International Journal of Astrobiology (2025), **24**, e18, 1–9
doi:10.1017/S1473550425100013

CAMBRIDGE UNIVERSITY PRESS

REVIEW ARTICLE

How can planetary back-contamination protection guidelines inform biosafety for synthetic biology?

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Received: 31 January 2025; **Revised:** 16 April 2025; **Accepted:** 16 April 2025

Keywords: biocontainment; bio-risk; biosafety; biosecurity; planetary protection; synthetic cell

Abstract

Due to the nascency of synthetically derived biological systems, there is a need to develop protocols for safety and security management. These protocols can be adapted from existing safety and security protocols (e.g., Biosafety Level Classification of biological agents) as well as NASA's and ESA's planetary protection guidelines. Currently, NASA is preparing for its first sample return mission from Mars including determining how to manage the types of hazards that may be returned to Earth. Synthetic biology can look to risk management practices from related disciplines, and NASA can look to its established protocols from lunar exploration as it strives to minimize Mars sample return bio-risk. Notably, the biosafety concerns of synthetic cell research are very similar to those of planetary back-contamination from extraterrestrial samples. Thus, the measures taken to limit planetary back-contamination can serve to help develop biosafety protocols for synthetic cell research. We summarize existing tools used in planetary protection that can be repurposed to establish protocols for synthetic cell safety and security.

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Introduction

The goal of planetary protection is to protect Earth – as well as other planetary bodies, Moons and asteroids – from contamination caused by exploration activities including sample and spacecraft return to Earth. Samples of off-Earth bodies provide insights into the origins of both the solar system and life. Sample return missions from extraterrestrial bodies help us understand the origin of life by analyzing pristine samples for building blocks of life, potentially revealing how these molecules might have been

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https://doi.org/10.1017/S1473550425100013 Published online by Cambridge University Press

delivered to an early pre-biotic Earth. In fact, such samples may contain a truly new life form. A primary goal of synthetic biology is to design and build artificial biological systems that ultimately lead to the *de novo* synthesis of a viable cell that would have a variety of practical applications. Such a feat would mark a breakthrough in science. Although this was the domain of science fiction just a half-century ago, recent developments in synthetic biology will bring the bottom-up generation of a synthetic cell into reality. Key features of a living cell include the ability of the cell to metabolize, grow, and divide. The first steps in recreating these processes in a synthetic cell have been developed (Olivi *et al.*, 2021). If a truly synthetic cell were to be developed, it would constitute a new life form. For this reason, strategies for risk assessment should be developed in parallel with safety protocols to ensure appropriate biocontainment. Synthetic cells and samples returned to Earth from extraterrestrial bodies will provide important scientific information. Furthermore, they present similar hazards to Earth's biosphere that may overwhelm Earth's ecosystems, and as such, the hazards can be dealt with using similar protocols

Biosafety practices can prevent inadvertent or accidental harm caused by biocontainment breaches, laboratory incidents, or unexpected laboratory outcomes. Because the biosafety concerns of synthetic cell research are comparable to those of planetary back-contamination protection from extraterrestrial samples, the measures taken to limit planetary back-contamination can serve to develop biosafety protocols for synthetic cell research. With capabilities for building synthetic cells emerging, researchers performing the work and administrators overseeing it should take reasonable precautions to minimize risk. There are plans to return samples from Mars as soon as 2033 (Meyer *et al.*, 2022). Transparency regarding the development and use of biosafety protocols for synthetic biology and planetary protection is important to earn and maintain public trust so that approaches can be appropriately scrutinized by relevant experts. Notably, not all synthetic biology projects and sample return missions have equal risk. For example, NASA's Stardust mission collected samples from a comet and brought them to Earth in 2004 (Sanford, 2021). These samples were considered low risk (United States National Research Council, 2011). Similarly, some synthetic biological research can be done safely at BSL-1 facilities and with limited biocontainment consideration. In synthetic cell development and returned samples from space, however, researchers may not have a complete understanding of the activity of synthetic cells and returned samples; thus, sufficient safeguards should be ensured (Sanford, 2021; United States National Research Council, 2011; Long *et al.*, 2020).

Space exploration and samples returned to Earth

Historically, Earth was the only solar system body that could be studied in detail. Today, we have numerous bodies to study throughout the universe. By investigating these bodies and applying what we know from Earth, we can gain a better understanding of planetary formation and their environments, including their potential for the origin and evolution of life.

Although we have conducted numerous space missions that have photographed and analyzed surface material from the moons, planets, and asteroids in our solar system, these missions are limited by the technology that can be transported to the body under investigation. A preferable way to analyze a solar system body is to conduct a sample return mission to allow us to use the more sophisticated analytical equipment on Earth.

Synthetic biology

Synthetic biology involves the design and construction of artificial biological pathways, devices, and systems, or the redesign of natural biological systems. Advances in biotechnology such as DNA sequencing, DNA editing, and bioinformatics have promoted the development of synthetic biology. Significant progress in medicine, ecology, material science, and plant science has been made by synthetic biology (Andersson *et al.*, 2012; Liu and Stewart, 2016). In recent years, the in-depth development of cell-free systems has been changing the pattern of synthetic biology and eliminating

https://doi.org/10.1017/S1473550425100013 Published online by Cambridge University Press

some of the limitations of working with living cells (Tinafar *et al*., 2019). Recent advances demonstrated the potential for synthetic biology to revolutionize technologies across several disciplines, including biocomputing, living materials, electronic interfacing, therapeutic genome editing, diagnostics, cellular recording, third-generation biorefineries, microbiome engineering, and living biotherapeutics (Craig *et al*., 2022; Xie and Fussenegger, 2018; Grozinger *et al*., 2019; McBee *et al*., 2022; Kaushik, *et al*., 2023; Wang and Doudna, 2023; Rottinghaus et al., 2023; McCarty *et al*., 2020; Liu *et al*., 2020; Rottinghaus *et al*., 2022).

Synthetic biology sheds light on the origin and nature of life. The origin of life on Earth is one of the most puzzling, unanswered questions in science. Eighty years after Schrödinger's *What is Life?*, new insights have been gained into this ever more challenging question, but nothing close to a definite answer has been obtained (Schrödinger, 1945). Rather than looking back, synthetic biology seeks to engineer biological systems in novel ways to display new phenotypes and perform functions that do not exist in nature (Serrano, 2007). By engineering living systems and deconstructing and reconstructing novel life forms, synthetic biology approaches the boundary of living and non-living matter and allows us to better understand the emergent properties of life (Benner and Sismour, 2005) and their governing principles (Moon, 2023b).

Biosafety and biosecurity rationale

Biosafety focuses on preventing the accidental exposure to and release of potentially harmful biological agents, while biosecurity addresses the intentional misuse or theft of biological materials. The practitioners of synthetic biology need to be cognizant of biosafety and biosecurity at all stages of the research lifecycle if they ultimately hope their research is applied to solve real-world challenges. Such considerations can prevent or mitigate potential harms of the inadvertent or deliberate misuse of these technologies. For example, safety- and security-conscious researchers may think more critically about biocontainment strategies early in technology development. They may choose experimental designs that minimize risk to the greatest extent possible.

The technical researchers' community should proactively consider biosafety and biosecurity with multi-disciplinary stakeholders so that diverse perspectives and expertise can be leveraged to develop, implement, and iterate upon appropriate frameworks for risk assessment and mitigation. Through such processes, regulatory policies can be developed that attend to actual risk, ensuring that safe technologies are not overly constrained in the name of biosafety and biosecurity, while also ensuring that technologies with real risk of harm are attended to appropriately. From the potential for unleashing a pandemic pathogen to the potential for releasing a synthetic organism that replicates indiscriminately to the detriment of local or global ecosystems, the consequences of not attending to bio-risks associated with rapidly expanding synthetic biology capabilities could potentially lead to global consequences. Recently, a paper was published suggesting that mirror life (i.e., a life form that has the opposite chirality of life on Earth) that could be produced using synthetic biological techniques poses an extreme threat to life on Earth in that it is a novel form of life that has the potential to significantly disrupt the biosphere (Adamala *et al*., 2024). These authors suggest a discussion on whether a moratorium on such research is warranted. Additionally, the planetary protection program has addressed the potential of novel forms of life and how to protect Earth (e.g., Craven *et al*., 2021).

Planetary protection

Planetary protection refers to the policy and practice of protecting both the Earth's biosphere and other planetary bodies from harmful biological contamination caused by exploration activities and samples returned to Earth. Mitigating the risk of harmful biological contamination of the Earth (termed "backward contamination") and other celestial bodies (termed "forward contamination") supports a safe and sustainable Earth and space environment (United States National Research Council, 2011). New

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space missions will occur and will be performed by national space programs around the world and the private sector. While samples from Earth's Moon have been deemed non-hazardous, and their return to Earth has been unrestricted since 1971, both public and private entities are considering missions that would collect and return samples from other planetary bodies that have not been as thoroughly studied (Craven *et al*., 2021). Regulatory guidance is necessary to address the diverse challenges and manage the potential risk of biological contamination associated with further space exploration, while balancing international interests in promoting scientific discovery, human exploration, and the growth of private sector space activities.

One premise of planetary protection is that life may exist beyond Earth. If life exists off Earth, measures to avoid the potential harm from the introduction of external contaminants are necessary to protect life on Earth and ensure the validity of any scientific study related to such a discovery. In essence, planetary protection focuses on two aspects of space exploration. First, planetary protection aims to protect future scientific investigations by limiting the forward biological contamination of other celestial bodies by terrestrial life forms. Second, planetary protection aims to protect Earth's biosphere by preventing the backward biological contamination of Earth by returning spacecraft and their payloads. As stated in several NASA documents (United States National Research Council 2011; DeLoach, 2022), planetary protection has three primary objectives corresponding to forward contamination, backward contamination, and private sector coordination:

Objective 1: Avoid harmful forward contamination by developing and implementing risk assessment and science-based guidelines and updating the interagency payload review process.

Objective 2: Avoid backward contamination by developing a Restricted Return Program to prevent its adverse effects on the Earth environment due to the potential return of extraterrestrial life.

Objective 3: Incorporate the perspective and needs of the private sector by soliciting feedback and developing guidelines regarding private sector activities with potential planetary protection implications.

Details for Objective 2 can be applied to synthetic cells as well. Specifically, we can avoid backward contamination as well as contamination outside the lab from synthetic cells by developing a restricted handling program to protect against adverse effects on the Earth environment due to the potential return of extraterrestrial life or release of a synthetic cell, respectively. We can also develop a risk assessment framework to guide procedures, processes, and protocols to reduce the risk of backward contamination or unintentional release of a synthetic cell within acceptable levels and assess the effectiveness of proposed procedures for limiting contamination. This framework should be used to assess the risk of samples to human or animal health and to the environment as needed. We can also develop efficient and effective processes for the approval of the handling, transfer, and use of samples posing a contamination threat, including updates to existing policies and regulatory mechanisms to ensure appropriate oversight, preparedness, and risk mitigation. A registry, inspection, assurance, and certification programs to secure and ensure the safety of handling, transfer, and use of off-world biological materials should also be established. Notably, we have a Return Procedures Framework (Craven *et al*., 2021). The backward contamination group should define and direct the development of appropriate protocols for the return of Restricted Earth Return samples, including, but not limited to, protocols that address in-space transport and operations to break the contamination pathways between the samples and Earth, nominal and off-nominal landings using existing containment facilities to mitigate biosafety and biosecurity risks, and international collaboration and communication for sample return missions.

Biosafety considerations in synthetic biology

Biosafety has always been a core consideration of the synthetic biology community, tracing back to its origins in recombinant DNA technology. In 1975, following some of the first successful gene-splicing experiments, pioneers in the field gathered in Asilomar, California to establish biosafety principles to guide future research. They recommended three safeguards to mitigate risk. First, organisms utilized for

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gene-splicing experiments should have complex nutritional requirements to minimize their likelihood of survival outside of the laboratory environment. Second, containment measures should align with a given experiment's estimated risk level. They suggested that prokaryotic and bacterial DNA plasmids were generally low risk, plasmids created from animal viruses were medium risk, and plasmids created from eukaryotes were considered high risk. Third, research should aim to find safer vectors and hosts, laboratory personnel should train in biosafety protocol, and safety measures should be reassessed regularly to incorporate new knowledge (Berg *et al*., 1975).

The Asilomar conference laid the groundwork for proactive risk management in biotechnology and the current biosafety standards, the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines, 2024; Synthetic Biology and Occupational Risk, 2017). The NIH Guidelines continue to evolve as synthetic biology develops new capabilities. Most recently, in 2024, the NIH Guidelines were updated to address unique biosafety concerns raised by gene drives, a technique by which researchers take advantage of selfish genetic elements to enhance the heritability of desired traits within a population (NIH Guidelines, 2024). The Occupational Safety and Health Administration (OSHA) enforces biotechnology safety regulations in the workplace (OSHA, 2011). In 1986, in response to the burgeoning biotechnology industry, they reviewed their responsibilities in the context of biotechnology, concluding that existing standards and regulations, such as the general duty clause, sufficiently cover risks to biotechnology workers. Specific regulations address hazards such as bloodborne pathogens, toxic chemicals, respiratory protection, and hazard communication. These regulations are also updated regularly; for example, in 2024, updates were made to the hazard communication standards to ensure that chemical packaging clearly communicates critical hazard information (Synthetic Biology and Occupational Risk, 2017; Federal Register 1986; https://www.osha.gov/news/newsreleases/national/05202024).

The Convention on Biological Diversity (CBD) has long considered the impacts of engineered organisms on biodiversity. In 2000, the CBD adopted the Cartagena Protocol on Biosafety, providing guidance on the transfer and transboundary movement of living-modified organisms in the context of preserving global biodiversity. The Cartagena Protocol established the Biosafety Clearinghouse, which serves as an online hub for biosafety information, regulations, and contact points for CBD parties (Cartagena Protocol, 2000). A significant weakness of the protections provided by the CBD and associated protocols is that United States, one of the biggest players in the development of synthetic biology and bioengineering tools, did not ratify the convention. Debates continue to revolve around whether regulations, risk assessment processes, and management tools developed for established biotechnologies adequately address the evolving issues raised by modern synthetic biology research.

Biosafety in synthetic biology extends beyond technical challenges and includes important social and political considerations (Moon 2023a, b). Ensuring safe, ethical, and responsible research requires collaboration among researchers, policymakers, regulators, and the public. Established guidelines and risk assessment framework help ensure ongoing evaluation and adaptation of biosafety protocols as new technologies emerge. By maintaining this dynamic approach to biosafety, synthetic biology has the potential to address critical global issues while minimizing risks and maximizing benefits for society.

Biosecurity

The development of a wholly synthetic cell would give rise to unique security challenges. Our current containment and safeguarding infrastructure and procedures are built around known properties and constraints of basic biological processes on Earth. Fundamental properties of life, such as the genetic code, the replication competence of pathogens, and the physicochemical constraints on the growth of organisms, are all known parameters used to build safety procedures. For example, the genetic code defines the relationship between DNA sequence and protein identity, so screening for DNA sequences of concern is used as a proxy to prevent inappropriate individuals or organizations from obtaining toxic

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Table 1. *Some key specific elements for safely and securely handling extraterrestrial samples to prevent contamination of the sample and of the Earth applicable to synthetic biology*

Task	Extraterrestrial sample[a]	Synthetic biological cell[b]
Develop a restricted handling protocol to protect against potential adverse effects	Examples include breaking the chain of contact with sample source and sample transport to receiving facility on Earth	Examples could include breaking the chain of contact with the outside lab environment
Determine the level of risk or hazard	Source of sample and lab analyses	Lab analyses
Work Environment	Clean room environment (air filtering and personnel garments); if extremely hazard, BSL-4	BSL-2-4 depending on the potential hazard level
Biocontainment	Samples archived in a physically secure and non-contaminating environment with a vault for storage and a vault for sample data and records	Synthetic cell lines archived in a physically secure and non-contaminating environment with a vault for storage and a vault for sample data and records
Dissemination of samples	Proposals submitted outlining research questions, methodologies, and the specific samples needed. Proposals are evaluated based on scientific merit, potential impact, and feasibility by NASA	Proposals submitted outlining research questions, methodologies, and the specific samples needed. Proposals are evaluated based on scientific merit, potential impact, and feasibility
Sample transfer	Protocol developed based on risk level	Develop protocol based on risk level

[a]Based on NASA's planetary protection guidelines and NASA's lunar sampling handling protocol.
[b]Protocols to be developed for a restricted handling program to protect against adverse effects on the Earth environment due to the potential release of a hazardous synthetic cell as is done for planetary protection (e.g., Craven *et al*., 2021).

or pathogenic proteins (Hoffmann *et al*., 2023). Knowledge of temperature and UV constraints on the growth of pathogenic organisms guides sterilization procedures (Rutala and Weber, 2008).

A small but important portion of synthetic biology efforts – those that rewrite those basic rules of life – may result in engineered organisms or biological systems that do not obey those known rules and constraints (Moon, 2023c). For example, DNA sequence screening processes may need significant changes if synthetic biologists are able to engineer organisms that break the highly conserved, natural relationships between triplet codons and amino acids. Technologies such as the use of flexizyme currently enable charging of arbitrary amino acids to any tRNA, enabling translation that does not obey the traditional codon table (Goto *et al*., 2011). As this capability expands, DNA synthesis companies will not necessarily be able to know what protein a given DNA sequence will be used to make. Additionally, organisms could be engineered to withstand commonly used sterilization procedures. Different extremophiles can maintain metabolism under intense temperature, irradiation and salinity conditions (Rothschild and Mancinelli, 2001; Merino *et al*., 2019). Engineered polyextremophile organisms could potentially withstand several extreme conditions, enabling their existence after undergoing the most used sterilization procedures. The development of technologies that push the limits of robustness and versatility of biological systems thus may challenge some of our current security paradigms.

In the case of recoded genetic code, it is conceivable to imagine commercial DNA synthesis companies, obeying all the sequence screening guidance, still produce a DNA template that can be

https://doi.org/10.1017/S1473550425100013 Published online by Cambridge University Press

translated into a viral or toxin protein. While creating such novel systems is still very challenging and faces many technical difficulties, those systems are either already possible, or the current directions of research strongly implicate the possibilities arising within the next decades. It is impossible to discount the possibility of determined well-funded efforts intentionally bypassing those safeguards.

While careful planning with biological safety officers and physical laboratory containment procedures are sufficient to enable researchers to safely undertake synthetic cell research, the risks of intentional release or accidental exposure to synthetic cell systems should not be ignored. Those that bend or even break typical rules of biology might be an even greater risk than natural pathogens. Current detection and mitigation procedures might not work, and thus, the novel cellular chassis might, under specific circumstances, become difficult to control.

This creates an urgent need for developing safety and security procedures that take those new, expanded cellular chassis into account. One example of such preparedness efforts already possible with current technology is the updating of DNA sequence screening algorithms to account for the frequency of amino acids, rather than codon identity. DNA synthesis screening and environmental monitoring to detect sequences with codon frequencies that correspond to known proteins of concern, rather than comparing to current databases of sequences of concern based on natural codon tables, could decrease the possibility of bypassing this safeguarding protocol.

While it is impossible to know in advance all properties of novel organisms, a coordinated effort by the synthetic cell engineering community to maintain an updated database of known and realistically achievable properties of synthetic cells could help identify and develop appropriate screening and containment procedures, while also helping the field move forward overall. Because we do not know whether life systems elsewhere abide by the rules and laws here on Earth (Moon, 2023c), by recognizing and mitigating security concerns arising from new synthetic organisms or systems, we can also be better prepared to respond to any non-terrestrial live forms or systems that may be found on a Planetary Return Mission.

Summary and conclusions

Here, we illustrate the commonalities between the apparently disparate disciplines of planetary protection and synthetic cell research to create the foundation for synthetic biology biosafety and biosecurity protocols. Examples of these commonalties are presented in Table 1. Both face hazards ranging from little or no hazard to the potential for an extremely high hazard risk. Planetary back-contamination brings back to Earth samples that may contain substances or organisms that may or may not be harmful, and because of the potential harm, relevant protocols have been formulated and put in place for handling samples. Similar approaches for formulating synthetic biology biosafety protocols could and should be taken. Both have the same goal that is the control of contamination of the Earth from samples returned from space in the case of planetary protection and samples made in the laboratory in the case of synthetic cells. Similarly, an intentional release of synthetic cells with the goal of harming people should be prevented by developing and implementing biosecurity measures. These efforts should be made by international collaboration and coordination because the potentially harmful impact of synthetic cells and samples from space might lead to global-scale disasters, as the COVID pandemic has shown for the past five years. We urge thoughtful consideration of appropriate biosafety and biosecurity protocols for synthetic cells.

Acknowledgements. We thank Maya Hey, Kathryn Brink, Baris Avsaroglu, John Glass, James Wagstaff, Anton Jackson-Smith, and Elibio Rech for valuable comments on this manuscript. The National Science Foundation Research Coordination Network award 1901145 to KA supported the Build-a-Cell meetings where the concepts for the paper were developed by the authors. We also thank the National Institutes of Health [RO1 AT009741 to T.S.M.], the United States Department of Agriculture [2020-33522032319 to T.S.M.], and the U.S. Environmental Protection Agency [84020501 to T.S.M.] for support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Author contributions. All authors wrote and revised the manuscript equally.

https://doi.org/10.1017/S1473550425100013 Published online by Cambridge University Press

Competing interests. The authors declare no conflict of interest.

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https://doi.org/10.1017/S1473550425100013 Published online by Cambridge University Press