large temperature range and use in low vapor pressure environments. The inner canister was shorter than the outer nested canister and was capped at both ends to provide a

sealed and isolated environment in which samples could be preserved with minimal risk of contamination.

Each canister rotation was controlled by a stepper motor and a worm gear (used to increase torque to overcome the friction between nested canisters). Sample collection was dependent on a 90 degree rotation of the inner canister in order to align air flow channels between the inner and outer chambers. A SolidWorks rendering of the canisters in the aligned position is shown in Figure 2. A hard stop was also in place in order to ensure the canisters remained sealed.

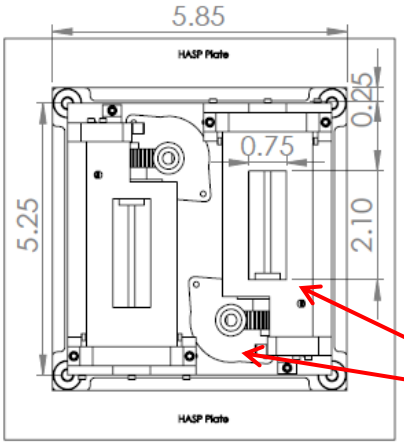


Figure 2) SolidWorks Rendering of payload top view.

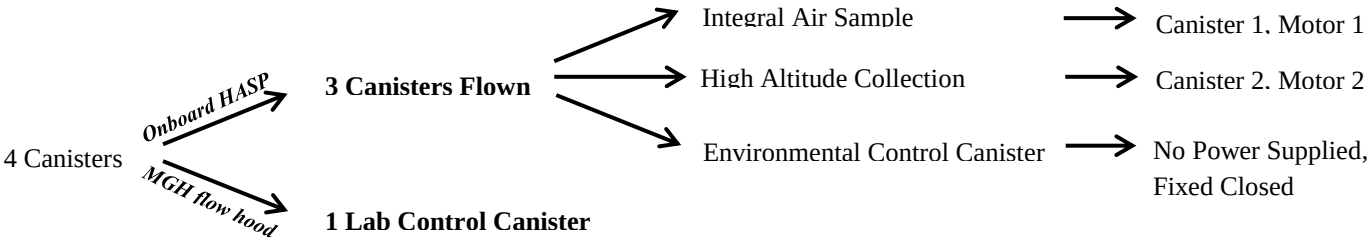


Figure 3) Delegation of canisters

We made a total of 4 collection canisters, as shown in Figure 3. Three of the four canisters flew aboard the HASP payload; however, one canister was an environmental control canister, and remained closed during flight. The fourth canister remained in a controlled flow hood in Massachusetts General Hospital.

Most of the parts were custom fabricated by hand, mill, lathe, waterjet, or laser cutter. The stock material for the nested cylinders was PTFE, a thermoplastic that withstands a range of temperatures from -100^{o}F to 400^{o}F (-73^{o}C to 204^{o}C). We also created support structures out of glass-filled (and regular) Delrin, which was stiff and has a temperature range of -40^{o}F to 185^{o}F (-40^{o}C to 85^{o}C). We chose material that has low outgassing properties and can withstand the low temperatures exhibited across the atmosphere.

Our system functioned as follows: During launch, both canisters were in the closed configuration. Once the desired sampling altitude was reached, a stepper motor then rotated the inner canister to align the airflow channels of the nested cylinders. A nominal voltage of -500 V was then applied to the electrode for the duration of the sampling time. Upon sampling termination, the inner canister was rotated 90 degrees and high voltage was no longer applied.

POST Flight Mechanical Inspections

The payload withstood the shocks of the landing. Since the HASP payload remained upright during landing, the ASTRO payload was almost unharmed upon landing. All insulation was in tact and worm gears were still fully engaged such that the canisters were in a closed position upon retrieval. This was essential, since canisters in a ***closed*** configuration upon landing reduced the amount of surface level contaminants found in the samples.

The only noticable difference was the dislocation of a blue ground wire leading to canister 1, as indicated by Figure 4. This wire was assumed loose upon impact with the ground.

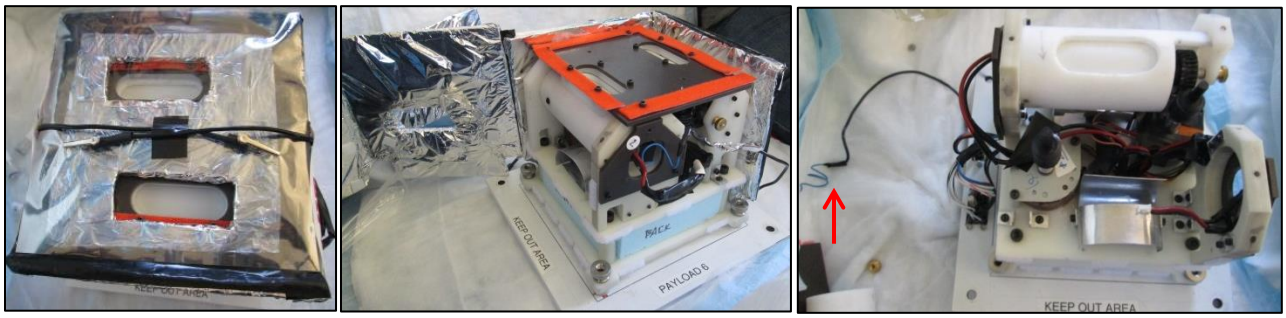


Figure 4) Left to Right: 1. Canisters closed upon landing; 2. Payload without thermal insulation; 3. Payload without canister 2. The red arrow indicates the loose ground wire from Canister 1

Electronics Subsystem

A complete electrical schematic is found on page 22.

Overview

The electrical design was centered on a TI MSP430F2274 microcontroller. The MSP430 military-grade family was selected for its low power consumption, high reliability, and survivability at extremely low temperatures. The HVDC sources, heaters, and steppers were powered by the MSP430's general-purpose digital outputs, buffered by discrete FET switches. A TI MAX3221 RS-232 line driver/receiver was used to convert HASP serial data to MSP430 3.3V logic levels. A 32.768kHz crystal oscillator was used to keep an accurate clock.

HVDC Sources

Two HVM Technologies UMHV0505N HVDC sources were used to charge the electrodes to -500V. These parts have been used in CubeSat payloads, so vacuum compatibility was not a major concern. The greatest measured voltage on the UMHV (Ultra Miniature High Voltage) chips was -570V, and their output was extremely stable with input voltage and temperature (variation in their output with pressure was not assessed). Assuming a 5% altitude margin, the worst case Paschen breakdown voltage was 637V, giving a 9.7% voltage safety margin. The HV sources were housed in the end caps of the cylinders, with external wires

carrying only 5V signals. Each collection tube was fitted with its own source so as to avoid switching high voltage. The return path for HV current was routed to a large foil electrode on the outside of the payload to dissipate static and tie the ground to the ambient potential of the atmosphere.

Thermal Control

The central circuit board and the steppers were fitted with Kapton heaters. Three thermistor-based temperature sensors were used. One, attached to the board, was input to the MSP430's ADC and used for automatic thermal management; the heaters were turned on if the board temperature dropped below a set threshold. The other two measured the motor temperatures and were attached to HASP analog channels for ground data collection.

Power

A TI PTN78060 switching regulator stepped the approximately 30V HASP power bus down to 5V for use by the outputs. A TI TPS780 LDO linear regulator stepped the 5V rail down to 3.3V for use by the microcontroller. Precision voltage references with outputs at 4.096V and 3.3V, respectively, were used for the temperature sensors.

The maximum current draw from the HASP bus occurred when a stepper motor was running. The continuous current was as high as 390 mA if the motor stalled, but 300 mA was more typical during normal operation. The motor heaters drew around 230 mA, meaning the steppers and their heaters could not be run at the same time while adhering to the 500 mA limit for small payloads. This did not present a thermal issue, though, because the steppers had to run only infrequently and for short periods. When the motors and heaters were not running, continuous current was 10-20 mA. The capacitive transient on power-up was measured at 5.5A for 150μs, which was empirically verified to not blow the HASP fuses.

Flight Performance

As HASP reached its cruising altitude, the current draw of the payload increased sharply. Based on prior HVDC shorting tests we identified arcing as a likely candidate for the current increase. One HVDC source that was known to run more negative than the nominal -500V was shut down and the current dropped back to normal levels. On recovery, the HVDC source was nonfunctional and had burn marks, which is consistent with the output being shorted. Most likely, the voltage output exceeded the Paschen breakdown voltage of air at the low atmospheric pressure and electrical arcing occurred. This may have resulted from changes in the UMHV voltage under low pressure, or other factors not taken into account during the safety margin calculation. Fortunately, canister 1, the canister in question, had already almost finished collecting its sample, so the failure did not have a major scientific impact. All other components functioned nominally, including the other HVDC source. If a similar collector is flown again, the voltage should be dynamically scaled to the ambient air pressure, with a substantial safety margin on the breakdown voltage.

The choice of MSP430 carried great benefits in terms of compactness and efficiency; the entire electrical subsystem, except the HVDC sources, fit on a single 3¾" x 2" PCB, and the

power draw when no outputs were running was only about 3% of the total power budget, most of which came from inefficiencies in the power supply. However, the minimal specs of this architecture were quite limiting on the software side, especially when driving serial communication. It was sufficient for the ASTRO 2013 payload, but a payload requiring more processing power will need to look for an alternative.

Software

State Features

The software functioned as a state machine. It kept an internal state accessible from all processes that contained the following features:

State of Motor 1: Binary variable that described whether the motor motor's position corresponded to canister 1 being open or closed.

State of Motor 2: Analogous to the state of motor 1, but for canister 2.

Time Since Start: Internal time keeping, used to control the duration of certain events.

GPS Time: Variable that contained the time given by the GPS data.

Temperature: Variable that kept the raw data from the ADC reading of a resistive temperature sensor.

State of heater 1: Variable that described whether or not heater 1 is supplied with power to heat up the board

State of heater 2: Analogous to the state of heater 1, but for heater 2

State of UMHV 1: Variable that described whether or not the UMHV 1 was supplied with power to collect samples in canister 1.

State of UMHV 2: Analogous to state of UMHV 1, but for UMHV 2 (for canister 2).

Safe Mode: Binary variable that indicates whether the system should work autonomously (events happen based on GPS data) or human-controlled (events happen in response to signals from serial).

Altitude: Variable that keeps the altitude reported by the GPS.

Overview

We divided our code into four parts: processing inputs from serial, sending output through serial (a description of the current state), actuating events in the real world (driving motors, turning heaters on/off) and interpreting events such as altitude change and temperature drops and using them to decide what real world action should happen, such as starting to sample with a canister or turning on the heaters.

Processing inputs from serial was further divided into two parts: ground commands and GPS data. The ground commands were distinguished by making sure the commands were made up of bytes that are illegal in the GPS data. Since we needed a small number of commands, we used a

simple error detecting code, sending the same byte twice, to have high confidence that we read the correct command and that the GPS data would not accidentally become corrupt and issue a command. The commands we implemented are listed in **Appendix A**.

To interpret the GPS data, we wrote a custom parser according to the specifications of the restricted protocol in the interface manual to be able to retrieve altitude and time. At the low level, this parsing was done byte-by-byte, where the arrival of a byte would trigger an interrupt that would cause it to be stored in a buffer and the parser would be queued for execution in the main loop, outside the interrupt. After receiving a command or a GPS packet, the parser always sends back a status report. This is done by sending the bytes of the state variable and a checksum for error detection. At the ground level, this information was parsed live, and produced output as shown in **Appendix B**.

Commands would cause actions to be executed, such as turning the motors or turning on the heaters if the safe mode flag was set. We had two special commands: one to set the safe mode flag, and one to completely shut down the payload. The latter command would completely turn off the processor, turn off the UMHVs and the heaters and close the canisters, so it would not even be possible to turn it on again unless it was power-cycled. This command was supposed to be sent at the end of flight.

The actuation portion was simple to design and develop, since it just consisted of changing the values of certain pins. The only special case was for controlling the stepper motors, since these require sending a pattern to spin them. The order in which the pattern is sent determines direction, while the frequency with which we send determines the speed. We controlled the motors at a fixed speed and used the internal timer to choose that speed. When changing the pattern the motor was receiving, the internal timer would be told to call a function to change that pattern in a specific amount of time, until enough steps had passed for the canister to be open or closed. We kept track of the motor's state by keeping track of the step number it was at and rotating until it reached the desired state (closed was 0 steps, open was dependent on the motor and canister).

The logic behind keeping the payload autonomous was very simple: we set threshold altitudes to start sampling on specific canisters, and threshold temperatures for turning on the heaters. During actual flight, there was a short circuit that caused one of the UMHVs to malfunction, so we had to turn on safe mode and completely stop autonomous mode. Safe mode was only used for a short amount of time, but it worked well: it enabled sampling on both canisters and turned on the heaters when necessary. When in safe mode, the team decided to manually close the canisters and keep the heaters on since there wasn't a risk of overheating due to our minimal design. A diagram of how the logical system makes decisions can be found in **Appendix C.**

Valuable Insight

- Keeping track of the current step of the motor was useful so that if, for whatever reason the canister had to be closed while in the process of opening, the motor would not

overspin (which was what used to happen when we had a controller that just spun the motor for a specific amount of steps, every time it was given a command).

- We kept receiving bytes in an array that we used as a cyclic queue (when we overflowed, we would place the new byte at the start). This worked very well, as long as the queue size is set properly as a function of how long it can take to parse each byte, and how often serial data is expected to come in (we determined this empirically).
- Initially, all of the code was run within interrupts. This was a bad decision, since the functions that took a longer time made interrupts trigger later than they should, which caused issues including: timing not being kept properly (since this was done by updating a variable every time a periodic interrupt triggered), and some serial bytes being dropped or corrupted (since the interrupt signaling the arrival of a byte was not triggered). To solve this, we kept a queue of functions to be executed in the main loop. Every time that queue was updated, the CPU was brought out of sleep and the functions, for example: parsing GPS bytes out of the queue, were executed in the main loop. When the queue was empty again, the CPU would go back to sleep. We also used optimization flags in the compiler to make our code run as fast as possible.
- We decided to not rely completely on the fidelity of GPS data, so in order to make sure we were above a certain altitude, our logic code had to see a series of consecutive measurements declaring that we were above (or below) that altitude before deciding to open or close the canister and start the sampling process.
- The initial reason for keeping track of the current step for each of the steppers was because we were trying to maintain a persistent state variable. To do this, on every change we would save it to the flash memory of the microcontroller. In case of system failure and reboot, the microcontroller would know the current state of every variable, and in case it rebooted when turning one of the motors, it would be able to just resume turning without overturning. It was never determined why this did not work, but we leave this as a possible strategy to handle system failures. Our new strategy was to make sure everything was in the default position when the system failed by having the motor overturn against a hard-stop screw that would limit how much it could actually rotate.

The code is publicly available for review at: https://github.com/jdkaplan/ASTRO

Biological Procedure

Overview

This section of the report will first review the protocol, procedure verification, and later biological analysis and overall system analysis. A schematic of the biological procedure can be found on page 23.

Prior to Flight

Prior to flight, each canister was sterilized at the same time in a UV treated bench top laminar flow hood. DNA Zap, a 2 part solution, was then applied to the canister surfaces to cause the degradation of residual genetic material in order to reduce potential sources of contamination.

Post Flight Field Collection

As shown in Figure 5, samples were collected by wiping the desired surface (including the electrodes, inner canister inner wall, and air inlets) with a sterile flocked swab to collect cells. Flocked swabs were then broken off into a solution of RNA Shield present inside of a 5 uL ZR 0.5 mm bead bashing tube. RNA Shield is a cell lysing solution that permits the preservation of genetic material. The use of RNA Shield would cause cell rupture and elution from swab, allowing for easier removal of genetic material.

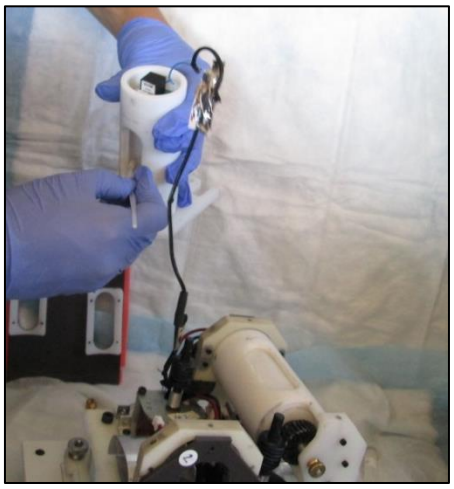


Figure 5) Scene from field collection of samples. Includes use of flocked swab to collect samples.

Collection took place in the field on sterile absorbent pads. The payload was disassembled and canisters were swabbed within this setting. The most pressing samples, the electrode and inner canister inner wall, were obtained first. Less crucial samples were obtained after these initial samples. After sample collection, each bead bashing tube contained one flocked swab and was labeled specific to the region of the canister. Therefore, we were able to track from which locations our samples originated.

We then sampled the soil of the landing site in order to provide a comparison of the land-based species and aerosolized microbes found by our collection device. Each soil sample was added to a ZR Bead bashing tube and 800 uL of RNA Shield.

As a positive control "(+)", 150 uL E. Coli cells from a culture held at 37^{o}C were lysed in a bead bashing tube filled with 800 uL RNA Shield, and the same downstream procedures were carried out. The E. Coli culture had a measured OD of 1.633 at the time of sample retrieval from the culture for use as (+) control. OD counts can help determine the state of replication the E.

Coli cells were in at time of harvest in order to estimate the number of chromosome replicates the E. Coli cells have. An OD of 1.633 might correspond to a chromosome count of 2n (2 copies of each chromosome). This number was crucial in our rough yield calculation below.

In-Lab Procedure

1) Additional Cell Lysis Step

Mechanical lysis was performed in lab by vigorously shaking the Zymo bead bashing tube in a specialized device (Zymo Xpedition™, a modified impact hammer). Mechanical lysis was performed in order to ensure complete cell rupture.

Zymo gDNA MicroPrep was then used to elute the genetic material from each sample bead bashing tube. Before use on flight samples, the method of gDNA (cell chromosomal DNA) extraction was tested on E. Coli cells that were cultured at 37^{o}C with an OD of 1.633. The result was an estimated yield of 60 percent from the use of Zymo gDNA MicroPrep. A lower yield was expected.

2) Distilling the genetic material from each sample

- gDNA was extracted from each flocked swab sample using the same method of Zymo gDNA MicroPrep. gDNA extraction from our samples consisted of filtration and washing of the genetic material of salts and proteins. We observed that there was a considerable amount of Krytox present beneath the beads of each bead bashing container. Presumably, all genetic material would be eluted into solution. However, this observation leaves room for potential questions regarding the potential of Krytox to retain cells or genetic material. However, these Krytox remnants were not seen in the most pressing of samples, the inner canister inner wall and the electrode.
- gDNA was extracted from the soil samples using Zymo Soil/ZR Microbe DNA MicroPrep for which 0.25 g of soil was used.

Upon elution of the genetic material from the filter, the gDNA was soon diluted in 1 part gDNA: 2 part PCR grade water (*Biological Protocol Schematic*, page 23). PCR grade water is free of nucleases and nucleic acid contamination that could cause false-positives in genetic material amplifications.

3) Amplifying the amount of genetic material present - PCR

Polymerase Chain Reaction (PCR) amplified a single part of the gDNA extracted from each sample. If sample bacterial ribosomal DNA was present in a certain well, we would expect to see a band at 400 bp in our gel electrophoresis run, as described below. PCR was run twice, once for *all* samples including E. Coli (+) control, soil samples, and all canister swabs. The first PCR run was run at 35 cycles. The second PCR reaction only focused on E. Coli (+) controls, the canister

electrodes, and inner canister inner wall ("IC.IW"). The second PCR was run at 40 cycles for increased sensitivity, suitable for amplification from single cells under good conditions.

Important PCR setup aspects

PCR was run according to protocol outlined by the Earth MicroBiome 16S rRNA program. Earth Microbiome 16S is specialized to bacterial and archaeal primers (515 Forward and 806 Reverse) and therefore amplifies regions specific to prokaryotes. In order to distinguish samples, each reverse primer contained a "barcode" region specific to that individual reverse primer. Therefore, upon sequencing, we will be able to determine which sample location on the canister a species was found.

Each sample was run in "triplicate," meaning each sample was amplified in 3 replicate 25 uL PCR reactions. Also, a Master Mix was created, composed of forward primers, 5 Prime Hot Master Mix (containing necessary nucleotides and enzymes), and PCR-grade water. Concentrations of these ingredients, in addition to the concentrations of reverse primers and templates, were fixed in order to ensure consistent results. PCR was then run using a thermocycler and programed to allow temperatures for strand separation, polymerase replication, and annealing.

4) Electrophoresis of PCR Product

Electrophoresis allows for the separation of DNA based on size, which is analogous to base pair ("bp") content. Genetic material is added to gel agarose wells and migrates across a gel once a voltage is applied to the system. For consistency, we used Lonza Flash Gel 16 +1 cartridges with a 1.2% Agarose content. Genetic material with higher bp content will migrate more slowly than smaller fragments. Our desired region for DNA migration was 381 base pairs, which accounts for the length of amplicon and attached forward and reverse primers

5) Cleaning Up PCR Products

Zymo Research Oligo Clean and Concentrator was then used in order to remove residual proteins, unused nucleotides, and other byproducts of the PCR reaction which could obfuscate the later downstream sequencing of our samples.

6) Sequencing

We are currently awaiting sequencing results. Sequencing is being performed at Massachusetts General Hospital and will be made available upon their completion and receipt of the sequences. We used a MiSeq Reagent Kit (500 cycles), which has an estimated output of 15 million reads. Sequencing was delayed due to a low genetic material yield from the 35 cycle PCR from all samples in PCR1. Therefore, sequencing will be run on the electrode and IC.IW samples from PCR2 and compared to background contamination. This will test to see if our samples are either contamination or atmospheric organisms.

Biological Results

PCR 1

The first PCR was less sensitive to single cells and therefore little genetic material was visible in any of the 4 canisters. However, the positive controls (3 wells of E. Coli amplicon) did form a band at 400 bp, implying that the PCR was viable (Figure 6). Negative controls were negative at the region of amplification of 400 bp. It was observed that there were two additional bands of genetic material at 150 bp and below 100 bp. A hypothesis surrounding the band at 150 bp is attributed to primer dimerization. The prevalent band at less than 100 bp is attributed to the presence of small, single forward and reverse primers. In regions where the 400 bp band was formed, the wells contained little to no bands at 150 bp and <100 bp. Electrophoresis of all 25 samples in addition to the two soil samples and positive and negative controls demonstrates the relative amounts of genetic material in the canisters in comparison to the positive control (E. Coli) and soil samples. E. Coli and soil samples came up with bands at 400 bp indicating that the PCR reaction did indeed work at amplifying the 400 bp region (Figure 6). However, the lack of a band or light band formation of the canister samples at 400 bp could signify (i) the low levels of contamination of our collection system, (ii) the low levels of microbes that are difficult to detect without using *more sensitive* isolation and amplification methods, or (iii) the loss of sample through our process of gDNA extraction.

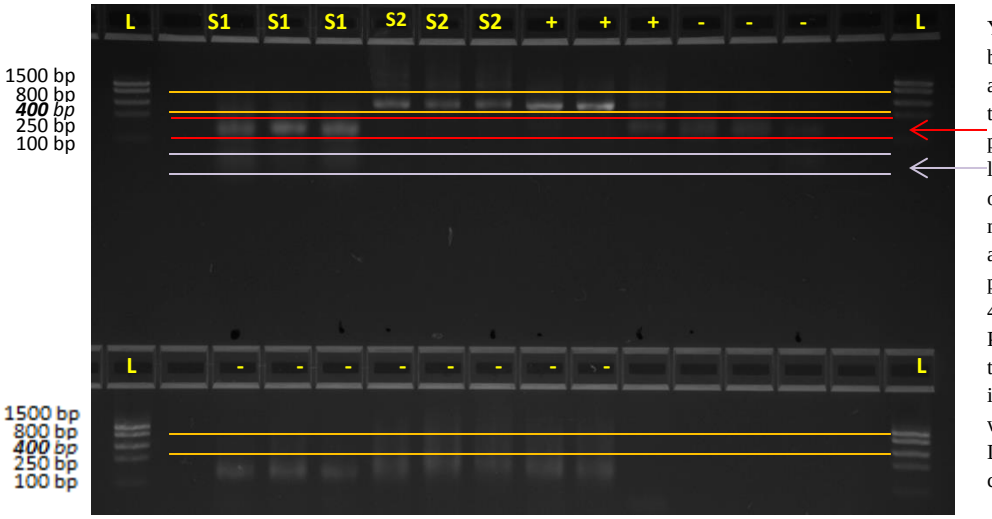


***Figure* 6)**

Yellow Region occurs at 400 bp, the desired region for amplicon. The red region is the region of hypothesized primer dimerization. The light purple region is region of remaining primer. The majority of positive controls and Soil Sample (S2) produced amplicon band at 400 bp, which verifies the PCR procedure. Also notice the little to no fluorescence in (-) controls at 400 bp, which is desirable. (L) is the DNA ladder with base pair count to the left of the image.

Figure 7) Light smear indicated at 400 bp (orange box) indicates little desired genetic material prior to size selection of 400 bp band

After using Oligo Clean and Concentrator, which essentially rids the PCR products of proteins and other undesirable byproducts, it was determined that there was not enough genetic material present at 400 bp to sequence. This is indicated by the light smear of genetic material at 400 bp (Figure 7).

PCR 2 – More Sensitive to Single Cell DNA Amplification

A second PCR at 40 cycles was run to visualize the relative amounts of genetic material present within high interest regions (electrode, IC.IW). PCR at 40 cycles is more sensitive, sensitive enough for *single cell* amplification. The results were favorable (Figure 8 & 9).

Electrode and IC.IW Samples

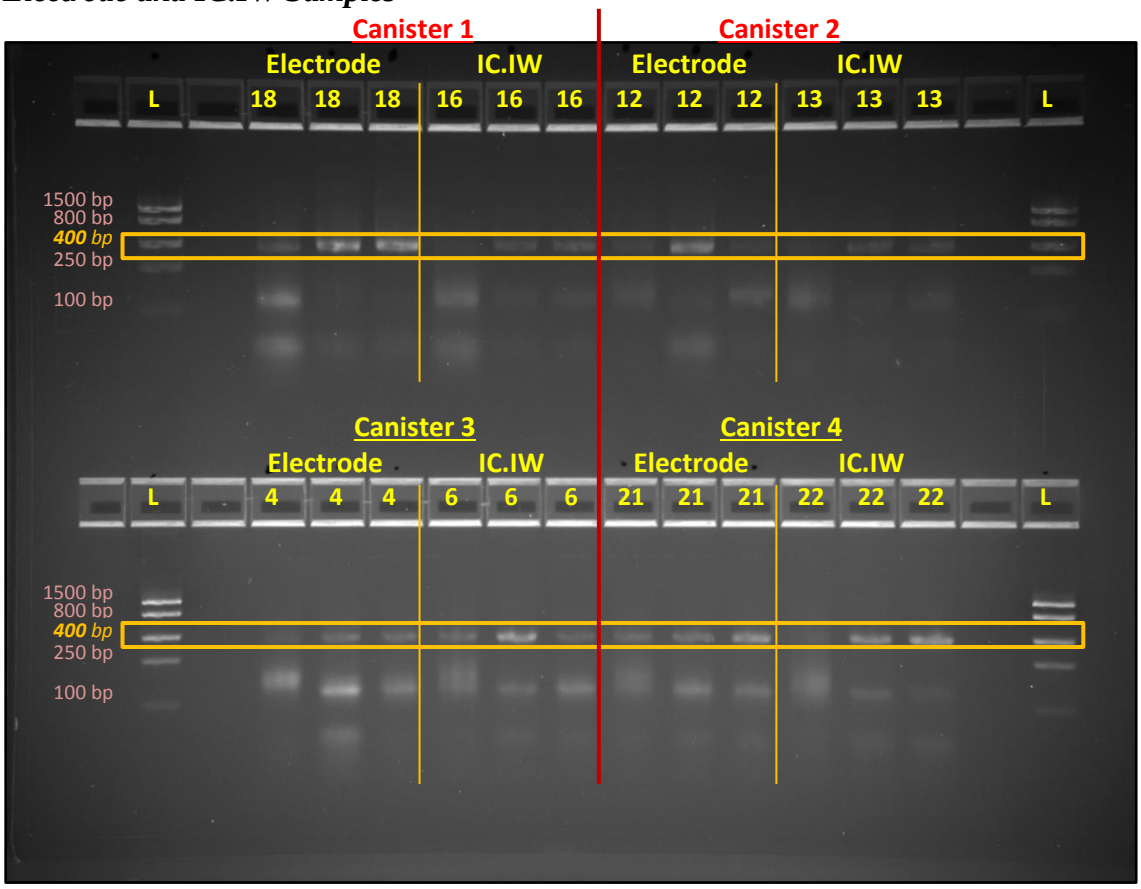


Figure 8) **Canister 1** collected integral atmospheric sample. **Canister 2** collected at 120,000 ft altitude. **Canister 3** is unpowered canister (environmental control). **Canister 4** is laboratory control. **IC.IW** is inner canister inner wall. **(L)** is the Flash Gel Quant Ladder Cat No. 50475.

Controls PCR2

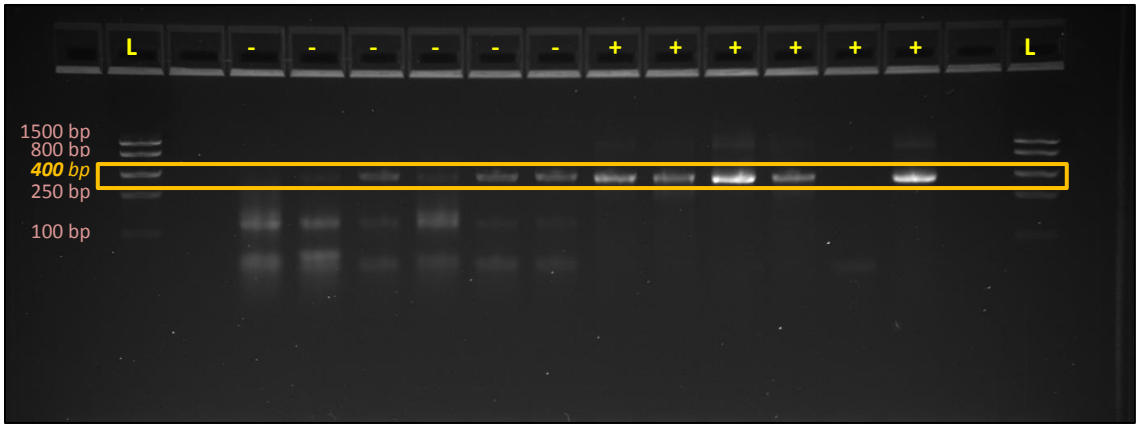


***Fig 9*) (-)** control used during PCR run. **(+)** control is based on E. Coli culture. **(L)** is the Flash Gel Quant Ladder Cat No. 50475.

Biological Analysis

Through these series of electrophoresis, we came to the conclusion that the electrostatic collection device did achieve its primary objective of collecting samples using electrostatic attraction. PCR is not qualitative so it cannot tell the amount of genetic material initially present, but rather indicates levels of genetic material present with respect to other samples.

Canister 1 - The relative amounts of amplicon fluorescence found from electrode samples from canister 1 is greater than the amount of amplicon fluorescence found on the inner canister inner wall (IC.IW). That is expected if electrostatic collection is the means through which sample collection is achieved. Therefore, the bright band found at 400 bp for the electrode with respect to IC.IW demonstrates that electrostatic collection was achieved. The 400 bp band is expected and indeed was brighter than those of canister 2, control canisters 3 & 4, negative PCR controls. This could be due to the duration of sampling; Canister 1 collected an integral atmospheric sample of the microbial populations. Therefore, it had sampled different densities of biological aerosols from altitudes of 28,000 to 100,000 feet, before premature termination at float altitude.

Canister 2 – The relative amounts of amplicon fluorescence at 400 bp found in electrode samples for canister 2 is greater than the amount of amplicon fluorescence found on IC.IW. That is again expected under the condition that electrostatic collection is achieved. The relatively low amounts of genetic material found on the electrode for canister 2 are expected because canister 2 was opened at float altitudes ranging from 100,000 to 120,000 feet altitude, where we expected little, if any sample, to be collected. The band at 400 bp for the electrode of Canister 2 also demonstrates that at high altitudes, electrostatic collection is still feasible. However, in order to determine if the sample collected by canister 2 is a high altitude bioaerosol, we must compare canister 2 sequences to those of the control canisters. Sequences not found in the controls would lend better to a bioaerosol sample specific to float altitude.

Canister 3 – The relative amounts of genetic material present on the control environmental canister that flew on HASP provides an interesting detail. The canister was unpowered and therefore no high voltage was applied to the electrode. Additionally, the amount of amplicon fluorescence found on the electrode is less bright than the amount of fluorescence found in the samples of IC.IW. This could further support our hypothesis that since the electrode has a smaller surface area than that of the Inner Canister Inner Wall, the electrode will have respectively less genetic material present than that of the IC.IW with a larger surface area.

Canister 4 – The relative amounts of genetic material were higher than initially anticipated. This could be because of inadequate sterilization or prolonged sampling time (on the order of weeks as opposed to days). But interestingly, the contaminants looked to be only slightly more present on the IC.IW.

Negative Controls – The negative controls are solely PCR grade water. The (-) controls that did show some amount of fluorescence were near areas of cross trafficking across the PCR plate. By having cross traffic over the PCR plate, it could increase the chances of free DNA becoming incorporated into the mixture. On a purely comparative basis, the canisters and (+) controls did indeed have fluorescence levels that were greater than that of the (-) controls.

Positive Controls – The positive controls came up with a relatively bright band at 400 bp except for the second to last lane. This is due to the fact that during PCR plate layout, the template DNA was not put into the reaction mixture as an omission on behalf of the experimenter.

Across all samples and controls, there were consistent bands again at 150 bp and <100 bp. The 150 bp is attributed to the dimerization of primers. The <100 bp fragments are attributed to the presence of primers.

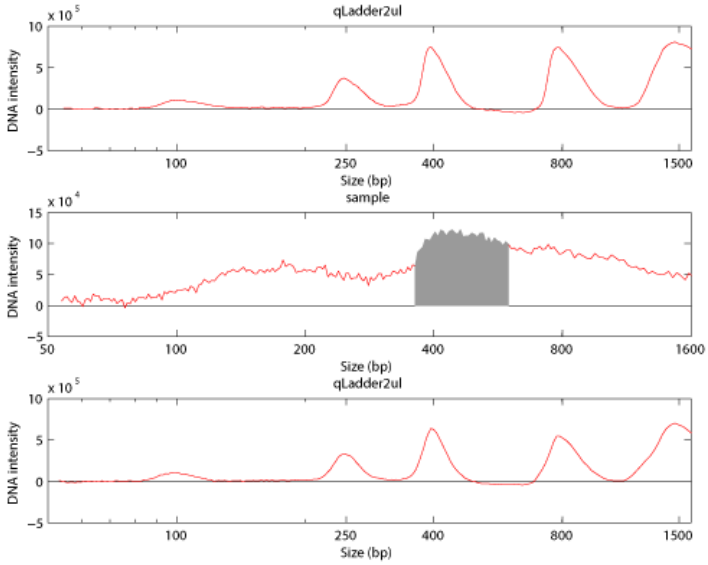


Figure 10) Gel quantification of sample for sequencing. The shaded region is the desired region to be sequenced. Plots labeled "qLadder2ul" are the quantifications of the ladder. Notice the shaded region is at 400 bp.

In all, the multiple PCR and electrophoresis runs confirmed a crucial point, the success of an electrostatic sampler at collecting microbial bioaerosols, even at low atmospheric concentrations. In order to determine if the band seen at 400 bp for the Canister 2 Electrode is indeed unique to only high altitudes and not contamination, it is crucial to next sequence the obtained PCR products using MiSeq Reagent Kit. We are currently awaiting sequencing results. The pooled

sample being sequenced has approximately 26.9 ng of purified and barcoded DNA, confirmed by NanoDrop and gel quantifications (Fig 10). The requirements for sequencing are 10 uL of 4 nM pooled and barcoded sample. Due to latency of sequencing, we do not have the sequences available for this final report but will write an amendment with the sequences, and respective phylogeny. It was desirable that our negative control canisters were amplified using single cell PCR with 40 cycles, because we now have a comparison basis off of which we can compare the organisms in the atmospheric samples to control samples. On the whole, the HASP flight was a success, because the ASTRO apparatus demonstrated the ability to sample low, atmospheric concentrations of microbial bioaerosols at points with low levels of atmosphere. However, by sequencing the bioaerosols' DNA, we can determine if the collected samples are above background and if so, which species are present at integral or high altitudes.

Areas for Biological Design Improvements

Although we achieved our initial goal of making an electrostatic bioaerosols sampler, there are many areas for design improvements in order to optimize the system, improve collection yields, and reduce the dependency on multiple downstream processing steps. Below is a list of areas for future improvement:

- Sample Volume - In order to qualitatively assess the concentration of microbial species for a given altitude sample, one would need to know a volume over which a sample was taken. This could be calculated by a flow rate sensor.
- Sample concentration – A more effective way of concentrating sample would avoid loss of sample during such events as swabbing of electrode and gDNA extraction processes. Such concentrating methods could be achieved through a microfluidic approach to sample collection off of the electrode.
- Larger diversity of sample analysis – In order to get a more revealing genetic profile of atmospheric bioaerosols, it would be advisable to test across more branches of life, such as using a primer that focuses broadly on eukaryotes and one specific to fungal samples. Therefore, in total, we would be testing for fungal and bacterial spores, in addition to whatever other eukaryotes reside within the atmosphere.

Lessons Learned

As a new team, we learned many lessons. We hope to come back to the HASP program next year. Below is a list of lessons learned for HASP personnel and future ASTRO teams.

Logistics

- Begin fundraising early and apply for department funding. It would be advisable to recruit team members for specifically fundraising or finance management. Team member specialization would allow each person to have his/her own role on the team.

Mechanical Design

- Start from the basic mechanical design and then get more detailed. If the design seems too complex, simplify to the essential elements. We recommend a modular design.
- During design brainstorming sessions, build rapid prototypes and later design in CAD.
- Tasks should occur in parallel. Avoid excessively changing existing mechanical or electronics systems.
- Design should take into account factors that are overlooked at sea level, such as vapor pressures (if using a fluid) and material survivability under cold temperatures and impact. Materials like PTFE or Delrin are appropriate at high altitudes. For thermal insulation, we recommend covering the payload with foam and Mylar.

Electrical Design

- Decide early on the controller board. For minimal power consumption, a MSP430 is advised. However, microcontrollers such as the MSPs and AVRs are slow and difficult to debug. A board such as a Raspberry Pi, Panda Board, Beagle Board, or even a Netbook, while requiring more power, is much easier to use. However, it might require design considerations such as maintaining a warm temperature for function.
- Debug with LEDs. During integration, you will not have live serial connection, but will have live visual feedback. So with LEDs, you can quickly determine the function of an unseen subsystem.
- If using a microcontroller, ensure you have an accurate timing component such as a 32kHz quartz crystal. Internal RC crystals tend to be inaccurate in varying temperatures, and that affects serial and other time-sensitive operations.

Programming

- Document the code and thought processes behind its development.
- Low-level Programming: Do not hard-code which pins are being used and do code with informative constants. Make a visual diagram of how the pins are connected.
- Implement a manual control mode for the software in order to perform unit testing. Write abstract functions that control individual components. With this, it is possible to write simulators in order to test without the need for electrical parts. Use this simulator to develop an autonomous control system.
- As code gets more complex, timing issues arise if using a microcontroller.

Team Member Demographics

Active Team Members over Time			
January 2013	**February 2013-May 2013**	**June 2013-September 2013**	**September 2013 – December 2013**
Christopher Carr Steven Gordon Jeremy Kaplan Jessica Sandoval Devon Sklair	Christopher Carr Ethan DiNinno Cheryl Gaul Rodrigo Gomes Jeremy Kaplan Jessica Sandoval Laura Standley Linda Xu	Christopher Carr Ethan DiNinno Rodrigo Gomes Jeremy Kaplan Jessica Sandoval	Christopher Carr Ethan DiNinno Cheryl Gaul Rodrigo Gomes Jeremy Kaplan Jessica Sandoval Laura Standley

MIT Undergraduate Student Profiles					
Name	**Role on ASTRO**	**Expected Graduation Year**	**Major**	**Ethnicity**	**Gender**
DiNinno, Ethan	Electronics	2016	Electrical Engineering	Caucasian	M
Gaul, Cheryl	Prototyping, Fundraising, Editing	2016	Aero Astro	Chinese, Caucasian	F
Gomes, Rodrigo	Programming, Website	2015	Computer Science	Hispanic	M
Gordon, Steven	Prototyping	2014	Aero Astro	Caucasian	M
Kaplan, Jeremy	Programming, Website	2015	Computer Science	Hispanic	M
Sandoval, Jessica	Team Leader, Design, Mechanical Build, Fundraising	2015	Biological Engineering	Hispanic	F
Sklair, Devon	Prototyping	2015	Aero Astro	Caucasian	F
Standley, Laura	Prototyping, Editing	2015	Mechanical Engineering	Caucasian	F
Xu, Linda	Prototyping	2015	Physics	No Response	F
Team Advisor					
Carr, Christopher Sc.D	Research Scientist, ASTRO advisor				

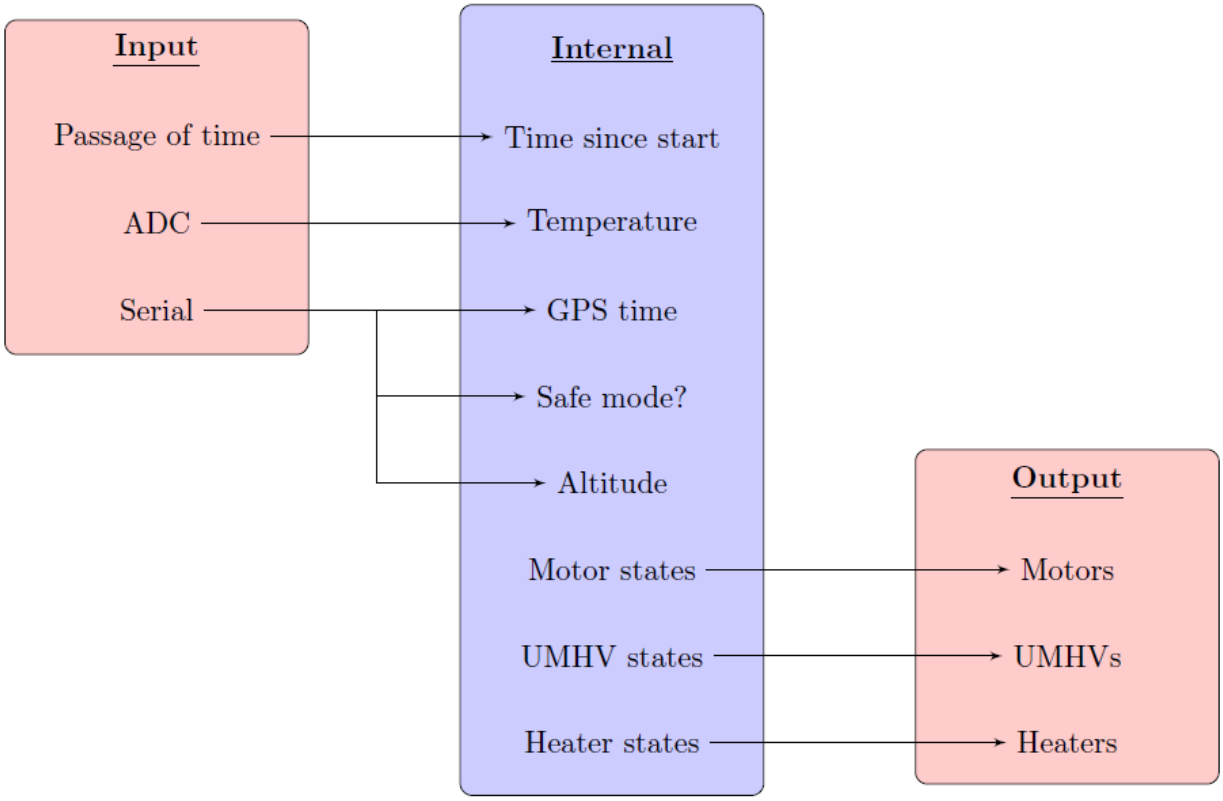
Appendix A
Uplink Command List

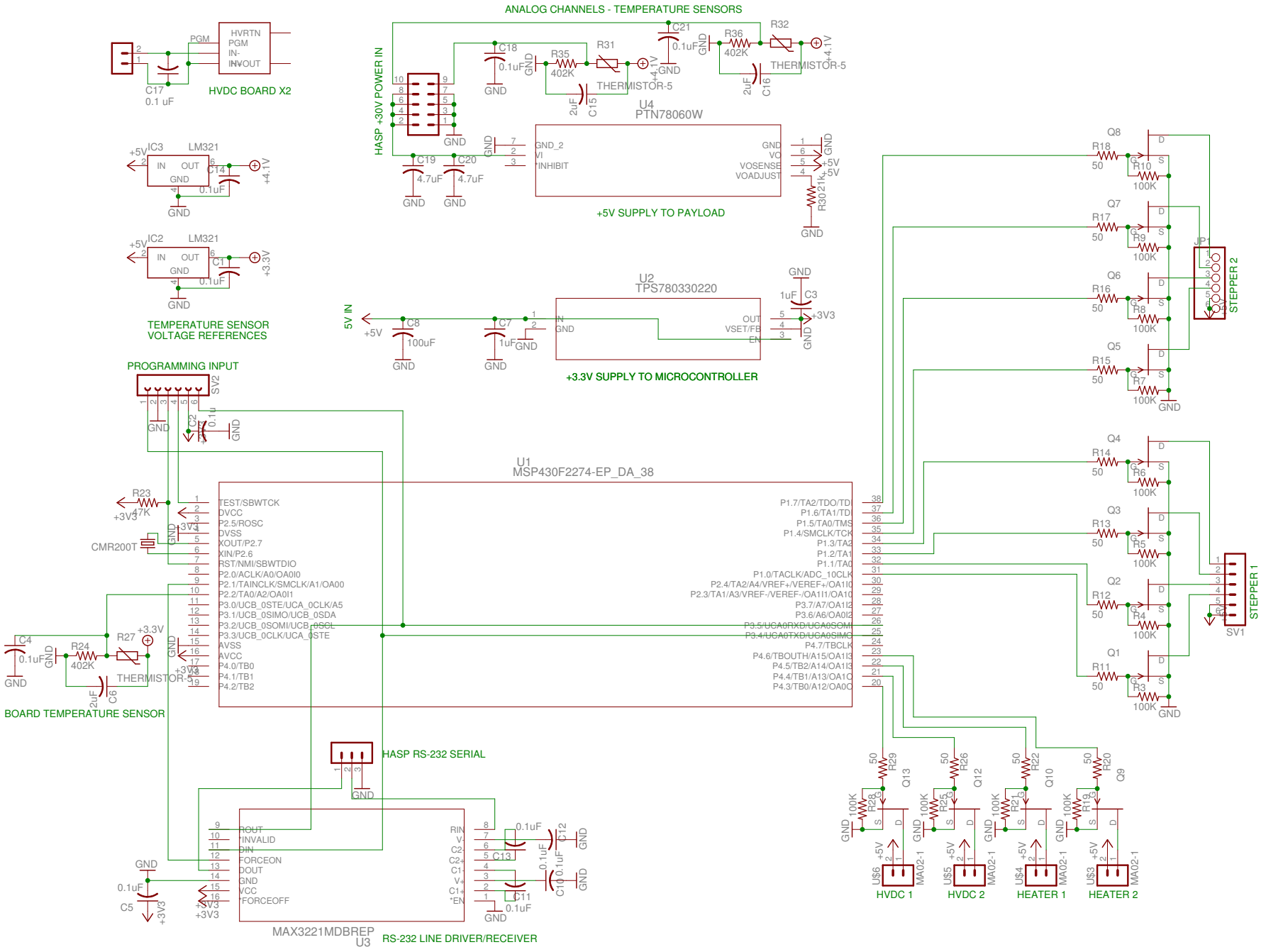
Name	Command	Description
PING	0x00 0x00	Send status response
RIDE	0x02 0x02	Enter manual override mode
OPEN1	0x04 0x04	Open canister 1
SHUT1	0x05 0x05	Close canister 1
OPEN2	0x06 0x06	Open canister 2
SHUT2	0x07 0x07	Close canister 2
LITE1	0x08 0x08	Turn on electrode 1
KILL1	0x09 0x09	Turn off electrode 1
LITE2	0x0B 0x0B	Turn on electrode 2
KILL2	0x0C 0x0C	Turn off electrode 2
HEAT1	0x0E 0x0E	Turn on heater 1
COOL1	0x0F 0x0F	Turn off heater 1
HEAT2	0x10 0x10	Turn on heater 2
COOL2	0x11 0x11	Turn off heater 2
DOWN	0x12 0x12	Shutdown payload / Power Off
AUTO	0x13 0x13	Power On / End override mode (Will NOT repower payload after a DOWN command)
HARD1	0x14 0x14	Drive motor 1 to hard stop for calibration (only use in case of failure)
HARD2	0x15 0x15	Drive motor 2 to hard stop for calibration (only use in case of failure)

Appendix B
State Information as seen on ground
command 0
internalTime 14411914 (approx. 14074.13s)
externalTime (18, 12, 24, 0)
height 37617
temperature 753 (approx. 25.19 C)

motorOne 180
motorTwo 300
safemode 0
heaterOne 0
heaterTwo 0
HVDCOne 1
HVDCTwo 1
checksum 247
Checksum correct?: True

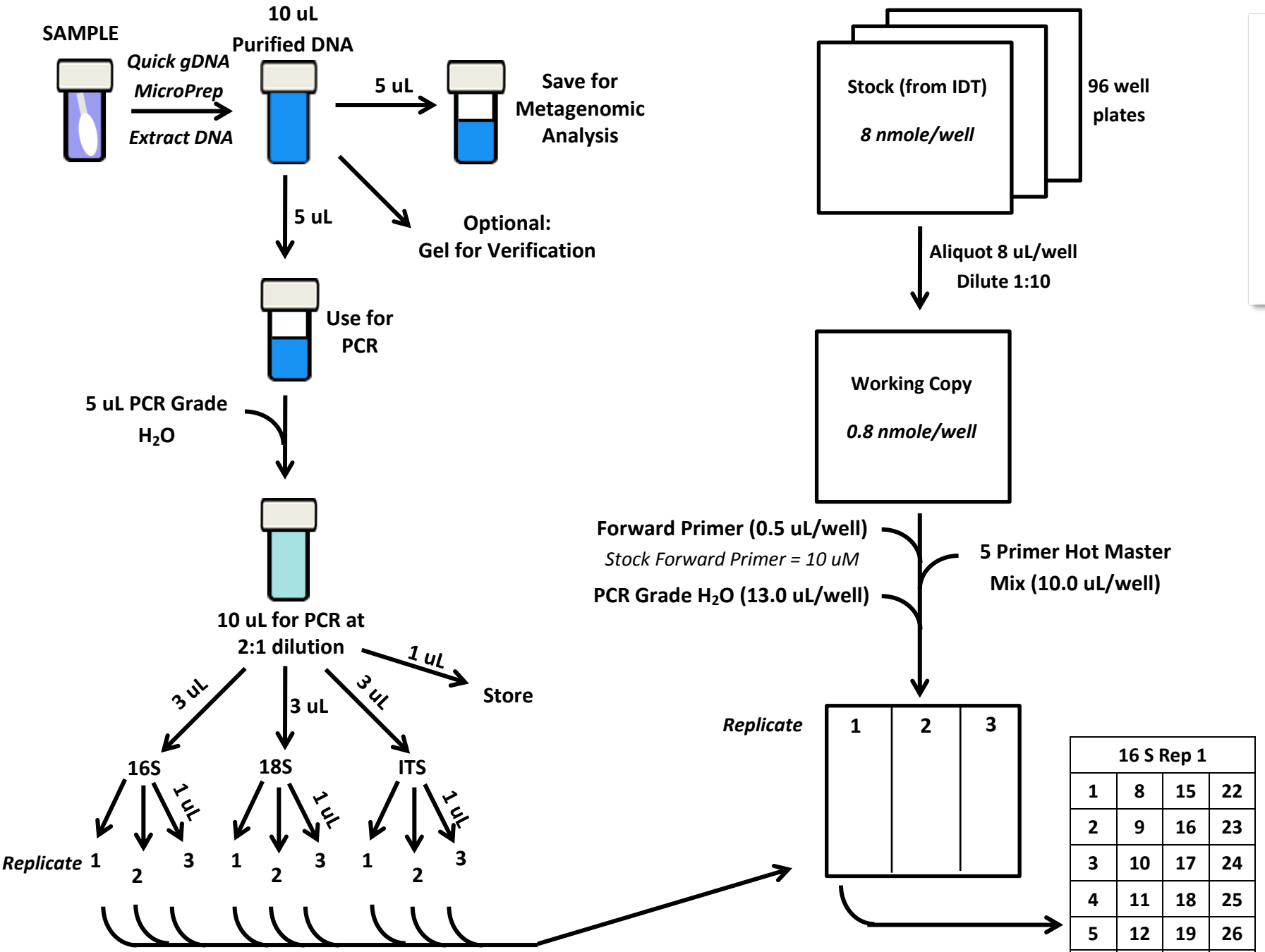
Appendix C
Logic Diagram
Red boxes are external to the code, while Blue boxes are the state variables. Directed arrows indicate what triggers what (for example, serial triggers a change in GPS time and Altitude).




ANALOG CHANNELS - TEMPERATURE SENSORS
HVDC BOARD X2
HASP +30V POWER IN
U4
PTN78060W
+5V SUPPLY TO PAYLOAD
IC3 LM321
IC2 LM321
TEMPERATURE SENSOR
VOLTAGE REFERENCES
U2
TPS780330220
5V IN
+3.3V SUPPLY TO MICROCONTROLLER
PROGRAMMING INPUT
U1
MSP430F2274-EP_DA_38
BOARD TEMPERATURE SENSOR
HASP RS-232 SERIAL
MAX3221MDBREP
U3
RS-232 LINE DRIVER/RECEIVER
STEPPER 1
STEPPER 2
HVDC 1
HVDC 2
HEATER 1
HEATER 2

Biological Protocol Schematic

From gDNA extraction through PCR



16 S Rep 1			
1	8	15	22
2	9	16	23
3	10	17	24
4	11	18	25
5	12	19	26
6	13	20	27
7	14	21	C4
C1	C2	C3	C5