

# 1 **Synchronous in-field application of life-detection techniques**

## 2 **in Icelandic Mars analogue sites**

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7 Keywords:

8 Astrobiology

9 Life detection

10 Mars

11 Analog

12 Mission simulation

### 13 **1 Abstract**

14 Field expeditions that simulate the operations of robotic planetary exploration missions at  
15 analogue sites on Earth can help establish best practices and are therefore a positive contribution

16 to the planetary exploration community. There are many sites in Iceland that possess heritage as  
17 planetary exploration analogue locations and whose environmental extremes make them suitable  
18 for simulating scientific sampling and robotic operations.

19 We conducted a planetary exploration analogue mission at two recent lava fields in  
20 Iceland, Fimmvörðuháls (2010) and Eldfell (1973), using a specially developed field laboratory.  
21 We tested the utility of in-field site sampling down selection and tiered analysis operational  
22 capabilities with three life detection and characterization techniques: fluorescence microscopy  
23 (FM), adenine-triphosphate (ATP) bioluminescence assay, and quantitative polymerase chain  
24 reaction (qPCR) assay. The study made use of multiple cycles of sample collection at multiple  
25 distance scales and field laboratory analysis using the synchronous life-detection techniques to  
26 heuristically develop the continuing sampling and analysis strategy during the expedition.

27 Here we report the operational lessons learned and provide brief summaries of scientific  
28 data. The full scientific data report will follow separately. We found that rapid in-field analysis  
29 to determine subsequent sampling decisions is operationally feasible, and that the chosen life  
30 detection and characterization techniques are suitable for a terrestrial life-detection field mission.

31 In-field analysis enables the rapid obtainment of scientific data and thus facilitates the  
32 collection of the most scientifically relevant samples within a single field expedition, without the  
33 need for sample relocation to external laboratories. The operational lessons learned in this study  
34 could be applied to future terrestrial field expeditions employing other analytical techniques and  
35 to future robotic planetary exploration missions.

## 36 **2 Introduction**

37 Extreme environments on Earth are used as analogs to inform both the science and  
38 operations of future planetary exploration missions (Amils *et al.*, 2007, Amato *et al.*, 2010, Billi  
39 *et al.*, 2013). In particular, Icelandic lava fields have an especially good heritage as Mars analog  
40 sites (Farr, 2004, Warner and Farmer, 2010, Cockell *et al.*, 2011, Cousins and Crawford, 2011,  
41 Mangold *et al.*, 2011, Ehlmann *et al.*, 2012, Cousins *et al.*, 2013). Lava fields are relevant for  
42 astrobiological science due to the presence of extreme conditions, including desiccation, low  
43 nutrient availability, temperature extremes (e.g. due to high elevation or close proximity to  
44 fumaroles), relatively young ages, and their isolation from anthropogenic contamination (Allen  
45 *et al.*, 1981, Bagshaw *et al.*, 2011). From an operational perspective, many Icelandic lava fields  
46 are remote enough to require that field expeditions address several sampling operational  
47 constraints that are also experienced in robotic planetary exploration (Arena *et al.*, 2004, Preston  
48 and Dartnell, 2014).

49 Terrestrial field campaigns designed to conduct scientific studies of planetary analogs can  
50 also serve as operational analogs for robotic planetary missions. Field campaigns typically  
51 involve *in situ* sampling, followed by preservation of any collected samples and subsequent  
52 return to an institutional laboratory where the samples can then be analyzed, analogous to  
53 planetary sample return missions. However, some field expeditions may carry limited  
54 instrumentation for *in situ* analysis (Ehlmann *et al.*, 2012), and like robotic planetary missions,  
55 these instruments must be chosen ahead of time. Limited on-site consumables further constrain

56 the amount that can be accomplished in the field by both terrestrial field expeditions and  
57 planetary exploration robots. Furthermore, sending samples to an institutional laboratory with a  
58 delay of potentially several months before full scientific analysis is possible. This may prevent  
59 results of prior sampling being available to influence sampling strategy throughout the  
60 expedition, and this applies to whether on Earth or elsewhere in the solar system. Although the  
61 results obtained might be available to assist in the planning stages of future field campaigns or  
62 missions, such follow-up expeditions might be weeks, months, years or decades in the future.  
63 The ability to maximize science return from limited in-field planetary exploration analyses is far  
64 more critical given that a sample return mission from Mars, or other astrobiologically relevant  
65 planetary bodies, is still decades away (McLennan, 2012), and raises significant planetary  
66 protection issues (Bridges and Guest, 2011).

67       The capacity for rapid sample analysis and interpretation can alleviate the problems posed  
68 by terrestrial or planetary expeditions. Firstly, it allows for the down-selection of sampling sites  
69 in the field. Rather than being dependent solely on previous mission data or remote sensing  
70 provided by partner programs, sampling choices can be made in the field based on near-real-time  
71 results. Secondly, it allows for 'tiered analysis', in which a single sample may be subject to a  
72 faster or lower-cost analysis (either non-destructively or by partitioning) to determine whether it  
73 is sufficiently interesting to warrant a second, more resource-intensive or more limited-capacity  
74 analysis. These features can be combined to maximize science return if a balance is struck

75 between the cost of carrying additional resource-light 'pre-sampling' instruments and the  
76 increased science return from more resource-intensive instruments.

77 Choosing the exact locations and samples that a field team, rover, or lander will analyze is  
78 critically important given the operational constraints. The planetary mission team must select a  
79 location to sample using the vehicle's remote sensing instruments (*e.g.* the ChemCam instrument  
80 on the Mars Science Laboratory (Meslin *et al.*, 2013)) and assume that this site is representative  
81 of the area of interest. If a difference in sampling location of a few meters, centimeters, or even  
82 hundreds of microns could make a significant difference in the results, it may mean that science  
83 objectives are not met. This will be especially critical when life-detection is the primary goal,  
84 given the inherent variability in the distribution of living things as we know them on Earth.  
85 Successfully characterizing multiple parameters across the multiple scales of a field site will help  
86 to reduce the number of initial sampling rounds needed.

87 We conducted a planetary exploration analog expedition to two recent Icelandic lava fields,  
88 Fimmvörðuháls (2010) and Eldfell (1973), with a specially developed field laboratory. Our main  
89 goal was to prove the feasibility of real-time sampling and site down-selection in a life detection  
90 robotic exploration context through quick-turnaround 'pre-sample' analysis and extrapolation of  
91 the likely presence of biomarkers. To inform the development of current and future *in situ*  
92 planetary missions, this was broken down into three interrelated operational sub-goals:

- 93 1. Demonstrate the feasibility of performing multiple rapid cycles of sample selection,  
94 sample analysis and interpretation in-field under simulated robotic exploration  
95 constraints.
- 96 2. Demonstrate the synchronous application of multiple life detection techniques  
97 within these multiple cycles.
- 98 3. Demonstrate the potential of fluorescence microscopy (FM), adenosine  
99 triphosphate (ATP) bioluminescence, and quantitative polymerase chain reaction  
100 (qPCR) assays as quick-turnaround terrestrial life detection techniques.

101 Here, we report upon the operational and logistic lessons learned during the expedition,  
102 which could influence the design of future field studies. Scientific results of the expedition will  
103 be reported separately (manuscript in preparation). Follow-up expeditions are planned and will  
104 be reported upon in relation to this work.

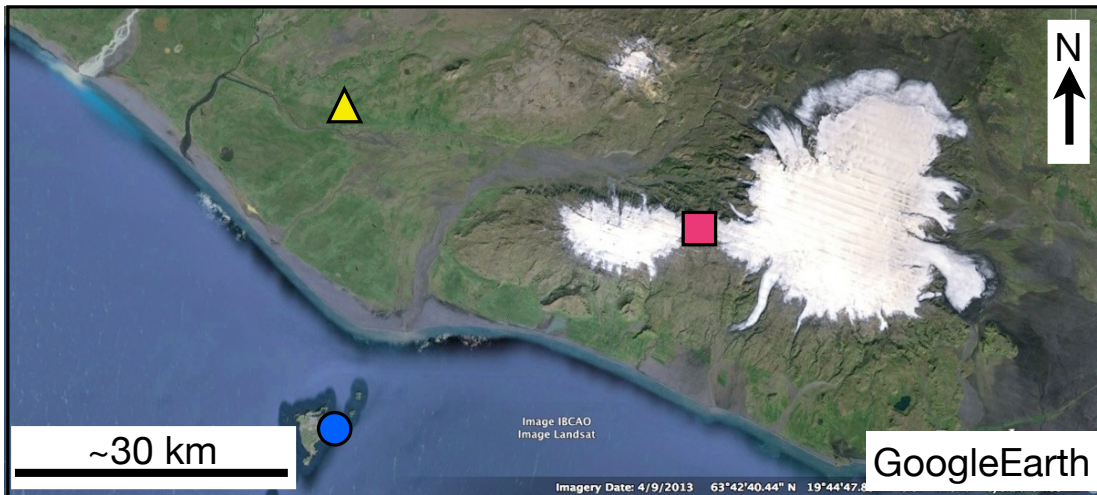
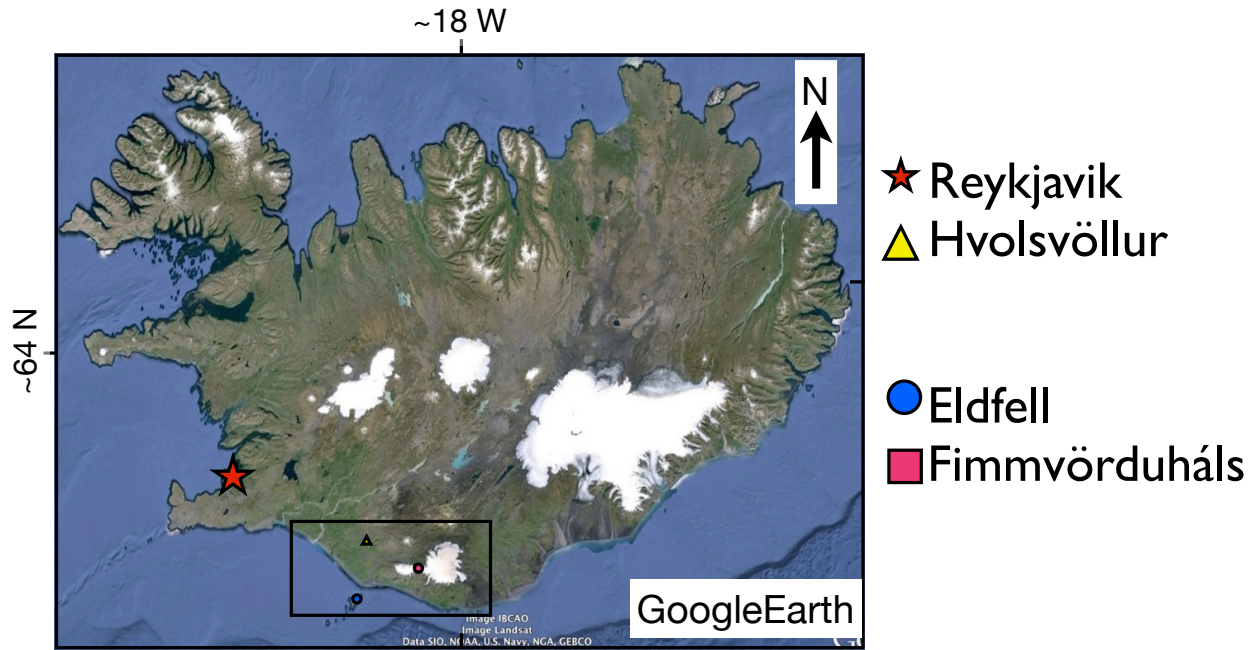
### 105 **3** **Methodology**

106 The expedition consisted of cycles of sampling, rapid preliminary analysis, and follow-up  
107 based on the results from the previous sampling and analysis cycle. The expedition personnel  
108 were split into two teams, allowing two of these repeated sampling and analysis cycles to be run  
109 in staggered parallel, thus increasing the expedition's throughput and ensuring that the field lab  
110 was neither idle nor acting as a bottleneck. After sampling cycles were completed all samples

111 were more extensively analyzed over three additional days in the field lab to address the more  
112 detailed question of sample site homogeneity.

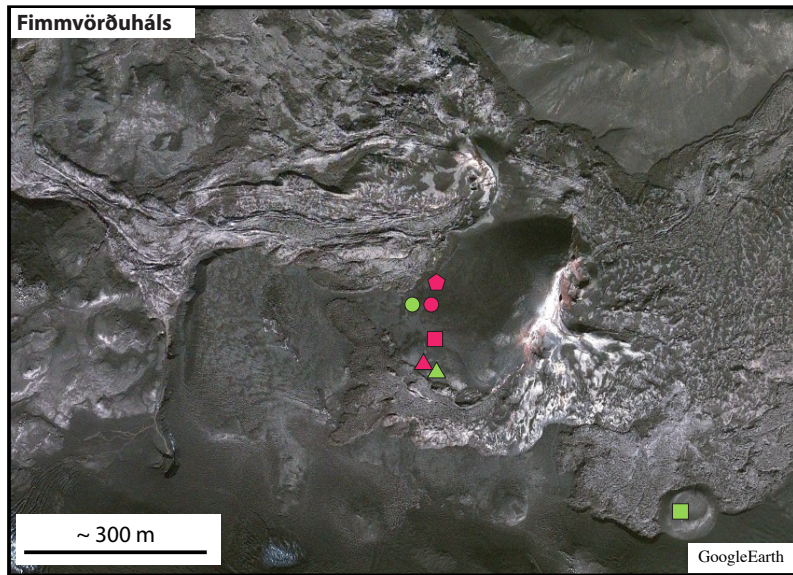
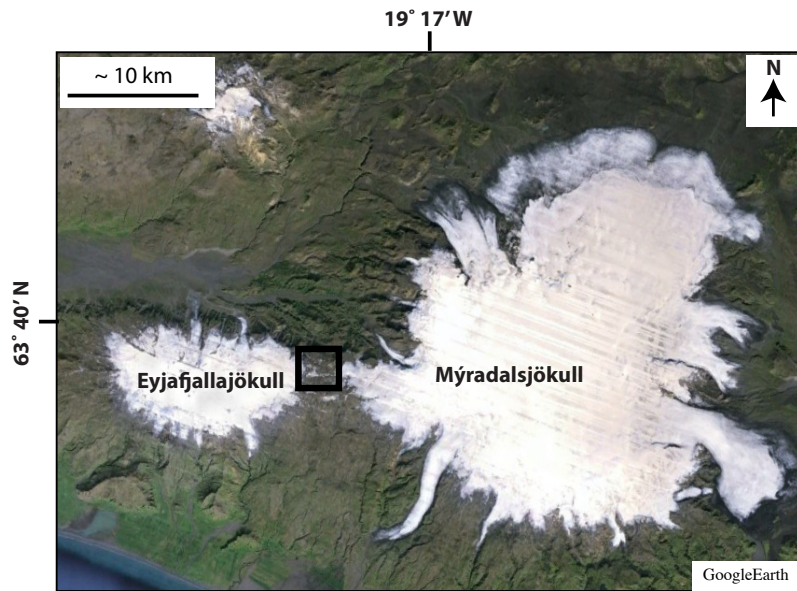
### 113 **3.1 Field Sites**








114 Two lava fields were chosen for our expedition: Fimmvörðuháls (63° 38' 12.30" N, 19° 26'  
115 49.20" W) and Eldfell (63° 25' 08.30" N, 20° 14' 38.70" W) (Figure 1). The Fimmvörðuháls  
116 lava field formed between 20 March and 12 April 2010, from a basaltic effusive eruption  
117 associated with the 2010 Eyjafjallajökull eruption located approximately 7.5 km away. The field  
118 site is located in a saddle between the larger Eyjafjallajökull and Myrdalsjökull volcanic  
119 structures (Figure 2) (Edwards *et al.*, 2012). The Eldfell volcano, associated with the  
120 Vestmannaeyjar volcanic system, began erupting on 23 January 1973 on the island of Heimaey.  
121 The Eldfell eruption had both effusive and explosive alkali basalt eruptions and lasted for five  
122 months, producing  $\sim 0.23 \text{ km}^3$  of volcanic material (Thorarinsson *et al.*, 1973, Higgins and  
123 Roberge, 2007). Both field sites have very similar basaltic tephra (unconsolidated volcanic  
124 material) sediment types with limited vegetation cover. The two sites are approximately 45.0 km  
125 apart.



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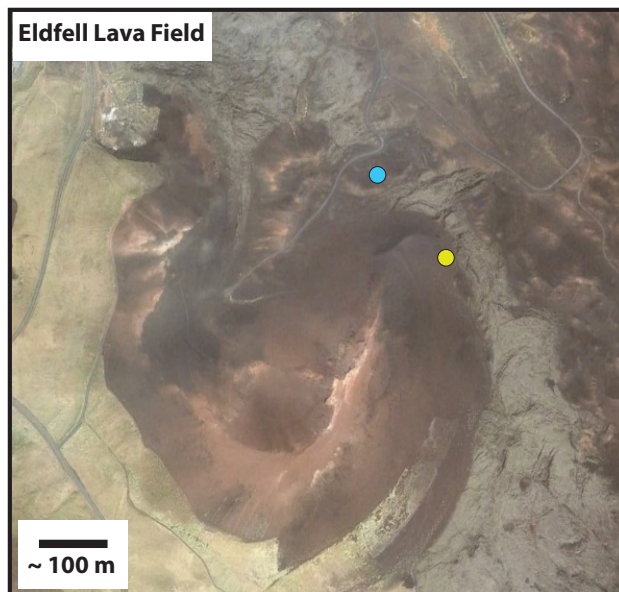
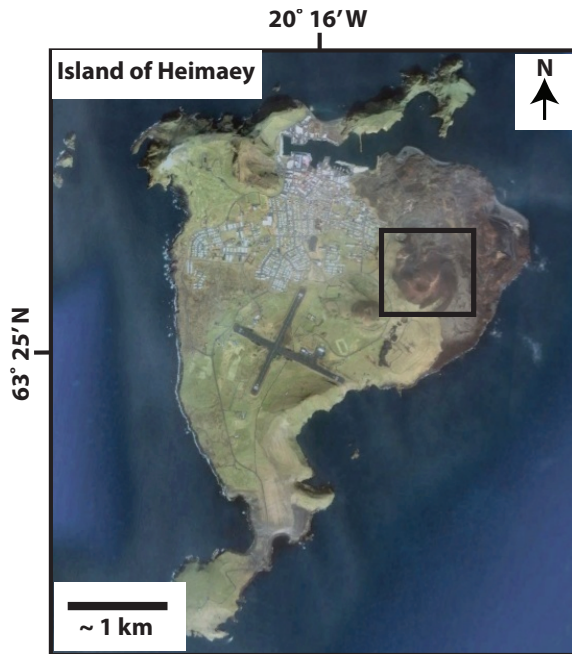
Figure 1 - A map of Iceland with the expedition's field sites marked.



July 27, 2013	July 29, 2013
 Fimm. 1 - (1-2) - (1-3)	 Fimm. 1 - (1-2) - (1-3)
 Fimm. 2 - (1-2) - (1-3)	 Fimm. 2 - (1-2) - (1-3)
 Fimm. 3 - (1-2) - (1-3)	 Fimm. D - 1 - (1-3)
	 Fimm. R - 1 - (1-3)

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Figure 2 - Sampling locations within the Fimmvörðuháls field site.



July 28, 2013

- HEI. 1 - (1-2) - (1-3)
- HEI. 2 - (1-2) - (1-3)

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Figure 3 - Sampling locations within the Eldfell field site.

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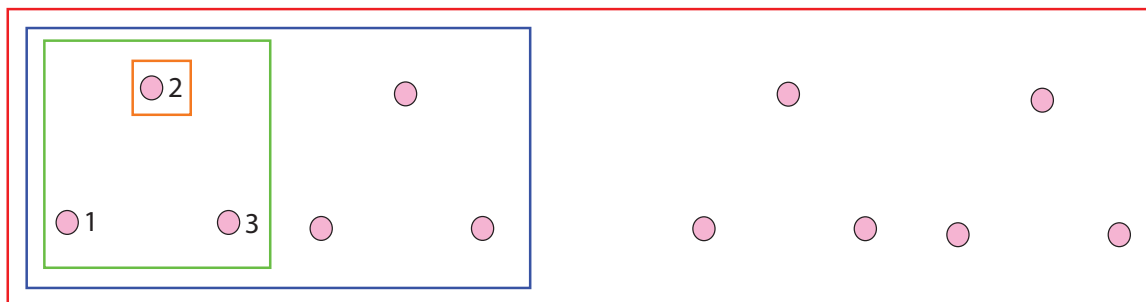
### 133 3.2 Sampling

134 A grid of sample locations spaced at 1 m, 10 m, and 100 m intervals (see Figure 4 and  
135 Figure 5) was established at each of the two main sites in an area where the basaltic tephra  
136 appeared to be visually homogeneous by color, morphology, and grain size. A triplicate sample  
137 set was taken at each grid point.

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#### Field Sampling and Naming Protocol

- Sampling Site      Naming Procedure: Field Site - 10<sup>2</sup> m scale - 10<sup>1</sup> m scale - 10<sup>0</sup> m scale  
Example: HEI. - 1 - 1 - 2



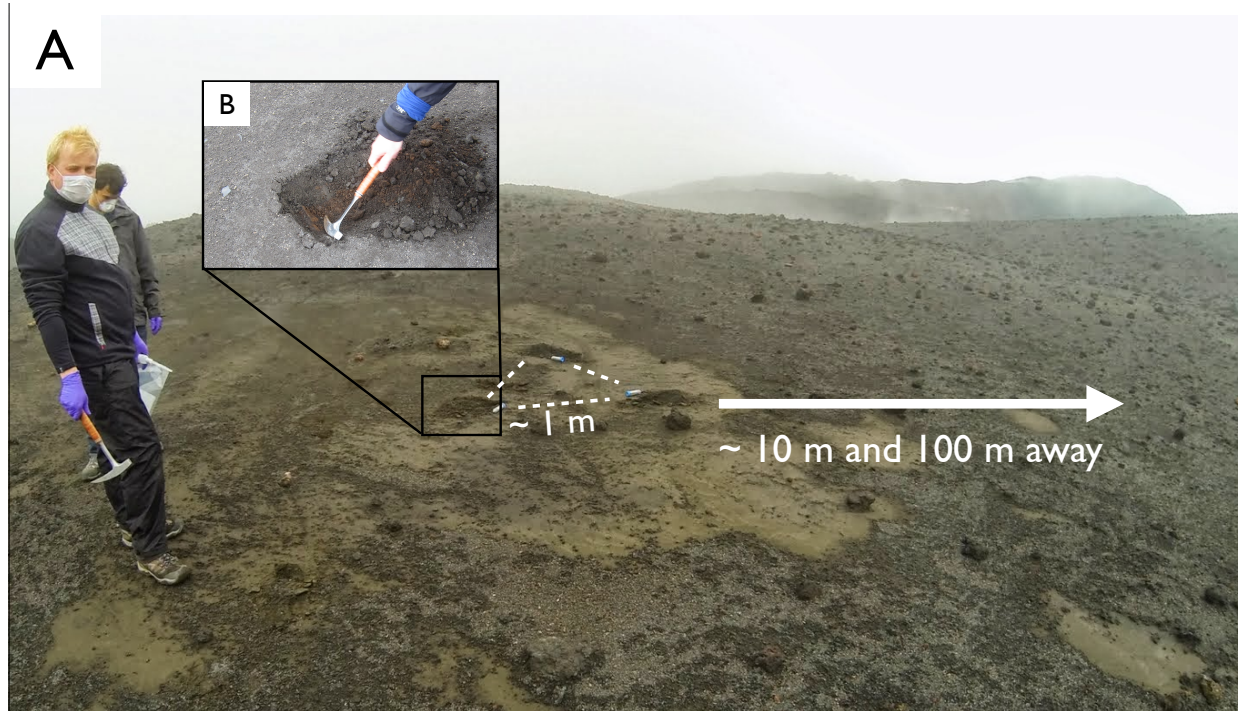
Note: For Fimvordulhas, where multiple samples were taken with this process, the 10<sup>2</sup> m scale region is subdivided into A, B, and C.

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Figure 4 - Field sampling and naming protocol.

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**Figure 5 - (A) Samples being collected at Fimmvörðuháls by A. Stevens (front) and E. Schwieterman (back) using the sampling protocol shown in Figure . (B) A closer view of how the samples were collected.**

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Each sample was taken from approximately 5 cm below the surface by digging with a rock hammer that was wiped with isopropanol before each sample collection (Figure 5) and scooping the uncovered tephra with a sterile 50 mL falcon tube, which was sealed and returned to the field lab. During sample collection, team members wore facemasks and gloves and approached the pristine sampling site from a downwind direction to minimize anthropomorphic contamination. Gloves were rinsed with isopropanol between samples. Caution was taken to avoid stepping on or otherwise disturbing any potential sampling site.



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**Figure 6 – Example of a sample collected from the Eldfell lava field.**

155 Fimmvörðuháls samples were collected along the base and in the surrounding area of the  
156 Magni cinder cone (Figure 2), which appeared to have had little foot traffic compared to the rest  
157 of the lava field. The basaltic tephra collected ranged from coarse ash to lapilli and reached up to  
158 50 cm in thickness. On 24 July, four samples were collected from a small area of the cinder cone  
159 using the general method described above. Along with these, a ‘positive control’ sample was also  
160 collected from the ash around the roots of a small tuft of grass, on the assumption that this would  
161 be a likely habitat for microorganisms. All other samples were deliberately taken at locations  
162 away from any vegetation. A separate positive control was also collected from the grassy area  
163 around the field laboratory. First-round analysis of these initial samples was used to inform

164 follow-up sample collection at Fimmvörðuháls (27 and 29 July), which were taken from the  
165 same general region (Figure 2).

166 The samples collected from the Eldfell lava field (28 July) were lapilli-sized or smaller and  
167 came from a large scoria cone associated with the main explosive eruption (Figure 6). As with  
168 the Fimmvörðuháls site, care was taken to collect samples from regions with little to no apparent  
169 anthropomorphic disturbance, but this was much more difficult given the status of Heimaey and  
170 Eldfell as a site for tourism. Further samples were taken from a site off the main eruption cone in  
171 an attempt to reduce the anthropomorphic contamination.

### 172 **3.3 Field Laboratory**

173 All analytical work was performed in a field laboratory located within a day's travel of the  
174 field sites at the Hvolsskóli school in Hvolsvöllur (Figure 1). While the classroom used had very  
175 little equipment itself, it provided the basis for a field laboratory with running water and access  
176 to power.

177 The majority of the scientific equipment used in the expedition was shipped directly to  
178 Hvolsvöllur in a single container. Some equipment (such as isopropyl alcohol) was purchased  
179 locally. A number of items required for the field lab were adapted from household items – for  
180 example, the autoclave used to sterilize equipment was a domestic pressure cooker. The  
181 equipment that was shipped to the field laboratory included a benchtop vortex mixer and  
182 centrifuge, sterilized pipettes and tips, a commercial water filtration system and ultrasonic  
183 cleaning system, Bio-Rad MiniOpticon™ real-time PCR system, the Partec CyScope®

184 fluorescence microscope and the Merck HY-LiTE® 2 portable luminometer used to quantify the  
185 ATP immunoassay. Consumables including sterile sample containers, gloves and masks, PCR  
186 and microcentrifuge tubes, filters, commercial qPCR assay kits, and the reagents for use with the  
187 ATP assay and FM were also shipped.

188 On arrival, the classroom was rearranged to form three analytical stations, one for each  
189 analytical technique, and each cleaned thoroughly with isopropanol. A second classroom was set  
190 up as a separate sample preparation room to reduce cross-contamination. A third classroom was  
191 assigned to be a darkroom for use with the FM.



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193 **Figure 7 - The three workstations as set up in our field laboratory. (l) DNA**  
194 **extraction, purification, and qPCR area, with general supply table in**  
195 **foreground. (c) ATP bioluminescence area, with FM staining area in the**  
196 **background. (r) 'Darkroom' established for fluorescence microscopy.**

### 197 **3.4 Analytical Methods**

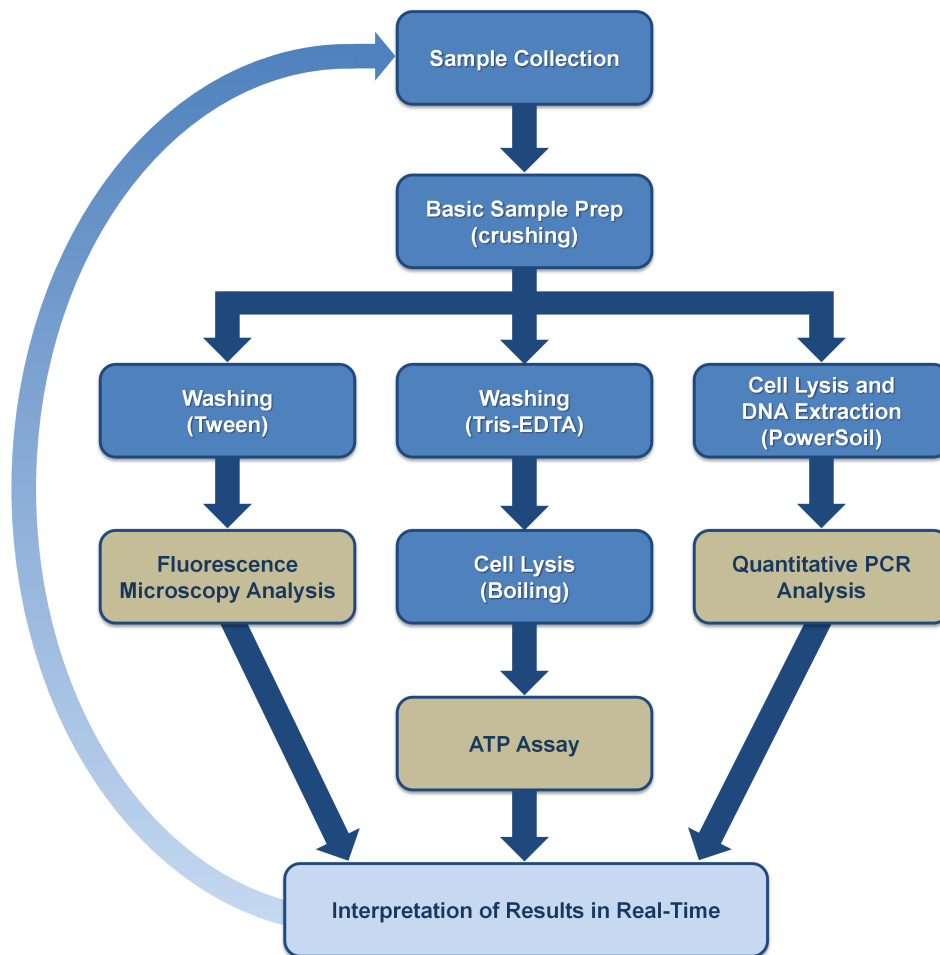
198 The analytical techniques used in the expedition were selected for their minimal laboratory  
199 overhead, low cost and consumables requirements, and established history in terrestrial life  
200 detection. Adenosine triphosphate (ATP) has long been considered a high-priority biomarker due  
201 to its indication of bioavailable energy and ease of detection via fluorescent dyes (Parnell *et al.*,

202 2007), and can be implemented with standard, commercially available solutions and a benchtop  
203 luminometer. Fluorescence microscopy (FM) offers the ability to directly quantify  
204 microorganisms under a microscope using a dye that binds to double-stranded DNA and  
205 fluoresces when illuminated under UV light. Counting cells via FM has been widely used in  
206 environmental samples (Kepner and Pratt, 1994), and can be implemented with standard,  
207 commercially available stains and buffer solutions and a microscope with appropriate  
208 illumination and filters. The quantitative polymerase chain reaction (qPCR) assay allows for  
209 simultaneous amplification and quantification of DNA that matches the set of primers used. By  
210 choosing an array of primers that correspond to different taxonomic groups, it is possible to  
211 assess both the quantity of DNA present in a given sample and the relative diversity (defined for  
212 our purposes as the relative ratios of the DNA recovery of different types of organism *e.g.*  
213 bacteria:fungi:archaea) of the organisms from which that DNA was extracted. The qPCR  
214 technique requires pre-synthesized primers, standard buffer solutions, and a thermocycler  
215 equipped with a well-plate fluorometer. We recognize that our chosen analytical suite is  
216 terrestrial extant-life-centric, and that other techniques could be more appropriate for  
217 extraterrestrial planetary expeditions.

218       The analytical workflow was designed to maximize overlap between techniques and  
219 stagger use of limited equipment. The same basic sample preparation techniques, including  
220 crushing using steel plates and a vice to visually homogenize particulate size, were used for all  
221 three life-detection techniques. Samples were then split into sub-sample groups for washing

222 (used for the fluorescence staining) and cell extraction and lysis (used for both the ATP and  
223 qPCR analyses). The lysate was then subject to a final DNA extraction step for qPCR only. The  
224 cell wash for the fluorescence staining required an additional filtration step.

225 Expedition personnel on laboratory duty for a given day were divided into sample  
226 preparation, qPCR, ATP and FM teams, as shown in Figure 8. The FM and qPCR teams took  
227 sample input directly from the sample preparation team; the ATP team was split into preparation  
228 and analysis groups to account for their additional secondary sample preparation step. Analytical  
229 protocols are briefly described below, and will be reported in full detail in a future manuscript.



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**Figure 8 - Sample preparation and analysis throughput chart. Data are analyzed in real-time, informing the next round of sample collection and analysis.**

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### 235 **3.4.1 ATP Bioluminescence**

236 The ATP bioluminescence assay was implemented using the Roche ATP Bioluminescence  
237 Assay Kit HS II following the manufacturer's recommendations for a tube assay, with some  
238 modifications as discussed in Barnett *et al.* (2012).

### 239 **3.4.2 Fluorescence Microscopy**

240 The FM protocol follows that of (Kepner and Pratt, 1994) with some minor modifications  
241 and serves to remove any microorganisms from the sample matrix and allow them to be stained.  
242 Briefly, a 1 mL portion of crushed sample was combined with 0.75 mL of PBS/Tween buffer,  
243 vortexed, sonicated, then centrifuged for 5 minutes at  $600 \times g$ . The supernatant was removed into  
244 a separate sterile tube and the extraction process was repeated. The combined supernatant  
245 (extract) was loaded into a 2 mL syringe, pushed through a Whatman Nuclepore Track-Etch  
246 Membrane 0.2  $\mu\text{m}$  filter, and flushed through twice with sterile water and twice with air. The  
247 sample filter was removed, stained with 100  $\mu\text{L}$  of SYBR Gold Nucleic Acid Gel Stain  
248 (Invitrogen), covered with a cover slip, and incubated in the dark for at least 15 minutes before  
249 imaging with a Partec Cycscope (fluorescence and transmitted light microscope) equipped with a  
250 455 nm (RB) “royal blue” emission light source and 500 nm (DM) “yellow/green” dichroic  
251 mirror long pass filter. The 100 $\times$  (oil) objective was used in conjunction with the imager to  
252 record digital micrographs of sample fields. The cell counts were documented with manual  
253 counting from five randomly chosen locations in the field of view, and images were recorded for  
254 later verification and analysis.

### 255 **3.4.3 Quantitative PCR**

256 DNA extraction and purification was performed on a 1 mL portion of crushed sample  
257 using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) following the  
258 manufacturer's protocol. Real-time qPCR was performed in duplicate for each sample on a 1:10  
259 dilution of purified DNA extract using a BioRad MiniOpticon™ real-time PCR system. Primers  
260 were selected for breadth of taxonomic range, and included primers to identify bacterial, fungal  
261 and Archaeal DNA. The primers chosen also had similar extensions to increase the amount of  
262 samples that could be analysed at the same time. Soil gathered outside of the field laboratory in  
263 Hvolsvöllur was used as a positive control; the negative control was sterile deionized water.

## 264 **4 Results and Discussion**

### 265 **4.1 Rapid Site-Selection Lessons**

266 One of the main objectives of the expedition was to demonstrate the feasibility of  
267 performing multiple rapid cycles of sample selection, sample analysis and interpretation in a  
268 field setting. To accomplish this, we performed several cycles of sampling and analysis, allowing  
269 methods and protocols to be developed throughout the expedition.

#### 270 **4.1.1 Preliminary Sampling and Protocol Testing**

271 After arriving at Hvolsvöllur and establishing the field lab, the first day of sampling was  
272 spent on a preliminary expedition to both potential sampling locations. The following day was  
273 spent analyzing these preliminary samples in the field laboratory. There were two goals: 1) to

274 survey potential areas for sampling in terms of accessibility and anthropogenic disturbance and  
275 2) to take preliminary samples with which to test our field protocols and ensure all participants  
276 were suitably trained on both field sampling protocols and the three analytical techniques.

277         After determining a common sample preparation protocol that could be used for all three  
278 techniques, preparation of all 22 samples took approximately two hours. However, samples were  
279 continually transferred into analysis as they were prepared, avoiding an initial two hour delay.  
280 All three analytical methods – FM, qPCR and ATP – were performed in parallel.

281         Fluorescence staining followed by microscopy proved to be the most rapid technique when  
282 used in a qualitative mode (i.e., gauging relative levels of microorganism abundance compared to  
283 the positive or negative controls). At this level of analysis, a day's sample set could be stained  
284 and measured in approximately one hour. The ATP bioluminescence assay took approximately  
285 two hours to produce a quantitative report regarding the activity of the microorganism abundance  
286 of the samples. The qPCR analysis required an entire day (~10 hours) to analyze the same  
287 number of samples as the other two techniques. Approximately five hours were required to  
288 perform the DNA extraction and purification; the remaining five hours were spent running the  
289 temperature cycling on a single qPCR instrument. On subsequent days, sample preparation for  
290 this analysis was given priority, and two team members were assigned to parallelize the  
291 extraction procedure in the morning.

#### 292 **4.1.2 First-Round Sampling and Analysis**

293 Our rapid site selection protocol was put into place starting on Day 1, with one team  
294 departing to gather samples at the Fimmvörðuháls site while the other implemented and tested  
295 protocol changes resulting from our preliminary analyses the previous day. Day 1 samples were  
296 then analysed on Day 2 while the second team took samples from the Eldfell site. The results of  
297 samples collected on Day 1 were reviewed in the evening of Day 2 to inform decisions about the  
298 follow-up sampling trip to Fimmvörðuháls on Day 3.

299 The samples from Fimmvörðuháls taken on Day 1 and analyzed on Day 2 showed  
300 generally low cell counts and low levels of ATP. The qPCR assays were used with both 10x and  
301 100x dilutions, and it was determined that a 10x dilution factor was the most effective; however,  
302 due to the complexity of qPCR data return, these results were not immediately available to guide  
303 first round analysis. The Eldfell samples, as expected given the older age and increased regional  
304 vegetation, showed a higher level of ATP than the results from Fimmvörðuháls, but still a lower  
305 level than the positive controls. Cell counts were also generally very low, but difficult to  
306 distinguish in magnitude from the Fimmvörðuháls samples. The results from the qPCR assays  
307 were mixed, indicating that the diversity of different cell types in our sampling area was not  
308 consistent.

309 A final change was made to our FM procedure after Day 1. Quantitative cell counts for all  
310 samples were proving to be infeasible in a single day's time frame under the constraints of our  
311 field laboratory and number of personnel. Fluorescent micrographs of the stained filters were

312 taken for later detailed counts, and qualitative-level comparisons were used to guide future  
313 sampling decisions.

314 A final change was made to our qPCR procedure after Day 2. By reducing the number of  
315 replicate samples for qPCR analysis each day, the analysis could be completed in a shorter  
316 timeframe (about eight hours). The resulting data therefore could be made available in time for  
317 high-level judgments, and additional replicates could be run later from the same DNA extract.

318 These initial results from both sites showed that assessment of heterogeneity in the field  
319 sites was crucial. The purpose of our follow-up sampling was at this point defined as measuring  
320 internal site variance at each area for each of the three life detection techniques.

### 321 **4.1.3 Subsequent Sampling and Analysis**

322 Samples were prepared as in the first round of analysis, with minor changes in protocols to  
323 maximize throughput and minimize material use. Qualitative FM and ATP assays were  
324 performed in the morning while a reduced set of samples was selected for qPCR analysis. After  
325 the final round of on-site sampling, an additional day was included in the schedule to allow for  
326 follow-up analysis of particularly interesting samples and any additional work that early results  
327 indicated should be performed. The qPCR analysis was completed on this day, as was initial top-  
328 level analysis of site homogeneity.

## 329 **4.2 Field Laboratory Lessons Learned**

330 Our experience of establishing a field laboratory for real-time on-site life detection provides  
331 several valuable lessons, which could be applied more generally to similar expeditions to  
332 planetary analogue sites, or the future in-field testing of life detection instrumentation.

333 The minimum requirements for locating a field laboratory are power, water, protection from  
334 the elements, and control over entry and exit (to minimize contamination). This can be cost-  
335 effectively provided by any reasonable local structure and has been achieved in far more modest  
336 locations than our expedition (see Barnett *et al.* (2012)). Appliances for low-temperature  
337 preservation (refrigerator and freezer) and heat (stove top) are highly recommended. At least  
338 two separate areas (in our expedition, two classrooms) are strongly recommended to allow  
339 sample preparation to be physically separated from analytical measurements.

340 The requirement that all three techniques were run in parallel meant that some of the  
341 expedition participants were trained in their use before the expedition. Those without experience  
342 of the techniques were training during the expedition. The time spent developing protocols and  
343 training participants before the expedition was critical for meeting our scientific objectives, as  
344 without pre-training several days in the field would have been lost. It also meant that it was fairly  
345 simple for one person to supervise the setup of each technique in the field lab. In-field training  
346 proved to be very effective, even for those with backgrounds in different fields, and helped to  
347 streamline all the protocols.

348 All necessary equipment and reagents can be shipped in a single trunk (in our case, a KA64  
349 Defender aluminium box, 1190 x 790 x 520 mm). Reagents can be shipped within the main  
350 container in a secondary insulating box with dry ice and will remain preserved for ~48 hours.

351 All consumables should be packed and shipped pre-sterilized whenever possible. The small  
352 autoclave (adapted from a consumer pressure cooker) was often a significant bottleneck.

353 Provisions should be made to take detailed data for later analysis. This was most notable with  
354 the fluorescence microscopy. The microscopes were excellent, but the low-cost camera  
355 attachments provided low image quality. A single higher-resolution camera and adapter would  
356 have been a worthwhile investment.

357 We have demonstrated the feasibility of performing complex life-detection analysis in a  
358 field-based laboratory in a single day, which enabled heuristic development of techniques,  
359 methods and protocols and offered significant flexibility over the expeditionary standard of  
360 bringing samples back to an institutional laboratory. While some highly sensitive techniques and  
361 instruments could not be used in this context and would therefore require sample transport,  
362 advances in miniaturization technology means that more analytical techniques are becoming  
363 viable to use in a field context.

#### 364 **4.2.1 Lessons Learned: Techniques**

365 Our initial FM protocol for cell quantification was impractical. The filters used for cell  
366 isolation and staining were challenging to use and to transfer between the filtering apparatus and

367 the microscope slides. A potential alternative for future work is to stain and view the cells in  
368 solution. Manual cell counting by eye also proved too slow for meaningful sample throughput.  
369 The use of this technique in a similar field lab could be substantially improved with the use of a  
370 higher-quality microscope camera and automated image-processing software. Alternately, given  
371 that qualitative comparisons were of adequate value for rapid-turnaround analysis, a low-cost  
372 fluorometer could be used to rank sample fluorescence levels post-staining without the need for a  
373 microscope.

374

375         The ATP bioluminescence assay proved generally robust and reliable under the conditions  
376 of our field laboratory. The protocols were easy to follow and required simple laboratory  
377 equipment (*i.e.* vortex, hot plate) beyond the ATP luminometer. The assay provided useful data  
378 in a relatively short amount of time, and therefore enabled the processing of a significant number  
379 of samples each day. ATP bioluminescence is definitely suitable for future use in rapid  
380 examination of biomass distribution during field expeditions to extreme environments.

381         The qPCR assay was largely successful as a field technique. DNA was successfully  
382 extracted from all samples. Amplification protocols for all three primer sets (bacteria, fungi and  
383 archaea) were successful as confirmed by Cq values of 17.5-27.5 in the positive controls (soil  
384 gathered outside of the field laboratory in Hvolsvöllur). The clearest need for improvement is  
385 the throughput, due to the serial-batch nature of the technique. Each batch of samples required at  
386 least 4 hrs to run on the qPCR instrument, enabling a maximum of 48 individual samples

387 (including replicates and different primers) to be run in a single day. Given the results of our site  
388 homogeneity analysis, it may be possible to reduce the number of samples taken in a given area  
389 to achieve the same level of confidence, though using more primers would require more sample  
390 wells. Alternately, the throughput could be increased by the addition of a second qPCR  
391 instrument or the substitution of one with a larger capacity.

## 392 **5 Preliminary results**

393 Here we present some initial results in order to illustrate more clearly how the different  
394 techniques were used in decision making and scientific investigation. A full statistical analysis of  
395 our results is currently in preparation.

396 The data collected suggest that sites that appeared to be homogeneous showed biological  
397 diversity at all spatial scales at but at different levels – *i.e.* there was diversity at a general  
398 microbial level (quantified by ATP), at a cell number level (quantified by FM), and at domain  
399 specific levels (quantified by qPCR).

### 400 **5.1 Fluorescence Microscopy**

401 Example micrographs are shown in Figure 9. There is a clear difference between (a) the  
402 positive control, (b) the negative control and (c) a sample taken from the Fimmvörðuháls field  
403 site. Micrographs showed very low cell counts across all field samples, but with variation at all  
404 spatial scales. However, the FM technique was generally only used as a qualitative estimate of  
405 the microbial levels in the samples, with the positive and negative controls providing the end

406 member cases of well- or poorly-populated material. These qualitative estimates allowed us to  
407 judge whether a sample was worthy of further analysis.



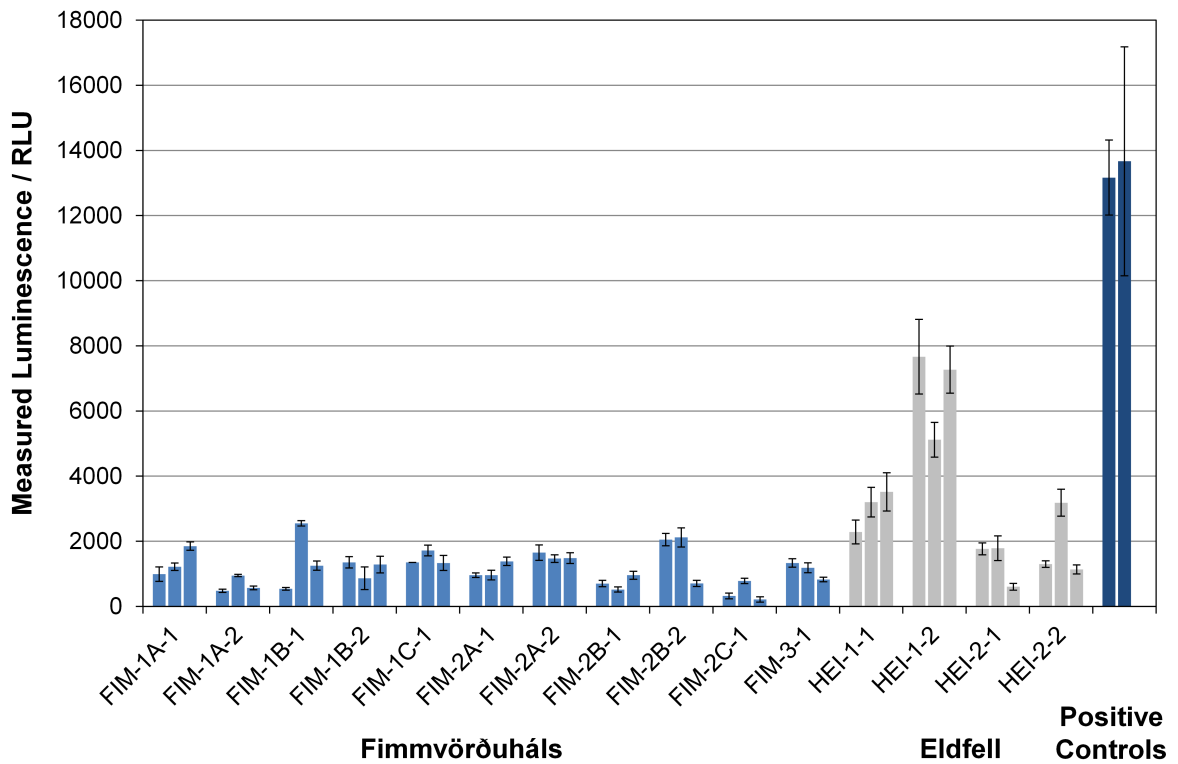
408

409 **Figure 9 - Fluorescence microscopy micrographs. Left: Positive control from vegetated soil. Center:**  
410 **Negative control from sterile extraction. Right: An extraction from a sample collected at the Fimmvörðuháls**  
411 **location. Micrographs were taken using a 100× oil immersion objective.**

412

## 413 **5.2 ATP assay**

414 Figure 10 gives a summary of the ATP levels across both field sites and for the positive  
415 control samples. There is a distinction between the very low levels of the Fimmvörðuháls field  
416 site and the slightly higher levels present in the samples from Heimaey, but both are low  
417 compared to the positive controls. There is also variation in the levels at different spatial scales  
418 within both field sites. Note that the RLU measurement produced by the assay is purely  
419 comparative and must be calibrated using a standard curve to provide details of ATP abundance  
420 in samples.



421

422 **Figure 10 – ATP assay luminescence levels for the Fimmvörðuháls and Heimay field sites and the**  
 423 **positive controls.**

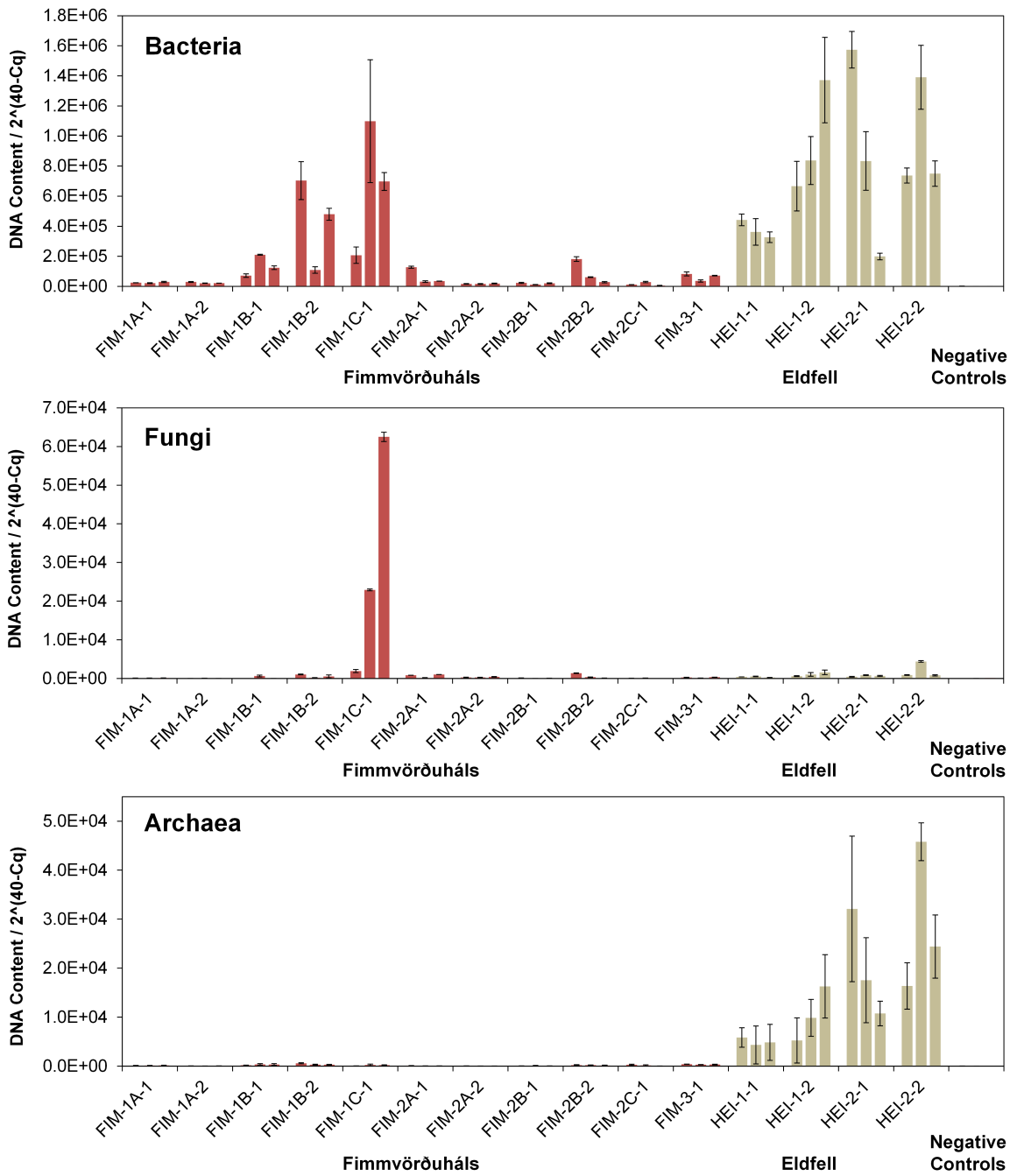
### 424 **5.3 Quantitative PCR**

425 Analysis of the qPCR data is more difficult as the instrument does not directly output DNA  
 426 abundance. The DNA content was calculated from the output data using the following equation, a  
 427 variation of the Livak method (Livak and Schmittgen, 2001):

$$E = 2^{Cq(r) - Cq(t)}$$

428 where  $E$  is the amount of DNA in a sample,  $Cq(r)$  is the quantification cycle value of the  
429 reference (equal to 40 in this case), and  $Cq(t)$  is the quantification cycle value of the target.  
430 Quantification cycle ( $Cq$ ) is the number of replication cycles needed to reach a set threshold  
431 fluorescence value. This method assumes that the target genes are amplified with efficiencies  
432 near 100%.

433         The results for the three separate domain-specific primers are shown in Figure 11. They  
434 suggest that while the results of the FM and ATP analyses show broadly consistent microbial  
435 levels at each field site, this consistency obscures high domain-level diversity in the sample  
436 locations. For example, one sample location contained far more fungal DNA than any other, and  
437 archaeal DNA was far more abundant in samples from Heimaey than Fimmvörðuháls.



438

439

Figure 11 – DNA content for all sample sites.

## 440 **6 Conclusions**

441 Our experience shows that rapid in-field analysis to determine subsequent sampling  
442 decisions is operationally feasible for planetary analogue expeditions. A single-day turnaround  
443 for biomarker analysis was achievable on a relatively low budget with the use of basic local  
444 facilities. Similarly, no additional difficulties were encountered in running analyses  
445 synchronously (*i.e.*, in parallel) and by using sample subdivision, results were acquired from  
446 three separate analytical techniques each day. With carefully chosen equipment and protocols,  
447 ATP bioluminescence and nucleic acid staining assays can be used to provide same-day input  
448 into sample down-selection for quantitative PCR, conserving the most expensive equipment and  
449 reagents without increasing the one-day analysis turnaround. The techniques chosen for this  
450 study were designed to characterize extant, metabolising life, whereas we recognize life  
451 detection missions to extraterrestrial locations cannot make the same assumptions and will most  
452 likely be looking for remnant biosignatures, thus requiring a different analytical suite. There is  
453 no *a priori* reason other analytical techniques could not be used in the same manner, so this  
454 decision making methodology could be used in other contexts.

455 These methods have a number of advantages over the standard sampling methodology of  
456 taking samples back to institutional laboratories, especially in the context of life detection, where  
457 changes over the course of even the days or weeks before analysis could severely affect the  
458 results. Additionally, assuming that all samples are partitioned appropriately while performing in-

459 field analysis, samples can still be retained for return to an institutional laboratory if they show  
460 particularly interesting results and would benefit from more extensive analysis.

461         Our expedition also has implications for robotic planetary missions. We have shown that  
462 with careful planning of experimental sequences and shared resources, multiple analytical  
463 techniques can be used synchronously and rapidly to provide information that improves further  
464 sampling. In the context of future biomarker detection missions, such as the ExoMars or Mars  
465 2020 rovers, instruments may face severe limits on their operation and the ability to increase  
466 their science return within those limits will be of extreme importance. Decision making as  
467 described here can help to increase science return. Future human planetary missions will also  
468 need robust decision making tools, though their sampling limitations will likely be reduced as  
469 compared to robotic missions.

470         The developments presented here are appropriate to the context of Icelandic lava fields, but  
471 may need to be modified for other analogue sites. For example, the analogue sites of Iceland are  
472 less remote than those such as the Haughton impact crater or remote regions of the Atacama  
473 desert, and it may be much more challenging to assemble a fully equipped field lab in some of  
474 these other analogue sites. Testing these methods in a variety of analogue sites will increase their  
475 robustness under varying levels of infrastructure and demonstrate their applicability to planetary  
476 missions.

477         The feasibility of implementing fluorescence microscopy with nucleic acid staining, ATP  
478 bioluminescence assay and qPCR as a set of quick-turnaround life detection techniques in the

479 context of a field campaign at a Mars analogue location has been demonstrated. With the use of a  
480 field laboratory such as that described here, these methods can be used and extended to increase  
481 the science return of terrestrial planetary analogue expeditions. Preliminary results from the  
482 expedition suggest that the diversity of microbes in extreme environments is a complex problem  
483 and further analogue investigations are required in order to enable the full exploitation of data  
484 from future planetary exploration missions. They also show that more comprehensive methods  
485 are required to assess the environmental homogeneity of sampling areas.

## 486 **7 Acknowledgements**

487 The authors would like to thank Lewis and Clark Exploration Fund (ESA, MLC and  
488 EWS), Oak Ridge Associated Universities (AMS), Cranfield University (DCC), and The Open  
489 University (AHS) for making the expedition possible. We also thank the community of  
490 Hvolsvöllur and especially Sigurlín Sveinbjarnardóttir, the headmistress of Hvolsskóli, for  
491 generously allowing use of its classrooms as our field laboratory, as well as the staff of the Hotel  
492 Hvolsvöllur, who helped a great deal with the arrangements for shipping our equipment.

## 493 **8 Abbreviations**

494 ATP, adenosine triphosphate; Cq, quantification cycle; DNA, deoxyribonucleic acid;  
495 EDTA, ethylenediaminetetraacetic acid; FIM, Fimmvörðuháls; FM, fluorescence microscopy;  
496 HEI, Heimaey; qPCR, quantitative polymerase chain reaction; RLU, relative light units; Tris,  
497 tris(hydroxymethyl)aminomethane; UV, ultraviolet.

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