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Synthetic Biology: Research Needs for Assessing Environmental Impacts

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Christopher M. Warner, Richard F. Lance, Fiona H. Crocker,
Edward J. Perkins, Taylor E. Rycroft, and Kaytee L. Pokrzywinski

Environmental Laboratory
Engineer Research and Development Center
U.S. Army Corps of Engineers
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Sarah R. Carter

Science Policy Consulting, LLC.
4109 33rd St. S.
Arlington, VA 22206

Heather N. Meeks

Defense Threat Reduction Agency
8725 John Kingman Rd. #6201
Ft. Belvoir, VA 22060

Bryn L. Adams

Adelphi Laboratory Center, Army Research Laboratory
2800 Powder Mill Rd.
Adelphi, MD 20783

Matthew L. Magnuson

U.S. Environmental Protection Agency
26 Martin Luther King Dr. W.
Cincinnati, OH 45220

Final Report

Abstract

Synthetic biology and its applications have the potential to greatly improve economic development, public health, environmental stewardship, technological advancement, and many other areas. In May 2017, sixty individuals gathered in Lexington, Massachusetts for a workshop sponsored by the U.S. Army Engineer Research and Development Center (ERDC) to discuss applications of synthetic biology with likely or intended interaction with the environment. Representatives from academia, government agencies, industry, and non-governmental organizations convened to identify knowledge gaps and research needs to assess potential environmental impacts from these technologies. The group discussed challenges in environmental risk assessment, regulation, and community engagement for emerging synthetic biology technologies. The workshop was structured around four hypothetical case studies, including the use of gene drive engineered organisms to control infectious disease vectors, engineered microbes for bioremediation, cell-free applications for advanced chemical production, and engineered viruses for water treatment.

Meeting these research needs will facilitate appropriate environmental risk assessment and informed decision making for the development and potential deployment of synthetic biology organisms and components in the environment.

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Preface

Acronyms

ADHB	Alcohol dehydrogenase II
CRISPR	Clustered Regularly Interspaced Palindromic Repeats
DIYBio	Do-It-Yourself Biology
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
EA	Environmental Assessment
EIS	Environmental Impact Statement
EL	Environmental Laboratory
EPA	Environmental Protection Agency
ERDC	Engineer Research Development Center
ESA	Endangered Species Act
FDA	Food and Drug Administration
FDCA	Food Drug and Cosmetics Act
FELIX	Finding Engineering-Linked Indicators
FONSI	Finding of No Significant Impact
HQUSACE	Headquarters, U.S. Army Corps of Engineers
IARPA	Intelligence Advanced Research Projects Activity
IBCs	Institutional Biosafety Committees
NEPA	National Environmental Policy Act
NIH	National Institutes of Health
NSF	National Science Foundation
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
RNA	Ribonucleic Acid
TSCA	Toxic Substances Control Act
UV	Ultra-violet
USDA	U.S. Department of Agriculture
3-D	Three Dimensional

1   Introduction

1.1   Background

Synthetic biology,[1] along with a wide range of emerging tools and techniques, will enable a new generation of biotechnology products of unprecedented scale and complexity over the next 5–10 years (NASEM 2017a). Within the U.S., synthetic biology and its applications are currently estimated to be a multi-billion dollar industry that continues to grow rapidly (Gronvall 2015) with significant private investment (e.g., SynBioBeta 2017). Many of these products are likely to have beneficial applications for military use, including new approaches to manage natural resources and ranges, produce fuels and other materials, and protect the warfighter. For this reason, the U.S. government, including the Department of Defense (DoD), has significant investments in synthetic biology (OTI 2015; Wilson Center 2015). While many of the next generation of products will be similar to existing biotechnology products, others are likely to be novel, including many with probable or intended release into the environment. These technologies may challenge our current regulatory and environmental risk assessment frameworks (Carter et al. 2014; Drinkwater et al. 2014; NASEM 2017a). The U.S. Army Engineer Research and Development Center (ERDC) is well-positioned to address some of the critical environmental questions that these new products will raise. In doing so, ERDC can help ensure that the U.S. DoD, regulatory agencies, broader government stakeholders, commercial entities, and others have the information and tools necessary to make informed decisions on development and potential use of synthetic biology organisms and components in the environment.[2]

In addition to posing challenges for environmental risk assessment, new biotechnologies raise broader regulatory and societal issues. For example, in some cases, there is uncertainty in the regulatory pathway that these

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[1] The term "synthetic biology" is used throughout this document when referring to a range of advanced biotechnologies. Synthetic biology refers to the design and construction of new biological entities such as enzymes, genetic circuits, and cells, or the redesign of existing biological systems (Keasling 2005). This capability is rooted in traditional molecular biology and engineering and incorporates newer techniques, including, for example, *de novo* deoxyribonucleic acid (DNA) synthesis, clustered regularly interspaced palindromic repeats (CRISPR)-based genome editing, and xenobiology.

[2] The term "synthetic biology organisms and components." are whole organisms that have been engineered using synthetic biology. Components in this context refers to synthetic biology constructs and circuits that may be used or deployed outside of a living organism.

products will have to traverse before they can be tested or deployed in the environment (Carter and Friedman 2016; NASEM 2017a). The potential for field testing and deployment of gene drive engineered organisms (a class of synthetic biology organisms that have intended interactions with the natural environment, see case study below), has generated much discussion about the need for community and stakeholder engagement (NASEM 2016; NASEM 2017a), with early engagement activities already underway in some contexts (Swetlitz 2017). These regulatory and engagement activities are likely to require time and commitment, with some types of products likely to face greater scrutiny and more challenges than others. A better understanding of these issues will be critical in the development and application of a wide range of biotechnology products.

1.2 Objectives

In May 2017, ERDC held a case study-based workshop in Lexington, MA that aimed to identify key challenges to the deployment of advanced biotechnologies. This 2.5-day meeting brought together sixty participants from a wide range of organizations, including ERDC and other DoD entities, universities, commercial companies, federal regulatory agencies, non-governmental and international organizations, and others (Appendix A). The agenda included context-setting plenary talks and five breakout sessions (Appendix B). Each breakout group focused on one of four specific case studies, including the following: gene drive engineered organisms to control infectious disease vectors, engineered microbes for bioremediation, cell-free applications for advanced chemical production, and engineered viruses for water treatment. The case studies (Appendix C) were chosen because they represent realistic biotechnologies that together reflect the wide range of synthetic biology technologies that could be submitted to regulatory agencies for consideration in the near future. To ensure balanced and cross-disciplinary discussions of these case studies, each group included participants with backgrounds in basic and applied research, including biological, engineering, social sciences, and regulatory processes. The breakout discussions within each case study were divided into topic areas including: 1) "Horizon Scanning" to identify the scope of technologies relevant to the case study, 2) "Environmental Impacts" to discuss potential environmental impacts, 3) "Safety and Regulation" to identify the current regulatory frameworks that apply to these

technologies, 4) "Community Engagement"[1] to identify broader societal issues, and 5) "Challenges and Opportunities" to identify the key themes and challenges to deployment.

1.3 Approach

Workshop discussions aimed to identify high-priority information, data, and capabilities needed to provide a basis for decision-making with respect to deployment of synthetic biology organisms and components in the environment. The focus of this workshop was primarily on environmental impacts, including potential hazards and risks.[2] Research and development needs related to understanding and monitoring potential environmental impacts are also described herein. Throughout the workshop, these needs were discussed primarily in the context of regulatory assessment and approval, but are also relevant in the context of non-regulatory (voluntary) assessment and mitigation measures that developers may choose to undertake (e.g., to reduce product liability). Some of these biotechnologies also have critical regulatory and societal uncertainties associated with their deployment, these information and capability needs are flagged as well. Without explicit and careful inclusion of these aspects in product development or release plans, beneficial applications of synthetic biology organisms and components could be delayed or never realized. Synthetic biology is also an important topic within the context of biosecurity and bioterrorism (NSABB 2010; NASEM 2017b). Although these are important discussion topics, and were briefly considered, these types of risks were not a focus of this workshop.

Section Two of this report describes themes that emerged from workshop discussions, and research, information, and capability needs that were identified. Many of these themes and needs were common across all case studies and represent opportunities for future high-impact research and development. Section Three includes discussion summaries from each of the four case studies. Section Four describes workshop conclusions,

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[1] The initial agenda for the workshop (Appendix B) refers to "Social License," but discussions at the workshop led to the conclusion that the term "Social License" is problematic because it implies one-sided, commercial interest in pursuing technologies. The term "Community Engagement" better captures the two-way learning process that is discussed here.

[2] In this document, the following definitions are used: risk is the probability of an effect on a specific endpoint or set of endpoints due to a specific stressor or set of stressors; hazard is a harmful effect; and impact is any effect which can be beneficial or harmful. Risk assessment is defined as the process by which all available evidence on the probability of effects is collected, evaluated, and interpreted to estimate the probability of the sum total of effects (NASEM 2016).

including the need for a strategic approach across the U.S. government for assessing the environmental impacts of synthetic biology organisms and components.

1.4 Scope

Although this report draws on the collected expertise of workshop participants and others (Appendix A), this is not a consensus report. The conclusions are those of the authors alone and do not represent any government position. Nevertheless, despite the wide range of perspectives provided at the workshop, there was a high level of agreement on many issues.

2 Common Themes and Research Needs

A number of common themes and research needs emerged from the consideration of the workshop case studies. Section 2 describes the research, information, and capability needs that were identified, including many that were common across all case studies. These needs represent opportunities for high-impact research and development.

2.1 Modeling

Because biological systems and their interactions with dynamic ecosystems are complex, development, refinement, and ongoing evaluation of models will be critical to understanding the characteristics and interactions of synthetic biology organisms and components, and their potential risks and benefits. The need to populate models with useful data will also provide an important basis for many of the research needs listed below. These needs include the following:

- Modeling of how synthetic biology organisms and components will interact with native populations and ecosystems, including scenarios of intentional release and escape.
- Modeling of how synthetic biology organisms and components may change or evolve over time in different contexts and environments.
- Experimental or observational evaluation of models, including the ability to ensure that relevant data are reliably generated and incorporated into models.

2.2 Fate and transport of synthetic biology organisms and components

The fate and transport of biological components, engineered or otherwise, has long been identified as a research need, but much work remains to be done. Needs include the following:

- Understanding of gene transfer for different types of nucleic acids (e.g., naked oligonucleotides, viral-encapsulated DNA and Ribonucleic acid (RNA), microbial plasmid and genomic DNA, and eukaryotic DNA) in both natural contexts and with synthetic biology organisms and components. This includes studies of the potential for hybridization of synthetic biology organisms with non-targeted, natural populations.

- Modeling (including evaluation of models) and measurement of the distance synthetic biology organisms and components are likely to travel within specific environments, and the length of time they are likely to persist in different contexts.

2.3 Control and stability of synthetic biology organisms and components

A key challenge for many synthetic biology organisms and components is ensuring that they are controllable, stable, and predictable in the environment. Needs include the following:

- Improved control of synthetic biology organisms and components. For example, gene drive engineered organisms that only survive where and when they are wanted with the characteristics that are intended, and microbial or viral systems that contain improved and stable intrinsic biocontainment mechanisms (e.g., kill switches and auxotrophic metabolism) to limit the spread of synthetic biology organisms and components in the environment.
- Development of predictive tools and methodologies to identify potential novel outcomes, such as genetic rearrangements, unintended enzymatic or metabolic activity, or unwanted reproduction.

2.4 Monitoring and surveillance

Discussions for each case study identified the need for improved monitoring and surveillance of environmental systems, both for improved baseline understanding of the naïve environment pre-release, and for tracking synthetic biology organisms and components following deployment. Needs include the following:

- Monitoring and surveillance tools for synthetic biology organisms and components in the environment, including development of metrics to track their spread, stability, and persistence.
- Baseline characterization of native environments into which synthetic biology organisms and components are likely to be deployed to detect and contextualize post-deployment changes.

2.5 Oversight, regulation, and community engagement

Several common themes emerged in discussions about regulatory oversight and community engagement for the four case studies. For regulatory decision-making, there was an awareness in each group of the need for case-by-case evaluation of synthetic biology organisms and components and potential environmental releases due to the wide variety of uses and contexts. There was also a desire for clarification of the regulatory process, including timelines and data requirements. Early and frequent engagement with regulators was identified as critical to successful navigation of the regulatory process. Another theme that arose was the need to evaluate the impacts of synthetic biology organisms and components against the impacts of alternative actions, including no action. Phased testing and evaluation of synthetic biology technologies were identified as a way to improve products and generate the data necessary to make decisions on eventual deployment in the environment.

The need for effective community engagement also emerged as a common theme in discussions of the four case studies, with one case study (gene drives) identifying it as fundamental to successful testing and deployment in the environment. When pursuing community engagement activities, product development teams should provide a means for community members and other stakeholders to impact decision-making. Such a process should include a well-defined intention, thoughtful analysis of who should be included, what information needs to be shared by the product development team, and how discussions with community participants can best inform decision making. By establishing engagement and building trust in the community early in the development and deployment process, decisions can be made with clarity and mutual respect. Throughout the workshop, there was discussion about whether and how engagement processes and deployment of the "first" synthetic biology technologies may impact perceptions and potential deployment of those that come later. Needs for regulatory and community engagement include the following:

- Development of processes to determine environmental endpoints of interest and clarity on how these should be measured/assessed. Regulators may already have some guidance, but it may be appropriate to clarify stakeholder roles and include community input in some cases.
- Characterization of and guidance for successful community engagement processes. This includes lessons learned from other types of community

engagement and best practices developed for similar types of products and technologies. It also includes understanding of how community perceptions and engagement processes are affected by previous and ongoing engagement on related technologies. Understanding whether successful deployment in the environment of one synthetic biology technology affects how the next is perceived and what factors influence these perceptions (e.g., type of technology, environment, or developer) is essential to successful community engagement.

- Improved communication tools, along with access to and awareness of potential collaborations with those experienced and skilled in community engagement, will empower scientists in fostering successful community engagement activities. This includes the development of more effective approaches for inclusion of stakeholder needs and perceptions throughout the development cycle.

3   Case Studies

This section includes discussion summaries for each of the following four synthetic biology case studies: gene drive engineered organisms to control infectious disease vectors, microbial engineering for bioremediation, cell-free technologies for advanced chemical production, and viral systems for water treatment (Appendix C). As mentioned in Section One, each discussion group included technical experts and those familiar with environmental, regulatory, policy, and other societal implications of biotechnologies. The groups met independently (with opportunities to report conclusions to the larger group), and the written reports below represent these separate discussions. Common themes and research priorities identified in discussions across case studies are outlined in Section Two.

3.1   Gene drive engineered organisms to control infectious disease vectors

3.1.1   Introduction

Gene drives are "systems of biased inheritance that enhance the ability of a genetic element to pass from an organism to its offspring through sexual reproduction" (NASEM 2016).[1] Naturally occurring gene drives have been studied for decades (Burt and Trivers 2006). However, in recent years, genome editing using CRISPR has overcome technical challenges involved in engineering gene drives. CRISPR allows insertion of genetic material targeted to a specific DNA sequence. Some types of CRISPR-based gene drive constructs can ensure that nearly 100% of offspring inherit that genetic material (NASEM 2016). A wide range of gene drive constructs and applications are currently being considered and developed in laboratory settings, but will require multiple stages of confined testing before being approved for field testing and deployment in the environment. It has been estimated that the first gene drive engineered organisms are likely to be ready for field testing and regulatory consideration in 5–10 years. Oversight and assessment of this process will largely be guided using

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[1] Throughout this document, we use the terms "gene drive," "gene drive constructs," and "gene drive engineered organism." Gene drive is the system of biased inheritance that enhances the ability of a genetic element to pass from an organism to its offspring through sexual reproduction (NASEM 2016). Gene drive construct refers to the engineered genetic construct that contains elements that are preferentially inherited by the progeny of an organism. Gene drive engineered organism refers to an organism that contains in its genome a gene drive construct.

frameworks already in place for genetically engineered organisms (WHO 2014; EFSA 2013; CBD 2012; FDA 2017a), though frameworks specific to gene drive engineered organisms are under development to address some of their specific governance challenges (NASEM 2016). The workshop case study involved a gene drive engineered *Aedes aegypti* mosquito for suppression of wild populations to reduce disease (Appendix C).

3.1.2 Horizon scanning

Gene drive engineered organisms can be developed for a wide range of purposes and applications (Esvelt et al. 2014). Most anticipated applications of gene drive engineered organisms at this time are for population suppression (i.e., decreasing numbers of an undesirable species). These include suppression of disease vectors (e.g., mosquitoes), invasive species (e.g., mice, rats, other mammals, cane toads, some invasive plant species), and agricultural weeds and pests (e.g., pigweed, screwworm, desert locust). Because gene drives require sexual reproduction, asexual or facultatively sexual species, such as many plants and fungi, may not be candidates for gene drives. To date, CRISPR-based gene drives have been demonstrated in fruit flies (*Drosophila*) and in mosquito species that are significant disease vectors (Gantz and Bier 2015; Hammond et al. 2016). Gene drive engineered organisms are also under development for management of invasive populations of the house mouse (*Mus musculus*) on islands where this species devastates native fauna, particularly birds (Lewis 2017). A wide range of gene drive constructs for a variety of potential applications are currently under development (DARPA 2017; Target Malaria 2017).

In addition to population suppression, gene drive engineered organisms can also be used to replace existing populations with those that are composed of individuals that carry (and pass on) genetic constructs that express one or more desired traits. Such traits could include enhanced resistance (or susceptibility) to pesticides, enhanced immunity to disease, reduced capacity to harbor disease-bearing parasites or pests, capacities for environmental remediation of pollutants or contaminants, or others. The diversity of potential uses for gene drive engineered organisms is only beginning to be realized.

Regardless of application, gene drive engineered organisms can be categorized based on the way that they are predicted to function. They can be self-sustaining (i.e., designed to spread in a population unless and until

the population generates resistance) or self-exhausting (i.e., designed to spread in a limited way in time and space). If a gene drive engineered organism is threshold-independent, then only a small number of individuals may allow the gene drive construct to spread in a population (unless and until the population generates resistance). In contrast, for a threshold-dependent gene drive, the engineered organism must be present in sufficient numbers relative to the wild-type individuals in a population (i.e., at or above a certain threshold) before it is likely to spread in that population. Below that threshold, it will die out. By definition, releases of threshold-dependent gene drive engineered organisms are reversible. Gene drive engineered organisms can be outcompeted by releasing sufficient numbers of wild-type organisms. They are also dispersal-limited, if a gene drive engineered organism migrates into a largely wild-type population, the gene drive engineered organism would be at below-threshold levels and should be extirpated in that population.

The simplest CRISPR-based gene drive constructs (e.g., insertion of a single CRISPR construct targeted to one sequence within that genome) are self-sustaining and threshold-independent, which, modeling indicates, may allow them to spread and persist in the environment even when only a few individual organisms are released. When an area-wide application is intended, this type of gene drive construct could provide significant benefits. However, genetic stability is a major challenge for these types of gene drive constructs. A single nucleotide mutation (or naturally occurring polymorphism in the release site population) in the targeted sequence has the potential to reduce or eliminate the functionality of the gene drive construct, and prevent it from "driving." This could in some cases also confer a selective advantage on individuals with the mutation, which could give rise to population-level resistance to the gene drive construct and bring about the extirpation of the gene drive engineered organism in the population over time, thus decreasing the benefits of the product (Noble et al. 2017). This challenge may be addressed by using multiplexed CRISPR-based gene drive constructs, where multiple DNA sequences are targeted for insertion of the gene drive construct; this is an active area of research.

A major focus of current research in gene drive laboratories is on designing and developing gene drives with limited spatiotemporal spread. For example, as described above, self-exhausting gene drive engineered organisms are designed to spread in a wild population for a limited time. Another approach is to target specific subpopulations that have unique

DNA sequences so that the gene drive construct will only spread among those subpopulations. These gene drive constructs may be more appropriate for limited applications (e.g., when only a localized pest population or subset of a pest population is targeted).

All gene drive engineered organisms face a significant challenge when testing at scale. It is difficult to collect meaningful data from contained cage trials that would be applicable to populations at the ecosystem scale where the gene drive construct is designed to function. Such experimental systems cannot capture the full ecological and environmental complexity that will be experienced by gene drive engineered organisms upon release during field testing or deployment in the environment. Also, failure modes for many types of gene drive constructs (e.g., multiple mutations giving rise to resistance in a multiplexed system) are anticipated to be very rare events, thus, having a sufficient number of individuals in a cage trial to reliably detect the mutation rate and rate of drive failure is a significant challenge. Furthermore, an understanding of the population dynamics of target organisms (e.g., short-term dispersal and gene flow patterns) is often lacking, limiting the reliability of modeling efforts. To address these challenges, additional data on target organisms and their population dynamics, including environmental factors, are needed. Researchers can also draw on information generated and lessons learned from the release of previous generations of genetically engineered organisms, including non-engineered biological control organisms and pesticide applications. As the field progresses, data generated from these sources including contained laboratory and field trials of gene drive engineered organisms should feed back into models to improve their predictive power.

3.1.3 Environmental impacts

The potential environmental impacts of release of a gene drive engineered organism must be considered prior to release. Critically, effects associated with the release of a gene drive engineered organism must be understood in relation to the alternatives (e.g., the use of pesticides) and/or no action (e.g., continuing human disease burden). A key issue in environmental risk assessment is problem formulation, identifying the environmental endpoints (protection goals) that is cared most about. Because it is impossible to monitor all parts of an ecosystem, even at a small scale, there must be some prioritization of endpoints in order to evaluate risks and benefits. Although regulators can help define key environmental endpoints, engagement with those publics who might be (or perceive that

they might be) affected by testing and deployment of a gene drive engineered organism in the environment should be involved in setting these priorities (e.g., Roberts et al. 2017).

For all gene drive engineered organisms, monitoring of the environmental endpoints of interest and of the organisms themselves (e.g., spread of the organisms, gene drive phenotype, effectiveness, and stability) will be critical. Prior to release of the gene drive engineered organism, some baseline monitoring of key features of the ecosystem will be needed to increase the likelihood that the impacts of release (if any) can be detected and measured. Effective monitoring also requires access to sites and potentially affected habitats, including financial support for a sustained effort. The goals of any monitoring program (including endpoints of interest and types of analyses to be performed) should be explicit to best ensure that the program generates data and information that informs decision making. Tools for detecting and tracking the spread of the gene drive engineered organisms will also be required, both for understanding the impact of the gene drive construct on the ecosystem and its effectiveness at spreading in the target population.

As discussed previously, there are a wide variety of gene drive constructs with different characteristics under development. Specific environmental considerations for each potential gene drive engineered organism will have to be evaluated on a case-by-case basis.

For the specific workshop case study (suppression of *Aedes aegypti* mosquito populations in the U.S., see Appendix C), a number of potential environmental factors would need to be explored in greater detail prior to release. These include trophic interactions, potential for interbreeding with other species of mosquitoes, impacts on vector competence, and potential for niche effects on *A. albopictus* and other mosquitoes (e.g., the suggestion that *A. albopictu*s may spread more quickly in the absence of *A. aegypti*). Additionally, a better understanding of how the specific gene drive construct might spread within the local mosquito population through modeling efforts that take local conditions into account may be required. Research on wild-type populations of Aedes (and other pest control programs such as pesticide applications and sterile insect techniques) could provide data related to population dynamics, gene flow, and genetic diversity. Such information would strengthen existing models

and assessment of impacts from release of gene drive engineered organisms and help developers improve product design and effectiveness.

Studies on mosquito populations and on potential environmental impacts must be considered in the context of the specific ecosystem into which these mosquitoes might be deployed. For example, *A. aegypti* are adapted to living with humans, so their dispersal rates and methods are influenced by human movement in the area. Some populations of *A. aegypti* are invasive and have arrived relatively recently (e.g., those in the U.S.), eradication of those populations may be seen as restoring the native ecosystem rather than a perturbation in the ecosystem.

3.1.4 Safety and regulation

Laboratory biosafety and containment in the U.S. is overseen primarily at the institutional level by institutional biosafety committees (IBCs). IBCs are not required under any regulation, but are a term and condition of funding from the U.S. National Institutes of Health (NIH) and most other federal agencies. However, the NIH guidelines for biosafety, which provide guidance for IBCs, are primarily focused on human pathogens and potential impacts on human health. As such, membership and expertise on many IBCs may be inadequate to evaluate and address potential environmental impacts that may arise from gene drive engineered organisms. Laboratories that work on insects (especially insect vectors of disease) also follow Arthropod Containment Guidelines developed by the American Society of Tropical Medicine and Hygiene (ACG 2004). These, too, are widely applied, though voluntary. Internationally, there is no consensus on appropriate biosafety precautions for working with gene drive engineered organisms, with different countries taking very different approaches.

Within the U.S., gene drive engineered organisms will be regulated based on their intended use (OSTP 2017). An organism intended for pest suppression may be regulated by the Environmental Protection Agency (EPA) under their rules for pesticides. If it is intended to decrease human disease burden, then it is likely to be regulated by the Food and Drug Administration (FDA) under the Federal Food Drug and Cosmetics Act (FDCA). If it is a plant or animal pest, then it may be regulated by the U.S. Department of Agriculture (USDA) under its plant and animal protection rules. In some cases, the gene drive engineered organism will be regulated by multiple agencies with these three primary agencies working together to coordinate testing, approval, and oversight.

Regardless of its regulatory pathway, all gene drive engineered organisms intended to be marketed in the U.S. must undergo some environmental risk assessment to comply with a federal regulatory agency. Outside of the U.S., many countries have a "process-based" regulatory system whereby genetically engineered organisms are regulated under laws specifically designed to regulate products derived using biotechnology. These regulations will also apply to gene drive engineered organisms that are being registered for in-country use.

For the specific workshop case of an *Aedes aegypti* mosquito release intended to prevent the spread of diseases, including Zika and dengue, regulatory oversight in the U.S. would be provided by FDA. Under FDA regulations, a mandatory pre-market approval would be required and a product would be approved only if it is shown to be "safe" (i.e., causes no greater harm to humans, other animals, and the environment as compared to non-engineered *A. aegypti*) and "effective." Effectiveness is determined based on the claim that the applicant intends to make, which should be specific and supported by data (provided by the developer or publicly available).

In addition to meeting FDA requirements, product developers would also need to develop an Environmental Assessment (EA) in compliance with the National Environmental Policy Act (NEPA) as part of the product approval process. The FDA would evaluate the EA for investigational use and issue either a Finding of No Significant Impact (FONSI) (allowing product development and testing to move forward) or require that a full Environmental Impact Statement (EIS) be conducted. The EIS is typically a broader and more rigorous analysis than the EA. Once the EIS is published, along with a record of decision, product development can proceed. When all other FDA requirements are met, including the publication of a final EA/FONSI or EIS/record of decision, the developer can file for an approval. The NEPA process requires publication of the draft EA or EIS, including opportunities for public comment, when the agency action is without precedent (i.e., if the type of product has never been approved by FDA in the past, which would likely include gene drive engineered organisms). If the gene drive engineered organism is expected to spread near the range of a federally listed endangered species or critical habitat, then the product developer may also be required to provide data and information for an assessment under the Endangered Species Act (ESA).

Regulators from the U.S. and other nations are likely to have (and are working to develop) some common requirements for gene drive engineered organisms. These might include information on the organism's molecular biology and resulting phenotype, quality control, construct and trait stability, and safety, along with tools and assays for detecting and monitoring the organism once released. A major unmet need for the regulation of gene drive engineered organisms is an understanding of their phenotypic and genotypic stability over generations. The regulatory agency will need to have some confidence that the product will remain stable over time because approval is given for a specific product with specific characteristics. Gene drive researchers will have to work with regulatory agencies to help define the requirements for stability and product quality control in this context.

There has been some guidance specific to performing environmental risk assessments for testing and deploying gene drive engineered organisms in the environment (NASEM 2016), and extensive guidance has been published for earlier generations of genetically engineered organisms (EFSA 2013; FDA 2017a), including mosquitoes (WHO 2014; CBD 2012). Much of this guidance emphasizes a phased approach, with Phase 1 focused on laboratory studies, Phase 2 on physically and/or ecologically confined field trials, Phase 3 on unconfined release, and Phase 4 on post-release surveillance. However, risk assessment for gene drive engineered organisms may pose some challenges beyond those posed by earlier generations of genetically engineered organisms. For example, for some types of gene drives (e.g., those that are threshold-independent), even a small number of escapees from a confined field test could have a significant impact on native populations; best practices at this stage are not yet clear. Several groups, including the WHO, are working to develop guidance to address risk assessment challenges associated with gene drive engineered mosquitoes. The first gene drive engineered organism to be developed will likely help define the regulatory pathway and the appropriate milestones and precautions to incorporate into this phased approach.

3.1.5 Community engagement

Gene drive engineered organisms are typically designed to persist in the environment and impact wild populations, often at large scales. Although they hold great promise, they also hold some uncertainty about potential environmental impacts. These factors raise important issues about the responsibilities of product development teams beyond just environmental

risk assessment and regulatory approvals. Because gene drive engineered organisms have the potential for broad impact, decision making for their deployment in the environment should also be broad, and include community and stakeholder engagement from the early stages of development (NASEM 2016; Carter and Friedman 2016). These efforts will require significant dedication and commitment from funders and product development teams.

A well-organized engagement process should be designed by product development teams with the intention of involving local communities throughout the phased development process to help guide product design, site preparation, early testing, product development and deployment in the environment, post-deployment monitoring, reporting and communication, etc. The product development team itself should include social scientists alongside researchers and other transition partners (e.g., companies or non-profit entities). There are multiple other contexts (e.g., public health and agriculture) within which community engagement processes have been used to successfully guide decision making, and these may provide some lessons for releases of gene drive engineered organisms. Examples include the Eliminate Dengue Program in Australia (Kolopack et al. 2015), efforts in support of field trials of genetically engineered mosquitoes in Mexico (Ramsey et al. 2014; Lavery et al. 2010), and the "Mosquito-free Hawaii" initiative, which has brought together community members and scientists to evaluate options for eliminating invasive mosquitoes from the islands. This process has included discussion of gene drive engineered mosquitoes as a far-future possibility (Revive and Restore 2017).

3.2　Case study: microbial engineering for bioremediation

3.2.1　Introduction

Although genetically engineered microbes have been used for decades in laboratories and for commercial purposes, genetic and metabolic engineering of microbes has become both much easier and more complex in recent years (Chari and Church 2017; also see, e.g., Temme *et al.* 2012). Domesticated microbes are regularly genetically modified and utilized in high-throughput commercial services (e.g., Ginkgo Bioworks, Zymergen), however, these microbes function in precisely maintained and optimized bioreactors. Engineering microbes that can survive and function as designed in the environment remains a major challenge. Even so, an

increasingly wide variety of engineered microbes with intended uses in the environment are under development, including microbes used for bioremediation, biomining, and chemical production. The workshop case study involved a microbe engineered for bioremediation (see Appendix C).

3.2.2 Horizon scanning

Much of the current work on engineered microbes for environmental applications has been focused on designs to overcome challenges and limitations related to release. Most successful microbial engineering endeavors have used well-characterized microbes that have been cultured for generations in the laboratory (such as *Escherichia coli*, *Saccharomyces spp.*, and *Bacillus spp.*). Ensuring their survival in the natural environment will be an additional challenge. Furthermore, the engineered genetic constructs and tools developed in laboratory strains like *E. coli* are not universally functional in other microbes and the extent to which they can be adapted and transferred to other chassis is not yet clear (Adams 2016; Kushwaha and Salis 2014). This issue is difficult to study because there has been limited research on how to quantify functional fitness in the field. There is also a lack of understanding of how genetic and phenotypic traits are correlated with an organism's fitness in the environment. Survival and reproduction is also related to the variation of microbiome diversity and complexity. Adjacent ecosystems may also contain variable nutrients and toxicants (especially relevant to microbes developed for bioremediation applications), these may impact survival and reproduction.

To address these challenges, more studies are needed on natural microbial communities, including survival factors, interactions between microbes, microbial evolution, and transfer of genetic information among different microbial strains and species. Such data will allow better prediction and monitoring of the broader impacts of engineered microbes in the environment. These studies will also allow more effective and predictable outcomes in engineering microbes to express products that penetrate into natural systems (e.g., mobile genetic elements that can be passed to multiple types of microbes for enhanced effectiveness), which have thus far been difficult.

Another significant challenge in developing novel functional systems is the lack of predictive tools. In particular, bioinformatics capabilities are needed that will enable researchers to discover functional components from unexplored genomes in order to expand the range of tools that can be

utilized in the future. The development of machine learning and artificial intelligence will likely lead to more rapid advances in the future, but these approaches will require reliable structured datasets and significant improvements in our underlying understanding of relationships between primary sequence, macromolecular structure, and function.

One major theme for engineered microbes is engineering systems for biocontrol and biocontainment. A variety of methods are being pursued by researchers. For example, codons can be reassigned so that only the intended microbial host is capable of reading the engineered DNA, or novel promoters can be inserted into engineered organisms so metabolic activity is controlled through addition of a chemical typically not found, or quickly degraded, in the environment. Additionally, engineered microorganisms can be designed to exclusively utilize non-natural amino acids or nucleic acids that do not exist in nature (Mandell et al. 2015). Such organisms are orthogonal to natural systems, and may appear "invisible" to native organisms (Schmidt 2010).

Traditional biocontainment methods can also be incorporated into advanced engineered microbes, but these methods require additional development in order to be effective. There have been significant research efforts in the development of auxotrophic systems, where the microbe is engineered to be dependent on a specific chemical or nutrient. While highly effective in controlled laboratory settings, microbes in complex natural environments are often able to find the required nutrients or suitable alternatives directly in the environment. There is the additional challenge that genetic constructs conferring a growth and survival disadvantage places a selective pressure on the organism to evolve away from those constraints in order to increase environmental fitness. For auxotrophic systems, there is the potential for engineered microbes to overcome the nutrient dependence through natural genetic mutations or by acquiring the necessary genes by foreign genetic material uptake from the environment (Moe-Behrens et al. 2013). Similarly, kill switches face the same challenges. A typical kill switch system contains a continuously expressed toxin that is lethal to the host cell. By linking an external signal (chemical) to neutralization of the toxic protein or genetic repression, the engineered microbe will only survive in the presence of the specific signal or chemical. However, this also provides a strong selective pressure against the kill switch, and can escape (Moe-Behrens et al. 2013). Incorporating multiple biocontainment mechanisms will likely have a

greater chance of success in limiting survival and propagation of an engineered microorganism in the environment.

3.2.3 Environmental impact

The potential environmental impacts of engineered microbes will depend on the particular engineered function and will have to be evaluated on a case-by-case basis. In some instances, advances in synthetic biology may reduce potential hazards; for example, the use of xenobiology or orthogonal genetic systems to prevent the transfer of genetic material to native organisms. However, the complexity of synthetic biology technologies may, in some cases, increase uncertainty. Risk assessors have little experience with proteins composed of non-natural amino acids, and potential impacts on the environment will have to be determined. Also, it can be difficult to identify the secondary and tertiary metabolites in complex metabolic pathways and to understand how these pathways interact with the natural environment. In all cases, engineered microbes that closely resemble previously evaluated microbes will be easiest to assess for safety and environmental impacts.

A critical challenge in determining the environmental impact of engineered microbes is that current understanding of natural microbial communities is lacking. The undisturbed (baseline) state of microbial ecosystems is often unknown, and indicators of "healthy" or "pristine" microbial ecosystems are not defined. The most relevant timescale for detecting impacts from engineered microbes is also unclear, and it will be difficult to determine the cause of observed perturbations in a microbial ecosystem. Environmental applications of engineered microbes are designed to have a measurable effect, and it may be difficult to understand if observed changes in the microbial communities are beneficial or detrimental to the environment. This dilemma is particularly clear for the workshop case study of an engineered microbe developed for bioremediation. Sites where such microbes would be deployed are likely to be highly polluted, so environmental changes in this context are likely desirable. Furthermore, polluted sites will naturally give rise to unusual microbial ecology, thus complicating what might be considered a baseline state. Research on natural microbial ecosystems in a variety of contexts will help to define healthy ecosystems, thus providing critical context for understanding the desirability of perturbations in those systems, and indicators for gauging microbial community resilience.

Due to the diversity of microbial ecosystems where engineered microbes may be released, each release should be evaluated within its ecological context. For example, soil microbes have developed competitive strategies such as production of anti-bacterial metabolites. Native microbial products at a specific site would affect survival and activity of the introduced engineered microbes, including the natural population. Knowledge of these metabolites will both improve the design of the microbes and may also provide a better understanding of changes in the ecosystem. Critical information on these effects can be obtained from microcosm or small-scale field experiments, and data should be collected in phases from the lab to the field in order to evaluate predictive models. Development of models will be especially critical when engineered microbes are intended for use in multiple areas or in a broad area that may include multiple microbial-scale ecosystems.

There are tools available for monitoring and detection of intentionally released engineered microbes. DNA sequencing of environmental samples using next generation sequencing can provide knowledge of the existing genes at a site. These methods, combined with increased annotation of genetic information and detection of DNA markers/barcodes, can provide useful information on the survival of the introduced microbes and can provide data on changes in the microbial ecosystem. Although these methods detect DNA, including DNA that is part of an engineered pathway, they cannot determine if that DNA remains in an engineered microbe or has been incorporated into a native organism (or persists outside of a cell). As discussed above, genetic containment methods are under development, but require more research to become reliable. The possibility of gene transfer has been studied for many years, but key questions still remain. These include the probability of chromosomal integration of introduced DNA and factors that affect this probability, such as nutrient levels in the environment and competence factors for different strains of bacteria. Genomic differences and cellular factors affecting gene transfer among native bacteria are difficult to study because most microbes are largely unknown or not sequenced, and cannot be cultured in the lab. Furthermore, the impact of gene transfer on the recipient microbes is not clear. It is believed that, in most cases, the engineered genes are likely to confer a selective disadvantage for the recipient and will be purged from the population over time; however, potential impacts will have to be evaluated on a case-by-case basis. As discussed above, changes in microbial ecology at highly polluted sites may be desirable.

Advances in monitoring and detection of accidental releases of engineered microbes will also be necessary. Currently, engineered microbes are predominantly located in governmental and academic research laboratories or commercial production facilities, and are securely maintained in physical containment systems (e.g., bioreactors) with safeguards in place. An accidental release at such an institution is not likely, but could be significant. Engineered microbes, albeit with less complexity and at smaller scales, are increasingly produced by the Do-It-Yourself Biology (DIYBio) community[1] in facilities with variable oversight that could also produce accidental releases. DNA sequencing of environmental samples can be used to track releases, but the lack of environmental baselining may make it difficult to detect an engineered DNA sequence. More data on natural microbial ecosystems would aid monitoring efforts.

3.2.4 Safety and regulation

Engineered microbes will be regulated in the U.S. depending on their intended use. If they are developed as a therapeutic (e.g., an engineered gut microbe, Synlogic 2017; Garber 2015), then it will be regulated by FDA. Microbial pesticides will be regulated by EPA under its pesticide authorities (the Federal Insecticide, Fungicide, and Rodenticide Act). Other types of engineered microbes developed for commercial use, including microbes developed for bioremediation, will be regulated by EPA under the Toxic Substances Control Act (TSCA) (OSTP 2017). If the microbe produces a new chemical, then the EPA will separately regulate that chemical as well. In its current review process, the EPA considers all potential uses of a microbe and, if necessary, issues a "significant new use rule" under which it can impose new safeguards or restrictions on the developer for uses not initially proposed.

One challenge the regulatory system may face in the near future is its ability to keep pace with the speed at which new bioengineered microbial strains and compounds are developed. The current regulatory framework has been adequate to date, the numbers of applications and products has increased at a manageable rate for the EPA. As high-throughput synthetic biology approaches become more widely used and development times

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[1] The DIYBio (Do-It-Yourself Biology) community refers to those practicing biology outside of the traditional academic or commercial institutions. It includes hobbyists, amateurs, students, and sometimes trained scientists, often using shared community laboratory facilities (Grushkin et al. 2013).

become faster, the EPA and other regulatory agencies could be overwhelmed, and the review process could slow considerably (NASEM 2017a; Carter et al. 2014).

The lack of comparators for risk assessment presents another challenge for the regulation of engineered microbes. To date, the EPA has been able to conduct risk assessments on engineered microbes by comparing them to naturally occurring microbes and previous technologies. However, future engineered microbes may incorporate increasingly novel traits (e.g., synthetic genetic elements, unusual chassis, and non-natural nucleic acids or amino acids). For previous generations of engineered microbes, the "host" organism for the engineered DNA construct has been clear, but newer engineered microbes may combine critical components from many species. Data requirements for such products may be more rigorous than for previous technologies. Early engagement with regulators at EPA will help identify critical questions and knowledge gaps for specific engineered microbes.

The value of current biocontainment measures, such as auxotrophic systems, kill switches, and (even further into the future) orthogonal biology, remains unclear as they are still under development. However, if and when they are fully successful (i.e., are shown to adequately reduce the microbe's persistence in the environment and/or horizontal gene transfer), the EPA may consider them as an appropriate containment measure. Under the TSCA, there are some exemptions for well-characterized microbes that contain well-understood DNA constructs and are used in contained systems. Similar exemptions could be considered, far in the future, for adequately biocontained microbes, should such technologies prove operational. Such exemptions would, however, require new formal rule making, which can be a time consuming process.

3.2.5 Community engagement

The decision to deploy an engineered microbe in the environment should be transparent and should incorporate actionable input from community members. The focus of this engagement should be on defined applications and uses of engineered microbes and issues of concern such that specific risks, benefits, and concerns can be articulated and discussed. This process will require interdisciplinary teams that include experts in community engagement, including early and frequent collaboration and communication with stakeholders outside of the development team. The

range of stakeholders will be broad and will include local communities, funders, regulators, policy makers, and others.

3.3 Case study: cell-free technologies for advanced chemical production

3.3.1 Introduction

For the purposes of this workshop, synthetic cell-free technologies were defined as the suite of synthetic biology technologies that allow for the exploitation of transcription and translation systems outside of the cell. Many cell-free technologies are under development, including paper-based cell-free systems for detecting chemical threat agents or pollutants (e.g., Ma 2013) and for on-demand chemical synthesis using cell-free protein production systems (Carlson et al. 2012). These tools can be compact in size, fitting on a small piece of paper, and can rapidly analyze the environment for specific target molecules or produce chemicals of interest. The workshop case study included several possible applications of cell-free systems to best generate discussion (see Appendix C).

Cell-free systems may present a unique opportunity to serve as a proving ground to identify and answer foundational questions around hazard exposure, risk, and public perception of synthetic biology. Many of the safety and efficacy questions that stakeholders may have about complex synthetic biology technologies, such as engineered microorganisms and gene drive engineered insects, can be addressed empirically using simpler cell-free systems. Cell-free tools may enable researchers to make certain determinations much more quickly than they could in a living cell, although direct comparisons require evaluation. Cell-free systems are likely to face fewer regulatory restrictions because they are not living. Additionally, engagement with the public and other stakeholders for deployment of cell-free systems may be simpler than for other synthetic biology organisms and components.

3.3.2 Horizon scanning

State-of-the-art cell-free technologies include paper-based gene circuits and cell-free manufacturing reactors. In general, paper-based gene circuit tools incorporate cell-free extracts that power a gene-based indicator. The cellular components for a detect-and-report system are freeze-dried on a porous medium (paper) and, once hydrated, "boot up" the genetic

circuitry. After a few hours, the circuitry has created enough detectable product (i.e., protein, RNA, or other macromolecule) to indicate the presence or absence of a specific target. Current gene circuits are relatively simplistic, employing "if this, then that" logic with simple colorimetric reporters (e.g., green fluorescent protein). For example, Pardee et al. (2016) describes a paper-based Zika virus diagnostic tool that detects 24 different Zika RNA sequences, turning the paper from yellow to purple. In the future, paper-based diagnostic gene circuits will likely be more elaborate with multi-step logic circuits that are more sensitive to the target molecules, and that have more rapid reporting times and more vivid indicators. In addition, other matrix materials are currently being studied, including cloth, silk, hydrogel beads, plastics, and wax-printed channels on glass. Further into the future, living cells might be included in the hydrating agent to make the diagnostic tool capable of more complex detection, thus providing improved read-outs.

Paper-based systems are based on either whole cell extracts or pure cellular components. Systems utilizing pure cellular components are very stable, but are very expensive due to the process required to obtain the purified components. Whole-extract systems, on the other hand, are inexpensive to make but are less stable, in part, because proteases present in cellular extracts may degrade important machinery. The stability of whole extracts may be increased by using alternate matrices, listed above. For example, a three dimensional (3-D) assay may incorporate channels that move material selectively and allow for components to be added in a multi-stage fashion.

Cell-free manufacturing reactors are currently in use and under development for a variety of products, including targeted bio-pesticides, drugs, and systems for complex chemical synthesis (e.g., GreenLight Biosciences 2017). This type of manufacturing is conducive to both macro-scale production in a manufacturing facility (i.e., bioreactors) and micro-scale production in a field-deployed situation. In both cases, very little DNA is required to make these systems functional. Cell-free systems also have the potential to be used in other consumer products, such as customized face-creams and bioluminescent lip balm.

One key advantage of cell-free systems is that they are light-weight and more conducive to transport compared to other technologies. Active pharmaceuticals or chemicals needed in a remote location may be easier

and more cost effective to create onsite via cell-free methods than to transport, avoiding the complex logistics required for equipment security, component stability (e.g., refrigeration), and reliable power supply. Cell-free systems also offer shorter development timelines and greater modularity, this is likely to enable a wide range of applications to be developed by a wide range of actors, including the DIYBio community. These advantages may also, however, provide opportunities for nefarious uses, such as small-scale production of toxins or narcotics, or the transport of benign components across borders for later incorporation into or manufacturing of harmful products. It is also possible that cell-free systems may have security gaps, such as components that can be sabotaged by viruses or other exogenously applied DNA.

There are many limitations for paper-based diagnostics that must be resolved, including target diversity, reliability, sensitivity, stability, and speed. Development of recognition elements is still quite cumbersome, and all sensors must be designed for specific, known chemicals or biomolecules. Cell-free sensors are best suited for screening (i.e., environmental analyses or other high-throughput applications with many samples), and are not currently reliable or sensitive enough to use as confirmatory diagnostic tools for human health. Another challenge is that cell-free diagnostic readouts are largely qualitative, with precise quantification requiring sophisticated techniques such as mass-spectrometry analysis of well-defined and purified extracts. Lastly, current cell-free gene circuits take 90–120 minutes for optimal read-outs, and although there are techniques to reduce this time slightly, it is still too slow for many health or sensor applications. It remains to be seen if cell-free systems mimic the functionality of the organism from which they are derived, or if differences exist in biological activity. Cell-free systems are a new and emerging technology, with highly active research and development efforts underway. These efforts will likely address many of the challenges outlined above and bring this technology into wider use in the near future.

3.3.3 Environmental impact

There are likely to be many cell-free systems that are developed and deployed because of their ease-of-use and their potential for relatively low regulatory hurdles (particularly for environmental applications, as discussed below). The environmental impact of cell-free systems is likely to be less than that of other synthetic biology technologies (e.g., engineered viruses, bacteria, or gene drive engineered organisms) because

of the lack of self-replication, the minute amount of cell-free material used in each product, and the low likelihood of components interacting with living systems.

Despite the anticipated low environmental impacts of cell-free components, potential impacts still require investigation. Many of the environmental risks posed by cell-free systems are also posed by other synthetic biology technologies. For example, the DNA component of a cell-free system could transform native organisms via horizontal gene transfer (transformation or transduction) and could provide new functionality to the unintended host, potentially disrupting an ecological balance. The impact of such an event would depend on the function encoded by the cell-free system DNA (e.g., DNA encoding antimicrobial or cell lysing proteins may kill the transformed host). Engineered controls embedded in the DNA, such as irregular codons or artificial promoters not found in natural organisms, may limit genetic transfer, integration, and expression. Other strategies include tightly binding DNA to the matrix to prevent uptake by other organisms, engineering designs that result in the rapid degradation of system components if released from the matrix, or using DNA constructed using non-natural nucleic acids that cannot be easily incorporated into or replicated by native flora.

Cell-free manufacturing applications may pose some environmental hazard if they are designed to produce a hazardous end product, although these pose similar concerns to traditional chemical manufacturing. If the cell-free components escape containment in a form that remains functional, a potential would exist for those toxic substances to continue to be manufactured and released directly into the environment. The waste from producing cell-free extracts, rather than the extracts themselves, may also be an environmental hazard (again, this hazard may be similar to hazards posed by traditional chemical manufacturing).

Multiple studies are needed to qualify and quantify these risks. The following lab-scale and field-scale studies are needed to characterize: 1) the likelihood of horizontal gene transfer, 2) the efficacy of built-in biocontainment mechanisms as safety controls (e.g., programmed cell lysis if native organisms acquire genetic components from cell-free systems), and 3) the quantification of viral replication in cell-free extracts. Field trials could be conducted in facilities with controlled experimental chambers (e.g., mesocosms).

Many of the questions about environmental impacts have been identified previously (such as those pertaining to stability and horizontal gene transfer of genetic material), but have not been answered empirically due to limited funding and a lack of prioritization by funding agencies. There are models for tracking fate and transport of genetic material in the environment, but evaluation of these methods and empirical data is limited (Furlan et al. 2016). Cell-free systems may serve as excellent tools for measuring environmental impacts related to synthetic biology organisms and components before deploying more complex technologies in the environment. For example, cell-free systems could be used to study gene flow and the uptake rate of free DNA in the environment. Other data derived from cell-free studies could be used to inform models developed to assess the impacts of synthetic biology organisms.

3.3.4 Safety and regulation

Laboratory biosafety requirements for cell-free systems are similar to those for other biochemical and biotechnological facilities and protocols. Within academia, research in cell-free synthetic biology is typically overseen by IBCs under the NIH Guidelines. For commercial products developed with cell-free systems (e.g., paper-based diagnostics, manufactured specialty chemicals, etc.), current regulations in the U.S. are likely to provide adequate oversight. For diagnostics and other health-related applications, regulatory oversight will be provided by the FDA, with any necessary environmental assessment performed in compliance with the NEPA (see gene drive discussion summary). Other cell-free systems and components with novel genetic arrangements, including those intended for use in the environment, are likely to be regulated by the EPA under the TSCA as new chemicals. Internationally, restrictions on transporting "living modified organisms" across borders are not likely to apply to cell-free systems, allowing easier deployment of these systems compared to living synthetic biology organisms.

3.3.5 Community engagement

Relative to other synthetic biology organisms and components, cell-free systems might not pose as great a challenge for community engagement as these systems can be seen as incremental advances over currently used technologies (such as home chemistry sets or pregnancy tests). Furthermore, because these systems do not contain living organisms, public concerns about environmental impacts and the uncertainties

around those impacts may be reduced. Community engagement can help ensure that cell-free systems are pursued in ways that are welcomed and embraced by the public, but the level of engagement required for cell-free systems may not be as in-depth as that required for living synthetic biology organisms, such as gene drive engineered organisms.

3.4 Viral systems for water treatment

3.4.1 Introduction

Viruses are 'semi-living' entities composed of single or double-stranded DNA or RNA surrounded by a protein capsid. They function by infecting a host, harnessing host cellular machinery for replication, and then releasing new viruses. Although there many different types of viruses that infect a variety of host cells, the focus of the workshop was on bacterial viruses, also called bacteriophages (phages). Phages contain highly compact genomes ranging from 10^4–10^5 nucleotide base pairs and constitute the most diverse genetic entities on Earth. There can be as many as 10^7 viral particles in one milliliter of sea water, an order of magnitude larger than marine microbes (Parsons et al. 2012).

Viruses are an appealing system for engineering, as they are relatively easy to work with, well-studied, and can transfer genetic material into a host genome with varying degrees of specificity. Viruses have been used for many industrial purposes, including medical, agricultural, and veterinary, including the production of novel materials. The workshop case study focused on the deliberate release of engineered phages through wastewater or as a result of wastewater treatment to inactivate high consequence pathogens. In this context, "wastewater" includes sewage, storm water, precipitation run-off, firefighting run-off, and other ways that an aqueous solution of virus can be generated and potentially enter the environment (see appendix C).

3.4.2 Horizon scanning

Phage therapy (the use of phages to kill harmful bacteria) predates the use of antibiotics. In western countries, including the U.S., there are some approved phage-based agricultural products in addition to ongoing human clinical trials using phages (Vandenheuvel et al. 2015; Parracho et al. 2012). All of these products use cocktails of natural phage isolates cultured in traditional large-scale fermenters. Advances in technology over the last

decade, including next generation sequencing, droplet microfluidics, single-cell-omics, protein design, receptor docking and biogeochemical modeling/bio-cycling, have revolutionized the study and understanding of phages. DNA synthesis, genetic editing, cell culture systems, and transformation protocols have all advanced to the point that phage engineering has become a common laboratory practice.

Despite these technological gains, there are several challenges remaining for those that work with and engineer phages. A better understanding of the interactions between phages with their microbial hosts is needed, including the interaction of phage genetic material with the bacteria. Another need is a greater characterization of viral and bacterial communities (i.e., viral- and microbiomes) and studies of community dynamics. In order to gain this level of understanding, phage propagation is critical, but that in itself a challenge. Not only is there limited knowledge as to what comprises the microbial communities in wastewater, but the ability to isolate and culture non-model organisms under laboratory conditions is also lacking. Beyond simple aquatic, terrestrial, or aerosolized environments, complicated biofilms and microbial mats with three dimensional and asymmetrical variation present unique challenges to studying viral dynamics. Research has focused on ways to improve or circumvent host cell culturing, including the development of cell-free systems to produce phages. Engineering bacterial hosts for expanded range, developing mixed cell culture systems that more closely mimic natural environments, and increasing the number of microbial host strains that can be grown in the laboratory are ongoing efforts.

Phages have a complex life cycle that is poorly understood. They infect bacteria through interactions between viral capsid proteins and bacterial cell surfaces. Capsid proteins often target conserved cell surface regions (e.g., receptors or lipid rafts) of target bacterial hosts. Many current research efforts are focused on modifying these receptors, either to expand the phage host range or to more precisely target a cell type. Once inside the cell, questions remain about both the efficiency of incorporation of viral genetic material into host genomes and the process of phage-mediated cell lysis. By understanding, engineering, and optimizing these factors, a variety of commercial phage-based applications may be developed in the future.

The ability to monitor and contain engineered phages after release into the environment is critical. One approach currently under investigation is the incorporation of kill switches into engineered phages. Like bacterial kill switches, these genetic components can trigger cell death or halt reproduction (or other metabolic activity) in the presence of an external stimulus, such as pH, temperature, or the addition of an enzyme or chemical. There are many ongoing research efforts to identify pathways to improve kill switch efficiency and to develop alternative biocontainment methods for phages. The same challenges face biocontainment of viral systems as do bacterial systems discussed in the engineered microbes for bioremediation case study, above.

3.4.3 Environmental impacts

Phages impact the environment in a variety of ways, each of which are habitat-specific and should be evaluated on a case-by-case basis. Prior to the release of engineered phages, characterization of the complex and dynamic ecological interactions in the natural environment is critical. Endpoints of concern should be established so that monitoring efforts can be directed toward meaningful data. Phased testing may provide a better understanding of how introduced phages may interact with native wild-type organisms and ecological processes, and provide relevant data for modeling efforts.

The treatment of wastewater (the case study discussed here) is a likely use of engineered phages, although such an application would still require substantial development and controlled testing. For example, testing could be conducted on a laboratory- or pilot-scale and follow the typical protocol for wastewater treatment: 1) large particles are settled out naturally, 2) microbes degrade contaminants, and 3) advanced treatment including chemicals, filtration membranes, disinfection or elimination of microbes. This approach allows phages to be investigated in a contained setting and critical comparisons could be made, such as determining if modified organisms become more resistant to typical disinfection processes.

Many environmental concerns surrounding the use of phages in the environment mirror those of industrial use and release of chemicals or use of pesticides. Essential considerations for understanding environmental impacts include the persistence of phages over time, the likelihoods of phage infection in new or unexpected bacterial strains or species, and the potential for unintended toxicity (due to, for example, endotoxin release

from cell lysis). When using phages for wastewater treatment, assessments should be made of the potential for phage transport into and persistence in downstream water bodies (e.g., irrigation systems and wastewater byproducts). Aerosolization and transfer of phage to aquatic organisms should be evaluated (Withey et al. 2005). Potential exposure of humans to engineered phages should also be considered (although phages do not infect human cells, they may find suitable hosts in the human microbiome). In all cases, it will be important to develop models and evaluate them by gathering meaningful data from laboratory experiments and phased releases.

While some environmental concerns surrounding the use of engineered phages are similar to those associated with industrial chemicals, engineered phages present unique challenges, particularly in regards to their ability to both replicate and mutate. Mutations are usually deleterious and lead to non-viable viruses, yet occasionally can result in novel properties. Some of these mutations have been shown to expand the host range, introduce novel virulence factors, or decrease phage susceptibility to neutralization (e.g., via ultra-violet (UV) light, cold, or desiccation). Mutation rates vary both among viruses and among host bacterial strains. RNA viruses, in particular, benefit from high mutation rates that promote rapid adaptation. Mutations and the emergence of fitness-enhancing traits in phages can be extremely difficult to detect and model. Gene transfer mechanisms, including horizontal gene transfer (movement and incorporation of DNA segments among viruses and bacteria), can also confer new capabilities on phages and surrounding microbiomes. One particular concern, among others, is the introduction of DNA encoding pathogenic traits into a new host through horizontal gene transfer, followed by increased virulence in a previously non-virulent species. Methods for studying horizontal gene transfer have not been standardized, due in part to the lack of understanding of how it occurs. Basic research in this area is required.

Accidental and unforeseen risks (e.g., natural disasters that damage containment infrastructure) should also be considered when evaluating potential environmental impacts. Product developers should be encouraged to develop worst-case scenario plans, and include genetically engineered biocontainment strategies when possible. Such strategies could, for example, focus on impaired replication and/or reproduction. Disaster mitigation plans may also include materials specifically designed

to remove viruses from the water system, such as selective absorption filtration systems that use membrane-bound bacteria or specialized nano-materials.

3.4.4 Safety and regulation

Engineered phage products have yet to be addressed by U.S. regulatory agencies, but will be regulated based on their intended use. There are examples of non-engineered phage products that have been approved use for various applications in the U.S. For example, a cocktail of non-engineered phage isolates is used to treat *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections, and is regulated by the FDA (Kingwell 2015). Future engineered phage therapeutics for humans will be similarly regulated by the FDA under its human drug provisions. Likewise, engineered phages used as pesticides will be regulated by EPA under its pesticide provisions (similarly to AgriPhage, a non-engineered pesticidal phage cocktail). Engineered phages for wastewater treatment (the case study presented here) would be regulated by the EPA under the TSCA, this requires a pre-market review for engineered microbes (including those intended for environmental release). Researchers should engage with regulators early on to ensure that the regulatory agencies can anticipate upcoming products and that planning and experimentation is aligned with current regulatory standards. Development of standardized forms and questions for environmental risk assessment that are more relevant to a wider range of synthetic biology technologies would also help researchers anticipate regulatory needs.

A variety of non-regulatory mechanisms also contribute to biosafety and appropriate use of engineered phages. Trainings for researchers on safe use of engineered phages (above and beyond that required by the NIH Guidelines) could also contribute to overall biosafety. Regulations are legal requirements, but companies also comply with environmental risk mitigation measures aimed at reducing legal liabilities, as well as maintaining trade secrets. For example, insurance for wastewater facilities using engineered phages will likely be simpler for those including well characterized containment mechanisms.

In order to enhance safety and regulation for deployment of engineered phages there is a need to advance the science that guides regulation. For example, better detection capabilities are needed for phages, including rapid-result, field-deployable platforms. Ecologically-relevant animal

models are also needed in order to determine potential impacts of engineered phages on native animal species and their microbiomes. The potential role of animal vectors in uncontrolled phage dispersal is also an important research gap. In addition, studies should evaluate the limitations of laboratory or caged trials in fully capturing phenomena that occur at larger scales, and aim to develop more powerful methodologies for test chamber or mesocosm studies. Models that integrate data and information from multiple sources will also be critical.

3.4.5 Community engagement

As with any new and unfamiliar technology, engaging with the public early and providing information in an understandable way will help ensure that the decision to deploy engineered phages (or not) is made responsibly. Key challenges in community engagement include identifying the stakeholders that should be involved and finding the right communicators. Frequently, scientists are not trained in communications or stakeholder engagements with non-technical audiences, therefore, product developers should team with appropriate experts. In all cases, the benefits and risks of deployment of synthetic biology technologies, including the uncertainties of both, should be clearly articulated.

4 Conclusion

One theme that provided a key foundation for the workshop and resonated in each of the discussions about interactions between synthetic biology organisms and components and the environment was the need for research to support environmental risk assessment and regulatory decision-making. Such research will be critical to the development, testing, and deployment of synthetic biology technologies. Given the level of investment in synthetic biology and its applications by the DoD and the broader U.S. government, commensurate investments in environmental and regulatory science may be warranted. Indeed, one of the recommendations from the NASEM report on Future Products of Biotechnology (NASEM 2017a) is that those U.S. government agencies that fund advanced biotechnology should also allocate funds for advancing regulatory science. By addressing some of the research needs described in Section Two of this report, ERDC and DoD can set an example for responsible development of synthetic biology technologies.

Many technical hurdles identified at the workshop, such as the need for improved control and modeling of engineered organisms, and surveillance and monitoring tools, have been identified previously (NASEM 2016; NASEM 2017a; Drinkwater et al. 2014; Carter and Friedman 2016). DARPA's Safe Genes program is working to overcome some of these hurdles for gene drives (DARPA 2017), and the Intelligence Advanced Research Projects Activity (IARPA) Finding Engineering-Linked Indicators (FELIX) program aims to develop tools for the identification of genetically engineered organisms in the environment (IARPA 2017). The need for more information on natural environmental processes, such as horizontal gene transfer in microbial communities, has also been highlighted in earlier reports (Drinkwater et al. 2014). Many of these research needs, particularly for environmental baselining and potential environmental interactions of synthetic biology organisms and components, have not been prioritized and remain under-funded.

There is a strategic opportunity to meet these technical needs, not only for development and deployment of synthetic biology organisms and components, but also for a wide range of U.S. government goals. For example, more effective and efficient methods for monitoring and surveying the environment for engineered organisms or components, or for characterizing ecological communities pre- and post-deployment, will

be important for fielding synthetic biology technologies. These efforts could also support basic research (e.g., National Science Foundation (NSF)'s National Ecological Observatory Network), public health efforts for tracking the spread of vector-borne diseases (e.g., the Global Emerging Infections Surveillance system) and/or antibiotic resistance (PCAST 2014), detection of inadvertent or nefarious biosecurity threats (PCAST 2016), efforts to address invasive species (NISC 2017), and characterization of ecosystems and ecosystem services (PCAST 2011, USGEO 2016). Monitoring efforts already underway within the U.S. government could be adapted and coordinated to better serve these multiple purposes. Improved control of the persistence and spread of genetically engineered organisms and components will yield benefits not only for potential environmental applications, but also for medical advances and countermeasures (DARPA 2017; PCAST 2016).

Many of the common themes identified at this workshop and in other venues address the critical need for regulatory and community engagement before, during, and after development and deployment of synthetic biology organisms and components. Case-by-case evaluation of products and environmental risk assessment have been hallmarks of the U.S. Coordinated Framework for the Regulation of Biotechnology since it was established in 1986 (OSTP 2017). Even so, these newer products raise well-described challenges for the U.S. regulatory system, with options and recommendations available (NASEM 2017a, Carter et al. 2014). The need for broader community and stakeholder engagement has also been identified repeatedly, especially for more complex engineered organisms, such as those that contain gene drive constructs (NASEM 2016; Carter and Friedman 2016). Establishing research priorities for synthetic biology organisms and components, including research providing the basis for environmental risk assessments, should be done in a coordinated way that best supports regulatory and community engagement needs. International perspectives should also be included where international deployment of synthetic biology technologies is either intended or may be possible. The U.S. State Department is active in multilateral fora where synthetic biology is a topic of interest, including the Convention on Biological Diversity and the Biological Weapons Convention.

One challenge that the U.S. government faces with the development of synthetic biology organisms and components is the dual-use nature of the technology. Biosecurity risks were not the focus of this workshop, but have

been topics for other meetings, workshops, and publications (PCAST 2016; Regalado 2016; NSABB 2010; NASEM 2017b). However, there is a repeated emphasis, even within the biosecurity and biodefense communities, on promoting innovation and ensuring that the benefits of synthetic biology can be harnessed (including development of countermeasures and other applications that may improve security). As these technologies are developed and research is prioritized, it will be important to include biosecurity and biodefense perspectives.

The wide-ranging applications and the promise of synthetic biology organisms and components will require a strategic and cross-disciplinary U.S. government approach to ensure that they are developed in a way that meets multiple objectives. Such an approach should include prioritizing research that underpins environmental risk assessment of the technologies, integrates with other relevant research and surveillance efforts, supports regulatory decision-making, limits the potential for unintended and nefarious use, and is guided by community and stakeholder needs.

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Appendix A: Acknowledgements and Workshop Attendees

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Name	Affiliation
Bryn L. Adams (Chair, Organizer)	Army Research Laboratory
Robert Bastian	Environmental Protection Agency
Blake Bextine	Defense Advanced Research Projects Agency
Camilla Beech	Cambea Consulting Ltd
Steve Bradbury	Iowa State University
Patrick Boyle	Ginkgo Bioworks
Kathleen Buckley (Organizer)	Oak Ridge National Laboratory
Peter Carr	Lincoln Laboratory
Catherine Cabrera	Lincoln Laboratory
Sarah R. Carter (Chair, Organizer)	Science Policy Consulting
Jessica Coleman	Engineer Research and Development Center
James Comolli	Lincoln Laboratory
Fiona H. Crocker (Chair, Organizer)	Engineer Research and Development Center
Jessica Cussins	Harvard University
Brinda Dass	Foundation for the National Institutes of Health
Jason Delborne	North Carolina State University
Jed Eberly (Organizer)	Montana State University
Peter Emanuel (Chair)	Edgewood Chemical Biological Center
Kevin Esvelt	Massachusetts Institute of Technology
Megan Frisk	Department of State
Christy Foran	Engineer Research and Development Center
Clem Fortman	Office of the Secretary of Defense
Justin Gallivan (Chair)	Defense Advanced Research Projects Agency
Cpt. Derese Getnet	Army Center for Environmental Health Research

Name	Affiliation
Nicholas Guido	Lincoln Laboratory
JC Gutierrez	Synlogic
Geoff Hosack	Commonwealth Scientific Industrial Research Org.
Todd Kuiken	North Carolina State University
Rick F. Lance (Chair, Organizer)	Engineer Research and Development Center
Jeantine Lunshof	Harvard University
Matthew L. Magnuson (Chair, Organizer)	Environmental Protection Agency
David Martin (Organizer)	Engineer Research and Development Center
Stefani McGregor	Water Environment Research Foundation
Heather N. Meeks (Chair, Organizer)	Defense Threat Reduction Agency
Paul Miller	Synlogic
Richard Murray	California Institute of Technology
Lola Olabode	Water Environment Research Foundation
Megan Palmer	Stanford University
Pamela Peralta-Yahya	Georgia Institute of Technology
Edward J. Perkins (Organizer)	Engineer Research and Development Center
Rebecca Phillips	Environmental Protection Agency
Kaytee Pokrzywinski	Engineer Research and Development Center
Vatsan Raman	University of Wisconsin
Jay R. Reichman	Environmental Protection Agency
Melissa Rhoads	Lockheed Martin
Taylor Rycroft	Engineer Research and Development Center
Royden Saah	Island Conservation
Mark Segal	Environmental Protection Agency
Zach Smith (Organizer)	Ginkgo Bioworks
Zach Sun	Synvitrobio
Chris Sund	Army Research Laboratory
Jessica Terrell	Army Research Laboratory
Karen Tountas	Foundation for the National Institutes of Health
Scott Walper	Naval Research Laboratory
David Walsh	Lincoln Laboratory
Chris M. Warner (Organizer)	Engineer Research and Development Center
Renee Wegrzyn (Organizer)	Defense Advanced Research Projects Agency

Appendix B: Workshop Agenda

Time	Agenda: Wednesday, May 17th 2017	Who's Responsible
8:00	Welcome and Workshop Overview, goals and purpose	Ed Perkins (ERDC)
8:40	Facility and logistics overview	Catherine Cabrera (LL)
9:00	Preparing for Future Products of Biotechnology	Richard Murray (Caltech)
9:30	Industrial Synthetic Biology	Patrick Boyle (Ginkgo Bioworks)
10:00	*Break Snacks and Coffee provided by LL*	
10:15	The Promise and Perils of Synthetic Biology	Peter Emanuel (ECBC)
10:45	Synthetic biology and scientific risk assessment	Geoff Hosack (CISRO)
11:15	Breakout participant Introductions	
12:00	*Lunch provided by LL*	
1:15	Breakout session charge: Description of task and roles/responsibilities	Chris Warner (ERDC)
1:30	Breakout Session 1: Horizon scanning of current and near future technologies	All
4:00	Present Day 1 Breakout Session Findings (5 min each group plus discussion)	4 Group leaders
4:30	Day 1 Debrief	(ERDC)

Time	Agenda: May 18th 2017	Who's Responsible
Evening	Activities TBD or on your own	
Time	Agenda: May 18th 2017	Who's Responsible
8:00	Arrival and check in	
8:15	Introduce today's activities and recap yesterday	Chris Warner (ERDC) and Catherine Cabrera (LL)
8:30	Toxic Substances Control Act and Genetically Engineered Microorganisms; EPA Responsibilities for Pesticide, Synthetic Biology Applications	Mark Segal (EPA)
9:00	Charge Session 2: Environmental Impacts	(ERDC)
9:15	*Break*	
9:30	Breakout Session 2: Environmental Impacts	All
11:30	Present Breakout Session 2 Findings	
12:00	*Lunch*	
1:00	Charge Session 3: Safety and regulation	(ERDC)
1:15	Breakout Session 3: Safety and regulation	All
4:15	Present Breakout Session 3 Findings	
4:40	End of day recap	(ERDC)
Evening	Activities TBD or on your own	
Time	**Agenda: May 18th 2017**	**Who's Responsible**
8:00	Arrival and check in	

8:15	Introduce today's activities and recap yesterday. Charge Session 4: Social License	(ERDC)
8:30	Breakout group 4: Social License	All
10:15	Present Breakout Session 4 Findings	
10:30	*Break*	
10:45	Charge Session 5: Define challenges & capabilities	(ERDC)
10:50	Breakout Session 5: Construct Road map for the path forward	All
11:45	Present Breakout Session 5 Findings	
12:30	Debrief	

Appendix C: Case Studies for Discussion

C.1 Gene drive case study

C.1.1 Background

A company has developed an engineered *Aedes aegypti* mosquito to combat dengue, Zika, and other mosquito-borne diseases. It includes an engineered trait that prevents female embryos from developing into adults and a CRISPR-based gene drive mechanism that results in nearly 100% of offspring inheriting this trait. Caged trials of these mosquitoes have been successful. Modeling suggests that release of a few of these gene drive mosquitoes will cause a dramatic decrease in the number of *A. aegypti* mosquitoes. As the gene drive mosquitoes spread among geographical areas, there is the potential for all *A. aegypti* mosquito populations worldwide to be decimated.

C.1.2 Hypothetical scenarios

What if the gene drive mosquito was engineered to have self-limiting population spread, either geographically or temporally?

What if modeling suggested that the subpopulation of modified mosquitos would die out before it spread more than five miles?

What if other limiting strategies were pursued?

What if a similar mosquito was developed for use in non-populated areas for conservation purposes (e.g., a Culex mosquito to combat avian malaria in endangered birds)?

What if the mosquito was developed by a different type of entity rather than a company (non-profit or non-profit consortium, public health agency, military, mixed funding)?

What if a similar gene drive were to be developed in mice for pest management, or weeds for agricultural purposes?

C.2 Microbial breakout session case studies

C.2.1 Bioremediation case study

Chemical contamination of the environment with xenobiotic compounds and products is a liability to current and future generations. In support of managing environmental resources for wildlife and training activities, DoD is conducting risk analyses on a variety of inorganic and organic emerging contaminants that are used in paints, electronics, fuels, explosives, flame retardants, deicers, and plastics. In addition, research to identify and provide remedial solutions to environmental contamination on military lands is ongoing.

Pseudomonas putida KT2440 is a very suitable host for synthetic biology applications, since its metabolic networks have been analyzed via flux based analysis and a dedicated set of molecular tools and genome editions are available. It is an important strain with many industrial and environmental applications since it has multiple pathways for the biotransformation of aromatic, chloro- , nitro-organic compounds, and produces polyhydroxyalkanoates etc for bioplastic production.

Current example: The obligate aerobic life style of strain KT2440 has been overcome in a recent example of engineering the strain to degrade a chlorinated xenobiotic under anaerobic conditions (Nikel and de Lorenzo 2013). Since strain KT2440 lacks an anaerobic electron transport chain and fermentative metabolism is impaired, the ability of the strain to generate ATP by substrate level phosphorylation was engineered into the strain by functionally complementing the native activity of the phosphotransacetylase enzyme (Pta) by adding the acetate kinase A enzyme (AckA) from *E. coli*. While improvements to the energy charge and ATP/ADP ratios were obtained, further adjustments to the strain's ability to manage the NADH/NAD+ couple were necessary. This was achieved by the addition of the pathway for ethanol synthesis from *Zymomonas mobilis* (pyruvate decarboxylase (Pdc) and alcohol dehydrogenase II (AdhB)). The recombinant strain carrying both AckA and Pdc-AdhB was metabolically active under anoxic conditions (96 h), but still unable to grow. The pathway for dechlorination of 1,3-dichloropropene (*dhA, caaD1,* and *caaD2*) was cloned from *Pseudomonas pavonaceae* 170 as a single transcriptional unit into the recombinant strain of KT2440. Under anoxic conditions nearly complete degradation of 1,3-DCP occurred and > 50% of the cells appeared to maintain physiological activity after 96 h. The

example given provides a rational design approach for the heterologous expression of genes from three different species.

For this exercise consider the deliberate release of an obligate aerobe engineered in a similar manner for the bioremediation of 1,3-dichloropropene in soil on a military installation. Also, consider the following as possibilities in engineering the strain: (1) the strain is metabolically active but incapable of growth under anaerobic conditions; (2) the strain is capable of growth under anaerobic conditions; (3) one of the plasmids is conjugative; (4) genes were introduced into the chromosome instead of on 1 or more plasmids.

C.3 Cell-free breakout session case study

C.3.1 Purpose

Synthetic biology offers novel ways to utilize cellular machinery. Breaking apart from cellular barriers while using cellular components introduces new challenges to risk assessment and technology development. The purpose of the cell-free breakout session is to discuss the development of these applications and identify major hurdles that are not identified in traditional risk assessment frameworks. Specifically, environmental release and ecological impacts that may affect the implementation of these technologies will be evaluated.

This session will consider possible impacts of introducing cellular components into naïve environments either intentionally or incidentally. Participants are encouraged to propose likely release scenarios based upon the particular application describes below, or of their own conception. Impacts could include added benefits, unintended consequences (whether beneficial or detrimental), and other effects which are actually or seemingly benign. To help frame discussion and analyses, a number of questions are posed following the "Background" section.

C.3.2 Background

Historians propose chemical production using cellular fermentation was one of the major drivers for mankind's shift from a nomadic to an agrarian lifestyle. The biotechnology revolution in the 1970s advanced fermentation technology to express heterologous proteins through recombinant DNA. Cells exquisitely draw resources and transform energy into useful

chemicals through complex metabolic pathways that are highly regulated. Cellular anabolism and catabolism is typically balanced so that the cell can survived and replicate in a myriad of environments. Advances In the last decade have broken free from the limitations of the cell while maintained our ability to harness biochemical production. Many of these "cell-free" systems are embryonic in technological sophistication and development, yet pose to solve many challenges we face.

Encapsulation of cellular devices has been identified as a major advancement in a variety of applications. For example, protein production machinery can be stored as freeze dried powders for on demand manufacture, which obviates the need for complex cold-train logistics. Other applications include:

DNA Storage. Synthesized DNA has recently been proposed as an information storage material. Synthetic DNA can last for thousands of years when stored appropriately. In addition, DNA can be stored in living systems that can replicate and mass-amplify the encoded message.

Cell free manufacture dip stick. Cellular extracts can be tailored for rapid prototyping of engineered circuits as well as for production of small quantities of proteins. Purification of cellular machinery and addition of nutrient solutions to power the production of a protein or nucleic acid from a template is possible in a variety of organism with varying levels of complexity. There exists an urgent need for advanced manufacture of complex organic macromolecules such as pharmaceuticals, vaccines, vitamins, and others. In low resource settings especially with limited access to cold chains, including remote facilities, forward operating bases, developing countries and disaster areas.

Protein or cell extract based sensor array. Paper based diagnostics show promise for rapid detection of pathogens and chemicals of concern. These system offer cost-effective, easily distributed platforms for customized analyte detection and quantification. In addition, the use of sensor arrays with genetically engineered sensor response elements provide passive sampling detection that can be used for remote sensing and environmental intelligence.

C.4 Viral breakout session case study

C.4.1 Purpose

Synthetic biology techniques can and have been used to modify viral properties. Such modifications can increase ability to use altered viruses and to achieve results that would not otherwise be possible. The purpose of the viral breakout session is to discuss applications that carry some risk, although the degree of risk may vary, for environmental release and impact. Several of the applications are discussed below with regard to the potential benefits they offer.

Specifically, this session will consider the possible impacts of introducing **engineered** virus or virus elements into naïve environments either intentionally or incidentally. Participants are encouraged to propose likely release scenarios based upon the particular applications delineated below or of their own conception. Impacts could include added benefits, unintended consequences (whether beneficial or detrimental), and other effects which are actually or seemingly benign. To help frame discussion and analyses, a number of questions are posed following the Background section.

C.4.2 Background

Several applications of virus are listed below. Whether the application involves natural viruses or those modified through synthetic biology, many are based on or utilize the specificity of virus to a specific host. For example, lytic bacteriophages (literally, "bacteria eaters") destroy bacterial cells by preempting host machinery to produce viral components and then lysing open the cell to release newly-formed phages. Receptor binding domains on the tail-spike or tail fiber assemblies provide for host recognition. Such domains afford a high degree of specificity that constrains the viral host to one or a few select bacterial species; thus, phages have found use as therapeutic agents for treatment of human diseases which involve bacteria.

Various research efforts have sought means to alter specificity of phages towards non-native hosts in order to broaden potential applications. For example, the receptor binding domain regions (which cause the phage to bind to one host but not another) of a phage known as T4 were modified using polymerase chain reaction (PCR) techniques. Although *E. coli* is the

natural host for T4 phages, T4 phages with this modification were found to propagate in both *Yersinia* and *Pseudomonas* species, which include human pathogens. Other methods have been applied to introduce deliberate modifications into phage genomes in order to provide desired characteristics for a range of applications from vaccine production to environmental biocontrol. Such applications include the following:

Human therapeutic applications. Phages can be modified to target specific cells where they can deliver a given payload or trigger an immune response to eradicate "invaders". Extensive work has examined use of phages for anti-cancer therapies that prevent indiscriminate delivery of chemotherapeutics and damage to healthy cells. Research likewise continues toward development of phages with enhanced antibacterial activity for pathogen killing, as well as microbiome engineering for mitigation of debilitating diseases like ulcerative colitis. Phages also show promise for use in vaccine development. Phage display vaccines can be made by expressing multiple copies of a given antigen on virion surfaces and may have superior immunogenic as well as safety profiles.

Industrial applications. Engineered bacteriophages have been used as scaffolds to promote self-assembly of structures which are difficult or expensive to generate in a reproducible fashion. In addition, phage capsids (i.e., their protein shell) with specifically designed functionalities have been evaluated for use as scaffolds in industrial biocatalysis where the dynamics of catalytic reaction cascades are tightly controlled through precise placement of enzymes.

Biodetection applications. Selectivity of phages for discrete bacterial hosts has been leveraged to develop pathogen detection systems for use in environmental and human sample matrices. For the majority of motifs, detection relies upon bacterial incorporation of phage-delivered genomic elements that either fluoresce or luminesce when expressed. Phage-reporter systems have been demonstrated for species such as *Mycobacterium tuberculosis, Yersinia pestis,* and agriculturally-significant *Pseudomonas* species.

Veterinary applications. Although genetically engineered phages have several potential applications in veterinary medicine, most efforts have focused on development of recombinant phages for vaccination (as

described in the human therapeutic applications section) against animal diseases.

Antibiotic alternative applications. There exists considerable interest in using bacteriophages for biocontrol -- as alternatives to antibiotics for pathogen control. Phage-based bactericides for plant pathogens (e.g., AgriPhage™) have been developed and are available for agricultural use. Similarly, FDA-approved Listshield™ is used for treatment of food products that are at high risk for contamination by *Listeria monocytogenes*. Phage therapies also could find application in aquaculture, where microbial diseases represent a significant threat to productivity, and as surface treatment agents to combat nosocomial diseases in hospitals and nursing homes. At present, licensed products are composed of naturally-occurring bacteriophages, but researchers are exploring ways to modify host specificity and to promote more efficient cell killing by modifying wild-type phages.

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14. ABSTRACT

Synthetic biology and its applications have the potential to greatly improve economic development, public health, environmental stewardship, technological advancement, and many other areas. In May 2017, sixty individuals gathered in Lexington, Massachusetts for a workshop sponsored by the U.S. Army Engineer Research and Development Center (ERDC) to discuss applications of synthetic biology with likely or intended interaction with the environment. Representatives from academia, government agencies, industry, and non-governmental organizations convened to identify knowledge gaps and research needs to assess potential environmental impacts from these technologies. The group discussed challenges in environmental risk assessment, regulation, and community engagement for emerging synthetic biology technologies. The workshop was structured around four hypothetical case studies, including the use of gene drive engineered organisms to control infectious disease vectors, engineered microbes for bioremediation, cell-free applications for advanced chemical production, and engineered viruses for water treatment.

Meeting these research needs will facilitate appropriate environmental risk assessment and informed decision making for the development and potential deployment of synthetic biology organisms and components in the environment.

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