ANALYSIS OF THE SCIENTIFIC VALUE OF THE MARS 2020 SPACECRAFT GENETIC INVENTORY TO MARS SAMPLE RETURN. The Returned Sample Science Board (D. W. Beaty[1], H. Y. McSween[2], B. L. Carrier[1], A. D. Czaja[3], Y. S. Goreva[1], E. M. Hausrath[4], C. D. K. Herd[5], M. Humayun[6], F. M. McCubbin[7], S. M. McLennan[8], L. M. Pratt[9], M. A. Sephton[10], A. Steele[11], and B. P. Weiss[12]), [1]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, [2]Univ. of Tennessee, Knoxville, TN, [3]Univ. of Cincinnati, Cincinnati, OH, [4]Univ. of Nevada, Los Vegas, NV, [5]Univ. of Alberta, Edmonton, Canada, [6]Florida State Univ., Tallahassee, FL, [7]NASA Johnson Space Center, Houston, [8]Stony Brook Univ., Stony Brook, NY, [9]Indiana Univ., Bloomington, IN, [10]Imperial College, London, U.K., [11]Carnegie Institution, Washington, DC, [12]Massachusetts Institute of Technology, Cambridge, MA.

Introduction. The primary astrobiological goal of Mars Sample Return (MSR) would be to assess returned samples for evidence of ancient life. However, the broader scientific community is also interested in whether these samples contain evidence of extant (alive or recently alive) martian life. A crucial aspect of potential MSR, therefore, would be to evaluate data that indicates that biological material has been detected and to determine its origin (i.e., as indigenous Mars-sourced versus contaminant Earth-sourced life forms). Missions that would be part of a potential MSR campaign should plan to acquire the following samples and information in support of this objective:

a) A set of witness blanks that would make the round trip,
b) A comprehensive genetic inventory and archive of trace Earth-sourced biological material collected from spacecraft surfaces during the spacecraft build,
c) A parallel genetic inventory and archive of trace biological material in the laboratory environments and related areas where samples are received, subsampled, and analyzed.

The planning for a) for the Mars 2020 mission is at or near finalization. However, there are unresolved issues regarding the detail and cost for the tasks outlined in b) and c). It is important to note that we have to assume Earth-sourced biological contamination would be detected in MSR samples, since it is implausible that the blanks collected as part of b) and c) would be zero.

To develop recommendations related to b) and c) from the perspective of science, the NASA Returned Sample Science Board (RSSB) solicited input from approximately a dozen experts in metagenomic techniques.

Hypotheses to be Tested. Although life as we know it on Earth would not survive for long in Mars' ionizing surface environment, it is possible that martian life has developed adaptions to live in such conditions. If so, evidence of martian life may be abundant in returned samples, and may be relatively easy to detect. However, indigenous modern life in martian surface samples may instead be present in trace quantities, possibly delivered by transport from a nearby habitable environment (including the subsurface). If the samples are low-biomass, sample preparation protocols could amplify tiny amounts of contaminating nucleic acids present in the reagents employed. It may not be possible to use MSR to test the hypothesis that modern Mars life is identical to that on Earth (and in fact, this hypothesis is scientifically unreasonable). It is likely that life on Mars can be identified only if its biology is significantly different than that on Earth. One metagenomics expert stated: "the chance of any indigenous life on Mars bearing a close relationship to spacecraft-contaminating organisms is infinitesimally small. … genome sequences and sequences of individual genes should show considerable innovation."

Important Case Histories. There are important lessons from the analysis of low-biomass samples from old and/or extreme environments on Earth. Examples include nucleic acids or microbes isolated from: Lake Vostok in Antarctica, 250 million-year-old salt crystals, fossilized amber, and Neanderthal and ancient hominids. All present analytical challenges due to low indigenous nucleic acid yields and attendant contamination concerns. Some reports of nucleic acid recovery from low-biomass samples have been discredited, some are viewed with skepticism, and some accepted as genuine. Criticisms include researchers isolating or sequencing contaminating material. Validated studies thoroughly and systematically excluded contamination as a possible source of their data.

Analytic Consideratons. To draw meaningful conclusions regarding possible extant martian life using MSR would require that scientific investigators have sufficient resources to constrain contamination as a possible explanation for the data. The bioburden and biodiversity of spacecraft assembly cleanrooms are typically measured on a routine basis, and these data and samples would be relevant to MSR. Should genetic material be discovered in martian samples, a close taxonomic relationship to known terrestrial contaminants would lower the credibility of an interpretation related to putative martian life.

An effective statistical assessment of any detected genetic material would require background information. Specific suggestions include negative controls in the form of the processing room genetic inventory, as well as positive, spike-in controls of synthetic nucleic acids to minimize the generation of random noise.

Finally, it would be important to store at least some samples for which the nucleic acids were not extracted prior to storage, to enable the future use of improved extraction methods, single cell genomics, and other analytical developments.

Genomic techniques are inherently geocentric and may not detect martian life. Scientists might have to rely on other methods, such as Raman spectroscopy, mass spectrometry, or atomic force microscopy to directly evaluate the presence of exotic nucleotides, proteins, cells, viruses, etc.

Population statistics, context, and position within a sample also matter for life detection. For example, if Staphylococci abundance in the cleanroom is high, the probability of having Staphlococci contamination in the sample increases. If Staphylococci abundance in the cleanroom is low and billions of signatures are observed in the martian sample, it would be harder to support an interpretation of terrestrial contamination.

The Importance of a Physical Archive. Some pre-launch swab/wipe samples taken from the Mars 2020 sample-collecting rover hardware should be analyzed immediately, and be archived to allow for re-analysis later, along with the returned martian samples. There are three primary considerations:

1) The analysis (or re-analysis) of samples from the archive could be performed after potential discoveries have been made in the martian samples, when it would be possible to tailor the specific way(s) in which the archived samples would be analyzed to support or discredit these results.
2) Future analytic methods will be better than they are now (especially for low-biomass samples). For example, sample extraction, sample recovery, nucleic acid sequencing methods, and sequence analysis algorithms are all expected to improve.
3) There are good reasons for analyzing samples of spacecraft-sourced contamination close in time to their collection. For low-biomass cleanroom samples, it is sometimes necessary to pool samples to obtain enough nucleic acids to measure, and the opportunity to collect at least some data while the opportunity to resample exists is valuable. However, it is a higher priority to archive the physical samples so that they can be analyzed later.

Degradation of DNA with time. The technology to preserve and store trace genetic samples such that the samples would be in a useful state over the time frame of interest (several decades) appears to exist. However, since organisms and nucleic acids degrade over time, preservation effectiveness can be a challenge for low-biomass samples, such as would be typical for a cleanroom environment. We know from long-running International Space Station experiments, that Earth-sourced nucleic acids will survive the radiation doses of interplanetary space for time periods longer than that of a round trip to Mars [1]. In any case, since the primary question would be whether any of the returning nucleic acids from the trip to Mars are Earth-based in origin, it should be safe to assume that any nucleic acids that make the trip would be at least as deteriorated as any nucleic acids in the genetic inventory.

The Receiving/Analysis Environment is Extremely Important. The sample processing/analysis facilities, instruments, reagents, and consumables are more likely to be sources of contamination than material on the spacecraft, excluding portions of the spacecraft in contact with the samples.

Summary. We envision the following scenarios, and the expected community reactions to them:

i) The sequences match those in the trace genetic inventory of spacecraft surfaces. Reaction: These were forward contaminated sequences/organisms.
ii) The sequences do not match those in the genetic inventory of spacecraft surfaces, but do match those in the processing lab(s) genetic inventory. Reaction: These represent contamination acquired from the receiving/curation/analytic environment.
iii) The sequences are found in neither genetic inventory but do match other known Earth-based organisms. Reaction: The genetic inventories were not sufficiently characterized; this would trigger a much more detailed set of analyses of the archived swab/wipe samples.
iv) The sequences are found in neither genetic inventory and do not match Earth-based organisms. Reaction: The data could represent random signal/priming from the sequencer itself (or some other sort of analytic artifact), or they could represent the discovery of martian life.

FINDINGS

1) An archive of spacecraft-sourced swab/wipe samples is required as a part of the scientific logic of MSR. Both the samples and all raw sequence data need to be archived.
2) Specifics of the analytic methodology can be decided once Mars samples have been received and analyzed, and any anomalous readings known.
3) If martian and terrestrial life are assumed to be distinct, then the entire terrestrial biome acts as a highly sampled genetic inventory.
4) Swab/wipe samples from at or close to the sample-contact surfaces are of higher priority than samples from other regions of the spacecraft.
5) A parallel set of samples would need to be collected from the sample receiving, curation, and sample analysis environments, although this is not a responsibility of the Mars 2020 Project.

References: [1] Horneck et al. 1998 Adv. Space Res. vol. 22, No. 3, pp. 317-326. 1998.