CREATING A RESEARCH AGENDA FOR THE ECOLOGICAL IMPLICATIONS OF SYNTHETIC BIOLOGY
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This report was prepared by the Wilson Center and the Massachusetts Institute of Technology Program on Emerging Technologies as a summarized record of discussion from the two workshops on Creating a Research Agenda for the Ecological Implications of Synthetic Biology. This report captures the main points and highlights of the meetings. It is not a complete record of all details discussed. The meetings were conducted under Chatham House Rule, and thus all comments are free from attribution. All included points represent the author’s interpretations of individual views of participants and should not be viewed as a consensus. Except where specifically noted, no statements in this report represent analyses by or positions of the National Science Foundation or any of the meeting hosts or participants.
Executive Summary

Synthetic biology is a field characterized by rapid rates of change and by the novelty and breadth of applications. It is also an area of basic research and application that encompasses engineering along with the natural, physical, and social sciences. In January 2014, the Massachusetts Institute of Technology Program on Emerging Technologies and the Woodrow Wilson Center convened workshops in Cambridge, MA, and Emeryville, CA, to develop a research agenda on the ecological implications of synthetic biology as part of National Science Foundation (NSF) grant #1337431. Varied applications were used to stimulate discussions among synthetic biologists, ecologists, environmental scientists, social scientists, as well as representatives from government, the private sector, academia, environmental organizations and think tanks. Projects included nitrogen fixation by engineered crops, gene drive propagation in populations of invasive species, engineered seeds and plants destined for distribution to the public, and bio-mining. A series of priority research areas were identified in Box 1.

Agreement among many participants was that in order to undertake the priority research agenda identified, it is necessary to establish, and sustain, interdisciplinary research groups. Addressing these complex questions and overcoming communication barriers across disciplines cannot be done on a short-term basis, and therefore long-term support is essential. In addition, a concomitant assessment of the economic and human social implications of applications is necessary to provide the widest possible context for the ecological impacts. It is possible to imagine, therefore, activities in research and education that cross all NSF directorates and involve other federal agencies. Synthetic biology offers a distinctive opportunity to support high risk, high reward research as recommended in the 2007 report1 by the National Science Board (“Enhancing support of transformative research at the National Science Foundation”). The fact that NSF is the only U.S. government agency dedicated to supporting basic research and education in all fields of science and engineering places the agency in a unique position to play a leadership role in this emerging area, although enhanced funding may be needed to ensure that NSF can fulfill this mission in relation to synthetic biology. However, the scale and scope of efforts required also demand targeted collaborations and strategic partnerships outside the agency. Democratic, deliberative processes will be challenging, but they should have a strong role, not just for the public to be informed, but consulted. Careful consideration should be dedicated to promoting and ensuring outlets are available for public input despite this hurdle.

Synthetic biology is poised to make non-incremental, transformative advances in basic and applied areas of research. To realize these goals, address the associated risks, and identify and mitigate potential ecological implications, a coordinated, prioritized research strategy should be developed by governmental agencies, academia, and industry.
Workshop participants identified the following areas as hurdles to understanding the potential ecological effects associated with the release of organisms modified using synthetic biology. In the remainder of the report, these sections will be more fully characterized.

1) **Comparators:** Synthetic biology’s pursuit of producing novel organisms challenges the established practice by risk analysts of comparing a modified organism to its wild-type “parent.” How does the lack of a wild type comparator affect risk assessments? What alternative testing schemes are needed for “no analog” organisms, possibly even in “no analog” ecosystems?

2) **Phenotypic characterization:** How can one identify and prioritize synthetic traits and/or synthetic organisms of concern? Which phenotypes are most relevant for assessing ecological interactions and consequences of such organisms in the short and long term?

3) **Fitness, genetic stability, and lateral gene transfer:** These properties contribute to and are affected by the interaction of organisms with their environments. How does one measure these properties and interactions in organisms produced using synthetic biology with consistency, reliability, and confidence? What metrics are needed for measuring these properties?

4) **Control of organismal traits:** What degrees of biological and physical control should be required in advance of deployment of a modified organism? How do environmental conditions affect the need for intrinsic and external controls for organisms produced using synthetic biology? Which types of environmental releases are likely to be irreversible?

5) **Monitoring and surveillance:** Is it feasible to monitor these organisms and their ecological/evolutionary effects? Should monitoring be broad-based, targeted or both? How can existing systems of monitoring and surveillance be used in this effort? What new systems of monitoring and surveillance are needed? What role should baseline data play in these efforts? Who manages and curates the data? Who manages access?

6) **Modeling:** What modeling tools exist for synthetic organisms and are they sufficient for situations where organisms produced using synthetic biology are released into the environment? Can existing models be combined across disciplines, or are new approaches needed to integrate natural, physical, and social sciences with engineering?

7) **Standardization of methods and data:** What research is needed in order to standardize testing methods, data reporting, and organism characterization for ecological evaluations? How should data collection and integration be handled? Who is responsible for developing, promoting, and enforcing standards?
# List of Acronyms

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<tr>
<th>Acronym</th>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>APHIS</td>
<td>Animal and Plant Health Inspection Service (USDA)</td>
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<td>COBD</td>
<td>Convention on Biological Diversity</td>
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<td>DIY</td>
<td>Do-It-Yourself</td>
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<td>ELSI</td>
<td>Ethical, Legal, and Social Implications</td>
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<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<td>GEO</td>
<td>Genetically Engineered Organism</td>
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<td>GIS</td>
<td>Geographic Information Systems</td>
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<td>GMO</td>
<td>Genetically Modified Organism</td>
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<td>HGT</td>
<td>Horizontal Gene Transfer</td>
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<td>I-Corps</td>
<td>Innovation Corps (NSF)</td>
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<td>IGERT</td>
<td>Integrative Graduate Education and Research Traineeship Program</td>
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<td>IPCC</td>
<td>Intergovernmental Program on Climate Change</td>
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<td>JBEI</td>
<td>Joint BioEnergy Institute</td>
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<td>MIT</td>
<td>Massachusetts Institute of Technology</td>
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<td>NSF</td>
<td>National Science Foundation</td>
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<td>NSTC</td>
<td>National Science and Technology Council</td>
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<td>SBOL</td>
<td>Synthetic Biology Open Language</td>
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<td>SynBERC</td>
<td>Synthetic Biology Engineering Research Center</td>
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<td>USDA</td>
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Introduction

Market research indicates that the global market for synthetic biology is likely to grow to over $16 billion by 2018, with the largest share of growth in the chemicals and energy sectors. A preliminary inventory recently compiled by the Woodrow Wilson Center indicates a broad array of products moving toward commercialization over the next five years. Economic viability of some of these applications, such as biofuels, may require the intentional release of synthetically engineered organisms into the environment, often on a massive scale. A range of organizations from the European Group on Ethics, civil society, and the U.S. Presidential Commission for the Study of Bioethical Issues has highlighted the need to address the ability of synthetic organisms to multiply in the natural environment and identify, as needed, reliable containment and control mechanisms. Before large numbers of synthetic biology applications move out of the lab and into the market and the environment, an opportunity exists to develop, fund, and execute an interdisciplinary research agenda that will enable a broader understanding of the evolutionary and ecological implications of synthetic biology.

In seeking to identify gaps in knowledge on the ecological effects of synthetic biology, to define a research agenda to improve the scientific and technical understandings of these ecological effects, and to provide input for NSF funding priorities in this area, NSF's Division of Molecular and Cellular Biosciences, Division of Environmental Biology, and the Engineering Directorate provided support (Grant # 1337431) for the MIT Center for International Studies and the Woodrow Wilson Center to organize two workshops. The meetings used current and future applications of synthetic biology as prompts for discussion on the following topics:

- Identification of potential ecological effects of synthetic biology applications;
- Identification of critical areas of uncertainty associated with potential ecological effects;
- Definition of technical research priorities to develop tools and methods to evaluate ecological effects of synthetic biology applications;
- Definition of scientific research priorities to improve understandings of and ways to mitigate ecological effects of synthetic biology applications.

To accomplish these goals, the workshops brought together synthetic biologists, evolutionary biologists, ecologists, environmental scientists, and social scientists as well as representatives from government, the private sector, academia, environmental organizations and think tanks. The applications of synthetic biology used to prompt discussion are discussed later. Dr. James Collins of Arizona State University, former Director of the Population Biology and Physiological Ecology Program and Assistant Director for Biological Sciences at NSF, Dr. Todd Kuiken of the Woodrow Wilson Center, and Dr. Kenneth Oye of the Program on Emerging Technologies at the Massachusetts Institute of Technology helped guide the discussions.
This project addressed a number of structural impediments to the proactive and anticipatory identification and management of the ecological risks associated with synthetic biology. The first is the separation between the “upstream” scientists developing applications and the “downstream” scientists focused on risk assessment. These groups often have diverging perceptions of risks and their associated novelty and uncertainties. The second is the lack of involvement of evolutionary and environmental biologists in existing funding models for synthetic biology. Finally, the disciplinary backgrounds of many synthetic biologists – engineering, physics, or computer science – embody notions of control, feedback, system linearity, and prediction that should be enriched by interactions with evolutionary and environmental biologists with the aim of developing environmentally benign organisms.

The project achieved two important outcomes. First, it strengthened the nascent, on-going collaboration between synthetic biology researchers and a wide range of evolutionary biologists, ecologists, and environmental scientists, with the intention of supporting a better assessment of the knowledge gaps and uncertainties related to the ecological impacts of synthetic biology as the field advances. Second, the project developed the beginnings of a research agenda for the ecological implications of synthetic biology based on existing, near-, and long-term applications of engineered organisms with general agreement and support for the research from key stakeholder groups.

Societal, regulatory and policy issues, while not a direct focus of the workshops, were consistently identified by a majority of the participants. Recommendations were made that a concomitant assessment of the economic and social implications of applications is necessary to provide context for the identified ecological impacts in order to develop sound public policies and regulatory structures governing the release of synthetic biology applications.
Researchers from academic and commercial ventures were asked to present on their work at the outset of the two January 2014 workshops. The selected applications were at varying stages of commercial viability at the time of the meetings, ranging from purely theoretical to entering regulatory evaluation. They were designed to stimulate conversations that could cut across disciplines and provide all participants with common points of reference. The case studies are briefly summarized here. The discussion topics raised following the presentations appear in Appendix 3.

Nitrogen fixation in non-legumes (Cambridge, MA)

Christopher Voigt, Massachusetts Institute of Technology

This project aims to minimize fertilizer application by conferring nitrogen-fixing abilities on agricultural crops via synthetic biology. The majority of work thus far has centered on restructuring and optimizing the nitrogen fixation, or “nif,” gene cluster from *Klebsiella oxytoca*. The cluster, which consists of approximately 20 genes, is responsible for nitrogen fixation processes in the native bacteria. By refactoring nif with entirely synthetic components, researchers have been preparing for the gene cluster’s migration to alternate host organisms. This shift is proposed via one of three possible pathways: 1) inserting the nif cluster directly into the target plant, with the plant’s chloroplast genome serving as the chassis; 2) inserting the cluster into the genetic structure of the plant’s root microbes, or endosymbionts, which live within a plant for at least part of its lifetime; or 3) inserting the gene cluster into soil microbes that surround the plant, such as pseudomonads.

Despite the varying proposed pathways, the three potential insertion destinations intend to achieve the same endpoint for the plant: conference of nitrogen fixation properties where there were none before. However, the potential ecological effects of the three pathways could be different.

Gene drive systems (Cambridge, MA)

Kevin Esvelt, Andrea Smidler and George Church, Harvard University

Gene drives are naturally self-replicating genetic elements capable of spreading through sexually reproducing populations by altering the odds that they will be inherited by surviving offspring. To date, research in the field has focused on mosquitoes. Researchers pointed out that the recent success of several laboratories in constructing various forms of gene drives combined with faster design-build-test cycles offered by DNA synthesis and genome engineering capabilities may lead to synthetic gene drives capable of editing genes in many different wild populations, not just mosquitoes. Researchers presented hypothetical gene drive systems as extensions of work demonstrated by other laboratories, introduced potential
applications, and highlighted the need for systematic analysis of potentially significant environmental and security implications in advance of development and testing.

Because they spread by biasing inheritance, gene drives could potentially be applied to most sexually reproducing species, though their effectiveness would be diminished in species with long reproduction cycles, like humans. Certain types of drive are unlikely to be blocked by natural mutations. In some cases, a subsequently released drive could in theory overwrite the effects of an earlier drive and may even restore the wild-type sequence. Some drive types might be made to spread exclusively through a subpopulation that bears a unique genetic polymorphism. Others under development, for now only in mosquitoes, would have the ability to alter the sex ratio of a population.

Possible applications included controlling invasive animal populations, eliminating disease vectors, and forcing speciation events. These applications were chosen to raise a wide range of potential ecological implications. This hypothetical case generated a rich discussion of the ecological implications of what could be, not just what currently is.
Bio-mining and bioremediation (Emeryville, CA)

Patrick Nee, Universal BioMining

Universal BioMining applies synthetic biology and genetic engineering techniques to the mining industry, with a particular focus on copper mining. At present, mining technologies are limited in their ability to reclaim copper from low-grade ore. One method involves allowing leachate, an acidic mixture, to percolate through low-grade ore heaps up to 750 feet in height. The leachate is then collected at the bottom of the heap, processed to liberate copper for recapture via electroplating, and re-circulated through the pile. Universal BioMining is attempting to increase the proportion of copper recaptured in this process by targeting an organism responsible for the intermediate step of influencing iron chemistry, which in turn increases the solubility of copper.
Current practice typically involves the use of microorganisms native to the mining site, but sometimes suites of organisms are shifted between sites. The organisms are typically acidophiles, a mix of heterotrophs and chemoautolithotrophs, and none are known to be pathogenic. Unlike many applications planned for field release, Universal BioMining’s organisms will not be engineered for maximized stability and fitness given the mining heap’s pre-existing re-inoculation system. Water-based leaching occurs in a closed cycle, with industrial mining effluent tightly monitored. The organisms will be engineered to thrive in the heap environment, meaning that they will struggle with viability at neutral pH.

**Glowing Plants and wide-scale transgenics distribution (Emeryville, CA)**

**Antony Evans, Glowing Plants**

The Glowing Plants project originated out of a Do-It-Yourself (DIY) community laboratory, and generated financial support via the crowd funding website Kickstarter. The project’s goal is to produce “glowing” *Arabidopsis thaliana* plants via genes from the bioluminescent bacterium *Vibrio fischeri*. Auto-luminescence has been conferred to other organisms in the past, and at least one other company will likely beat this project to market; however, what makes Glowing Plants unique is the massively distributed nature of its application. The campaign saw about 6,000 individuals contribute at the $40 level, which promises packets of Glowing Plant seeds in return. Other funding levels offer the receipt of live plants, DIY maker kits which will enable users to make their own seeds using more traditional gene transfer techniques, and the chance to imprint a personal message in the genetic code of the organism. Once Glowing Plants achieves its near-term goal, it plans to attempt the construction of a wider variety of glowing plants.

Over the course of product development, the Glowing Plants team investigated the applicability of various regulations to its products. Because it will be engineering its final product for seed distribution via a gene gun as opposed to *Agrobacterium*, it shouldn’t be regulated by the U.S. Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS). The DIY maker plant kits, on the other hand, will employ the latter method, and may be regulated. Neither product is expected to be regulated by the U.S. Environmental Protection Agency (EPA).
Priority Research Areas Identified

The workshops included participants from a wide variety of fields, and the resulting mix of perspectives allowed for significant knowledge exchange and collaboration. In discussions of identifying and prioritizing research needs for evaluating the ecological implications of synthetic biology, this variety of interests became particularly apparent, as each new idea brought to the table triggered the introduction and consideration of another. At the root of the discussions stood the motivating question of what makes synthetic biology unique, and how those unique aspects drive the need for a new or revised research agenda. In the debate that followed, two overarching traits distinguishing research and applications in synthetic biology emerged:

- **Novelty and speed**: Synthetic biology techniques push beyond incremental changes to organisms and the leap from old to “new” could transcend common evolutionary pathways. The speed at which these leaps could occur is unprecedented.

- **Eco-evolutionary dynamics**: Research should incorporate the simultaneous drivers of ecology and evolution, as opposed to progress in one area while holding the other constant. All new theory and data should take into account this dynamic.

Research questions based on the specific case studies are included in Appendix 3. Through the iterative discussion process, the participants eventually coalesced around seven major research needs, as well as an additional variety of points identified for further discussion. These main points are summarized in box 1 and in the subsections that follow the motivations are laid out for why these topics were identified as areas of primary research focus.

Importantly, the workshops were not intended to result in consensus; instead, they were designed to achieve material saturation, wherein the majority of unique areas of concern are discussed. The sections are broken out by topic area to allow for thematic discussion; however, there are undeniable links between and across topics, making the divisions at times somewhat arbitrary.
1) Comparators

Comparing a modified organism to its wild-type “parent” will be made increasingly difficult, and potentially irrelevant, by future synthetic biology practices. This shift draws attention to the need for new frames of reference when defining and assessing an organism. How should an application be evaluated if the organism has no present-day analogues to use as a basis for comparison? The implications of decreasing relevance of comparators also bear on the topics of monitoring and surveillance, modeling, and phenotypic characterization.

- **Emphasize causal pathways.** Modifications made to an organism result in changes to specific internal causal networks (cause and effect relationships). As a method for organization and prioritization, focus on comparisons relating to identifying what those causal networks are, what they are connected to, and how their effects may differ based on surrounding environments. Consider applying methods for evaluating complex socio-ecological systems, like fault tree analysis and hazard identification, to highlight key areas of focus.

- **Develop generalizable protocols.** In the current environment of continued relevance of baseline comparators, it is essential to design a systematic process for making these comparisons and sharing the resulting data. For example, in a future environment where such ease of comparison is lost, there may still be relevance in establishing a comparison across traits to examine potential new clades. A clade is a grouping that includes a common ancestor and all the descendants, both living and extinct, of that ancestor (e.g., distinct lineages of descendants).

- **Assess need for baselines.** Establishing an environmental baseline is challenging, but important (see Snow et al. 2005) for monitoring and surveillance efforts in order to measure deviations from a point of reference. However, deviations from that baseline do not necessarily measure the impact of that change. With the potential absence of comparators, how does the utility of a baseline change, and how might the attributes of a survey shift to meet those needs?

2) Phenotypic characterization

In the progression toward an increasing mix of modified traits within an organism, there is a concomitant increase in the need to be able to better identify and prioritize traits of concern. While this topic overlaps with multiple additional research categories, it received enough attention in both workshops to merit individual consideration. In particular, three major points are addressed. First, when evaluating ecological interactions, more should be done to understand which phenotypes are most relevant to ecological consequences over the short and long term. Second, an increased emphasis should be placed on understanding the function of a trait, as opposed to fixating on the origin of its DNA. This echoes earlier recommendations from the
U.S. National Academy of Sciences\textsuperscript{9} to focus on the product, not just the process, when evaluating GEOs. Finally, the degree to which context (e.g. ecosystem/environment in which the organisms are being introduced) affects the characteristics of a phenotype must be better understood and characterized.

3) Fitness, genetic stability, and lateral gene transfer

Fitness, the propensity of a gene to persist and spread across generations; stability, the integrity of genetic material across generations; and lateral gene transfer, the likelihood of a trait to be transferred between unrelated species, are three properties that contribute to, and are affected by, the interactions of organisms with the environment. Open questions remain regarding how to measure these properties and interactions with consistency, reliability, and confidence.

- **Examine pertinence of existing models.** Do environmental models currently exist for studying organism persistence and propensity for horizontal gene transfer in microorganisms and other taxa? Could data generated by such models be used to identify areas of potential ecological concern, and be used to evaluate effects on an organism based on different ecological conditions? Could these same models be used to evaluate the introduction of engineered traits into the studied organisms?
• **Engineer for reliability.** With environmental release and in laboratory experiments, there arises the opportunity for organisms to have unexpected interactions in addition to those planned and accounted for. Beyond developing methods for biological confinement, what should be included in engineering planning to ensure reliability in the face of unanticipated interactions? Further, how does this differ between applications favoring stability versus those that do not?

• **Develop standardized metrics.** Research should be directed at prioritizing parameters of concern, and understanding what degree of confidence should accompany such parameters. This could be achieved by undertaking a sensitivity analysis to highlight key areas of concern.

• **Develop quantitative thresholds.** Environment-specific measurements and standards are driven by the establishment of thresholds; how to derive these thresholds for properties of fitness, stability, and horizontal gene transfer remains an open question. How these thresholds could or should shift in the face of context-specific attributes should also be studied.

• **Couple research on fitness and stability.** Discussions of enhanced fitness sometimes assume the application of a biological containment or confinement device. However, characterization of fitness should be tied to genetic stability, as an organism that loses its containment mechanism may subsequently demonstrate very high fitness.

4) Control of organismal traits

Prior to the deployment of an organism, an application should be characterized in terms of expected levels of biological and physical controls. Determining a sufficiently protective level of control and understanding how the desired level of control can be shared between intrinsic and external control measures is of central importance when considering intentional and accidental field release scenarios. This work should be shared between researchers identifying the potential implications and policymakers developing accompanying risk prioritization frameworks.

• **Adaptive evaluation.** Engineered organisms will be designed to survive and deliver their designed traits and functionalities at various intervals depending on the application. For some, consistency and tightly regulated control are essential to maintain over time; for others, evolution of design in concert with surrounding environmental changes is fundamental for the organism’s survival and delivery of its designed outcomes (e.g. a fuel producer or a pollutant degrader). As such, any future evaluation scheme should be adaptive in order to capture varying designs in relation to the organism’s engineered purpose and function.
• **Prepare for instability.** Regardless of the degree to which stability is engineered into an organism, the possibility of someone purposely attempting to engineer out biological containment mechanisms exists, as has been previously documented (see section on gene drives). The ease with which this can be achieved should be characterized, and methods for preventing or monitoring such actions should be studied. Ability to stack containment approaches should be considered to develop redundancies.

• **Assess reliability of engineered reversibility.** The implications of engineering back to a previous state, the viability of such an approach, and the potential requirement of engineering “immunization” into a wild-type population to defend against engineered counterparts all should be studied further. The practicality of coupling the development of every application with that of a “countermeasure” should also be assessed.

5) Monitoring and surveillance

Synthetic biology is moving toward a future of assumed organism release, be it intentional or accidental. Monitoring and surveillance could be employed to track these releases, but the scope of the need may far exceed any present infrastructure. The focus of such monitoring efforts, and how to best track these indicators, requires additional research. Further, a system should be established for assigning an entity to manage, curate, and provide access to the data.

• **Characterize scope of challenge.** Should monitoring and surveillance efforts be reactive or pro-active? In other words, should they be developed to provide broad-based detection capabilities, or track the deployment and status of specific applications (or both)? With the possibility of irreversible effects to the environment in mind, should there be an approach that erects a wall and issues an alert anytime something new enters, or should the base assumption be one of organism release occurring throughout the environment?

• **Prioritize monitoring and surveillance needs.** What are the basic tools from molecular biology that provide advanced surveillance capacities? Metagenomics could be used to conduct baseline surveys. What should we be looking for, where should assays be performed, and how can assays be validated are key questions demanding resolution. Metagenomics also only tests at the molecular level; are there methods for attaining a phenotypic characterization of an environment? Current genomics approaches are increasing in their ability to detect and identify multiple kinds of microbial pathogens in the same assay. But, if the assessment target is uncertain or the application is designed to evolve and adapt over time, additional abilities need to be developed. Should a
“barcoding” system be developed and widely and consistently applied in order for any monitoring and surveillance efforts to be practical?

- **Determine requirement for baseline data.** If a characterization of the baseline environment is deemed necessary for effectively performing monitoring and surveillance tasks, then more should be done to understand which data are required to meet those needs. Can environments be generalized and baseline characterizations applied broadly, or should each scenario be uniquely considered? How can these methods be validated? With potentially decreasing relevance of comparator organisms, does establishing a baseline still make sense?

- **Assess potential for integrating existing systems.** The National Institutes of Health is directing significant sums of money toward metagenomic analyses with an eye toward human health; any monitoring and surveillance efforts developed here should work to collaborate with, rather than compete against, such efforts. The National Ecological Observatory Network (NEON) and the Ocean Observatories Initiative (OOI) are NSF-funded projects. Each is a planned network infrastructure of science-driven sensor systems. Collaborations with these networks could yield significant access to wide parts of the terrestrial U.S. and ocean systems. Additional potential partners include the Department of Homeland Security and the U.S. Department of Agriculture, their respective developed infrastructures, and their international counterparts. Expanding work performed by the biosecurity field to environmental issues could yield valuable insights, particularly with regard to controlled experiments studying diversity, selectivity, and the degree to which assays of near neighbors may create false positives.

- **Establish protocols for data curation and access.** The surveillance and monitoring process should be decoupled from private actors with the potential for conflicts of interest. To achieve the broad scope and scale needed for the task, multiple technologies with varying sensitivities will likely be employed. How will certain inconsistencies
be handled, such as differences in time until monitoring results are accessible, variances in false positive rates, and competing data prioritizations? Further, how will the needs of commercial, federal, and international actors be balanced, considering the likely international scope of future applications, and thus their required monitoring and surveillance needs?

6) Modeling

Models have the potential to deeply inform questions regarding the ecological implications of synthetic biology applications. The degree to which these insights can provide definitive findings or predictions, rather than simply directing researchers on where to focus their efforts more closely, remains uncertain. Can models from different fields be effectively integrated? If so, would their integration provide a sufficient system for assessing synthetic biology applications? If not, should new tools be developed, and what gaps would those tools need to address?

Non-computational modeling

- **Move beyond monocultures.** Organisms are typically tested and evaluated in monocultures, or highly limited mesocosms. Data collected from a mixed-population environment are far more applicable and relevant to current and future needs. Some effort has been made to develop synthetic communities. Can such communities serve as adequate stand-ins for true environmental diversity? What metrics are needed to validate these synthetic communities with their natural counterparts? Can data generated from these synthetic communities be used in larger ecosystem models?

- **Integrate organizational theories.** Assuming a standard conceptual framework of eco-evolutionary dynamics for synthetic biology, what can we learn from macroevolutionary theory and theory related to novel organisms evolving into novel niches? These theories should assume the simultaneous pressures of ecological interactions and evolution.

- **Design for hazard identification and prioritization.** A systematic framework should be designed to inform model development. This would ensure the adequate identification of, and attention to, priority hazards within the model. Such a framework should be developed through the contributions of a variety of expertise.

Computational modeling

- **Identify current modeling systems.** Many fields tangential to synthetic biology rely on models to inform their efforts. Few incorporate data and insights from across fields. Synthetic biology would necessarily draw from all of these fields, including ecological modeling, co-evolutionary modeling, computer science artificial evolution modeling, digital evolution computation, engineering optimization modeling, plant evolution modeling, and socio-economic modeling (e.g., BEACON).
• **Identify gaps in existing systems.** Few existing ecological models attempt to integrate concepts of evolution into their systems, with the exception of evolution-specific modeling. However, for synthetic biology, understanding eco-evolutionary dynamics is central to understanding how an application will behave in the environment overall. Therefore, a focused effort should be made to develop tools and methods for integrating meaningful evolutionary concepts into ecological models. Artificial evolution has a long history in computer science, but the attempted application of such efforts in ecological models has been limited in scope. Research needs include understanding and modeling the complexities of the translation of genetic variation into phenotypic variation.

• **Characterize confidence in models over time.** At present, some of the most advanced models incorporating evolutionary concepts struggle to predict beyond the 10-year horizon. Efforts should be made to lengthen this horizon; additionally, efforts should also be made to understand and assess where and when that horizon falls across and throughout modeling systems. Especially at distant time points, models become increasingly valuable for their ability to highlight sensitivities, not to predict the future.

• **Prioritize desired modeling capabilities.** As models are improved, there should be a prioritization scheme for determining which areas should get built out first. One approach could involve completely characterizing a select number of organisms and applications, and for each, studying the degree to which their modified traits could be expected to affect the surrounding ecosystem. This would build off of current agent-based models that assess the effect of individual traits on ecological communities. This process could be scaled, beginning with simplified systems, and then growing those into contained releases under careful monitoring, and surveying for the effects that should be studied, such as gene transfer, perturbations in the system, and so on.

7) **Standardization of methods and data**

Vast amounts of data are required for bolstering modeling and monitoring efforts; the speed at which these data are collected is at least in part a function of standardized testing methodologies and reporting procedures. Such standardization is essential for data collection collaborations, and subsequent data integration. What these standards are, who is responsible for developing them, and how they should be enforced across independent actors and national boundaries are all open questions.

• **Standardize notation.** Synthetic biology has begun this process through the development of the Synthetic Biology Open Language (SBOL)\(^\text{11}\). Support should be provided for its continued elaboration, and to ensure that its growth includes purposeful build-outs for all necessary applications. This will become increasingly important as data are gathered by a variety of sources on applications around the country, and the world. Ecologists are beginning to standardize efforts through Dryad\(^\text{12}\) and TreeBASE\(^\text{13}\), and efforts should be
made to facilitate communication across and between existing languages. Standardization of synthetic biology notation will ease the integration of other inputs, like geographic information systems (GIS) layers and remote sensing.

- **Standardize testing procedures.** A considerable amount of data is required to inform future modeling and assessment efforts. To expedite the collection process, there should be a distribution of labor. The usefulness of the data will be maximized if the data are derived from standardized methodologies and reported based on standardized metrics. Such testing and reporting methods should be developed, including the potential creation of a controlled set of test environments, or standardized test-beds that would allow for comparisons within specific ecosystems.

- **Standardize assessment rubric.** As applications move toward organisms with increasingly distant comparators, the development of methods for analyzing unknown pathways would be valuable. For example, for an uncharacterized novel organism, a standardized general suite of tests for assessing metabolic activity would be useful.

- **Incorporate and process existing data.** The collection, integration, and interpretation of environmental data from sources such as environmental impact statements—long limited to niche readerships—could yield important insights into ecosystem attributes if catalogued in an accessible database with standardized searchable terms. The standardization of future reporting could help to reduce the inaccessibility of such research and reports.

- **Characterize effects of common protocols.** Synthetic biology employs a range of methodologies and protocols; however, some practices are common across most applications. Research should be dedicated to understanding how these different practices may affect the properties of the final application.
Facilitating the Research Process: A Leading Role for NSF

Among workshop participants, a consensus developed that independent scientific disciplines attempting to identify and solve questions related to the ecological impacts of synthetic biology was poor practice. Such an approach would fail to include significant elements of concern, and would fail to take advantage of pre-existing accumulated knowledge within various fields of relevance. Therefore, a conscientious effort should be made to establish and sustain multidisciplinary research groups to address priority research areas. Additionally, because these complex questions introduce communication barriers across disciplines, they should take place over the long term to ensure a favorable outcome from the effort.

With NSF as the only U.S. government agency dedicated to supporting basic research and education in all fields of science and engineering, it is in a unique position to play a leadership role in this emerging area. However, the scale and scope of efforts required demand targeted collaborations and strategic partnerships with other federal agencies, academic institutions and industry. Additionally, because synthetic biology is poised to make non-incremental, transformative advances in basic and applied areas of research, the field offers a distinctive opportunity to support high risk, high reward research, as per the 2007 National Science Board1 recommendations. Finally, it is possible to envision activities in research and education applied to characterizing the socio-economic implications of applications, therefore crossing directorates within NSF and reaching out to other agencies, as well. The National Science and Technology Council (NSTC) may serve as a good forum for such conversations.

Recommendations

1. **Acknowledge evolutionary rapidity of synthetic biology as a research area.** Synthetic biology is largely defined by the speed at which it drives the shift from known to novel outcomes. This means that institutions, regulators, and researchers should be prepared to respond to rapidly changing situations in a timely way. Democratic, deliberative processes will be challenging but they should have a strong role, not just for the public to be informed, but consulted. Careful consideration should be dedicated to promoting and ensuring outlets are available for public input despite these hurdles.

2. **Build from existing models.** Several pre-existing systems for research were considered as examples, with the pros and cons of each discussed in light of currently identified needs. These examples included:
   - **BEACON:** The BEACON Center for the Study of Evolution in Action is an NSF Science and Technology Center. It tackles questions of evolution by bringing together biologists, computer scientists, and engineers to look at real-time evolution issues. This program was cited as an example of a center that actively works to involve and integrate a multitude of perspectives in order to best characterize the projects at hand.
• **CBD:** The Convention on Biological Diversity (CBD) Identification, Monitoring, Indicators and Assessments program indicates which components countries might need to focus on when designing biodiversity monitoring programs. These components include ecosystems and habitats; species and communities; and to describe genomes and genes of social, scientific or economic importance.

• **I-Corps:** The NSF Innovation Corps (I-Corps) is a public-private partnership that develops activities and programs for pushing promising basic research to market. Its primary goal is “to foster entrepreneurship that will lead to the commercialization of technology that has been supported previously by NSF-funded research.” Participants strongly supported the model, including its preference for offering small-scale grants, and suggested development of an analogous program devoted to helping researchers tackle questions of ecological importance when considering commercialization.

• **IPCC:** The Intergovernmental Panel on Climate Change (IPCC) is an international body charged with the assessment of climate change. Because climate change is global in nature, the governing institution was necessary. An analog could potentially be built out for synthetic biology, and study the means by which ecosystems and ecosystem services interact on a global scale, and how any perturbations could ripple, amplify, or dampen over time.

• **SESYNC:** The National Socio-Environmental Synthesis Center (SESYNC) is funded through a NSF grant to the University of Maryland and is dedicated to solving society's most challenging and complex environmental problems. As one of only a few U.S. trans-disciplinary research centers, SESYNC brings together different disciplines and stakeholders to increase knowledge on the complex interactions between human and ecological systems.

• **SynBERC:** The Synthetic Biology Engineering Research Center (SynBERC) is a federally-funded ERC that brings together primarily technical researchers from a handful of universities. While participants agreed that the program had successfully spurred collaborations, they also noted that a more interdisciplinary effort would benefit from being built out of an institutional setting where support for such a mission could receive direct attention.

3. **Collaborate with international partners.** NSF, as a lead agency, should ensure that conversations are initiated and supported with cross-border institutions. These partnerships will become increasingly important as applications proliferate and become multi-national in scope. While current international arrangements are heavily focused on
medical, health, and security issues, future efforts should also work to include environmental concerns as well.

4. **Build and sustain multidisciplinary research teams.** The ecological implications of synthetic biology are multi-faceted, with applications having the potential to involve a wide variety of fields. Complete characterization of these issues therefore requires involving the insights and expertise of just as many individuals. While this may be unsustainable over the long-term, determined efforts must be made to establish scalable, generalizable protocols that would guide the assessment of potential implications of an application. Such a process requires the careful inclusion of individuals spanning the appropriate disciplines.

- **Sustain teams over the long-term.** Multidisciplinary efforts typically require significant ramp-up periods in order to allow for the development of a common language and understanding between disciplines. If initiatives are only funded over the short-term, these powerful collaborations are disbanded soon after they become truly useful. Small pilot grants, while beneficial for funding a range of efforts, would require additional institutional support so as to mitigate the costs of the scale-up period and create a repository for the accumulated knowledge. The value to co-location should not be underestimated.

- **Promote interdisciplinary efforts.** Multidisciplinary work involves contributions from a variety of fields to solve pieces of the puzzle; interdisciplinary work involves collaborations across those viewpoints to push for new insights and understanding. The more the latter can be achieved, the deeper and stronger the findings coming out of these groups will be. Research on the ecological implications of synthetic biology should be paired with social science research in the same areas.

- **Initiate process through select case studies.** Case studies provide an opportunity to dive into a carefully selected research area; test assumptions, methods, and protocols; and report on identified gaps in current knowledge and tools. Further, such efforts can ideally yield the development of generalizable methods to use as best practices for guiding future approaches, especially when commonalities arise across cases. NSF’s Integrative Graduate Education and Research Traineeship Program (IGERT) could serve as a basis, though not exact model, for such programs. A conscientious effort should be made to begin with cases that project high levels of benefits and low levels of risk; some participants recommended focusing early cases on conservation and environmental issues. A high “failure” rate should be assumed early on, in so far as mapping a path to complete characterization of an application’s implications. A phased funding process could allow for those few that do succeed to proceed into deeper characterization efforts.
Examine methods of science and innovation policy. As synthetic biology advances, its tools will become increasingly accessible. The shift away from centralized research may result in changes to organizational structures, and potentially affect questions of public trust and project assessment. This research should loop synthetic biologists into a conversation typically limited to the social, behavioral, and economic sciences. One method could be to combine research efforts of those studying the evolution of organisms and consortia.

Endnotes


11. Synthetic Biology Open Language. Available at: http://www.sbolstandard.org/ (Last Accessed May 2014)

12. DRYAD. Available at: http://datadryad.org/ (Last Accessed May 2014)

13. TreeBASE. Available at: http://treebase.org/treebase-web/home.html (Last Accessed May 2014)
Appendix 1. Workshop Agendas

The January 2014 workshops were designed with the end goal of development of an actionable research agenda in mind. Therefore, while time was provided for open conversations, significant blocks of the meetings were reserved for moderated discussions explicitly addressing gaps in knowledge and research needs. These dialogues were rooted in common reference points through the use of case studies presented at the start of both sessions. The chosen applications were selected to illuminate near- and long-term research needs, and to provoke consideration of a wide range of possible ecological implications. They also facilitated the development of a shared language for attendees hailing from a wide variety of fields.

The January 8-9 (Cambridge, MA) and January 16-17 (Emeryville, CA) workshops followed matching agendas but for the use of different case studies, as outlined below.

**Day One – Facilitated by Dr. Todd Kuiken and Dr. Kenneth Oye**

Overview on Goals of Workshop and Project

Introduction of Participants

**Cambridge Case Studies**

- Nitrogen fixation in non-legumes (Voigt – MIT)
- Gene drive systems, with conservation applications (Church, Esvelt – Harvard)
- Summary of Ecological Society of America position paper (Snow – Ohio State)

**Emeryville Case Studies**

- Bio-mining and bioremediation (Nee – Universal BioMining)
- Glowing Plants and wide-scale distribution (Evans – Glowing Plants)
- Open technologies for plant synthetic biology (Haselhoff – Cambridge)


- Research questions - What jumps out?
- Instrumentation/Metrology needs
- Database needs - gene sequencing data
- Gaps in methods
- Scope/Scale of projects
- Areas of expertise needed
- Costs - beginning to frame/triage costs

**Day Two – Facilitated by Dr. James P. Collins**

Recap Day One

Determine whether consensus exists on research themes and scope

Prioritize areas of research and funding sources
Appendix 2. List of Participants

Massachusetts Institute of Technology, Cambridge, MA
January 8-9, 2014

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### Joint BioEnergy Institute, Emeryville, CA
### January 16-17, 2014

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Appendix 3. Workshop Case Studies and Discussions

Information below represents the questions raised during the discussion of the case studies presented.

Nitrogen fixation in non-legumes (Cambridge, MA)

Christopher Voigt, Massachusetts Institute of Technology

The nitrogen fixation case study presented an opportunity for participants to compare and contrast the potential implications of a series of application endpoints because the research is still undecided regarding where to confer the nitrogen fixation abilities. Major discussion topics included planned methods of control and implications of research decisions on regulatory oversight.

Species characterization

- Is it practical to characterize the components of the nif gene cluster, or does it only make sense to consider the suite of genes as a whole?
- What happens when the system is placed under pressure?
- What is the stability of the system in the environment? What is its potential for horizontal gene transfer?
- How would the application perform in a microcosm study when assessed in combination with other introduced genetic materials?
- How is the time-scale for the application defined?
- Is it possible to understand the function of the organism in the broader ecosystem?

Organism control

- Could the refactored suite of genes be broken into sub-units and distributed throughout the genome as a means of reducing likelihood of wholesale transfer of function? Does data exist to inform this opinion?
- Can anything be learned about potential interactions and likelihood of control based off the trait’s historically tight regulation in nature?
- How difficult must horizontal gene transfer be for the application to be deemed “safe”? Alternatively, if the trait being transferred is deemed “safe,” does the frequency of transfer matter?
• What factors should be considered upstream of the effects of gene flow? For example, does the organism lyse, and must a phage be present to internalize the material?

**Environmental interactions**

• How will other components of the soil biome react to the addition of the nif gene cluster?
• What are the consequences of the nitrogen fixation system transferring to another organism?
• Do any of the nif genes have the potential to change the microbial landscape? What about the potential plant pest environment and impacts on non-target species?
• Are there any detrimental effects associated with the organism’s survival post-expected lifespan?
• How would interaction questions change if the project used artificial amino acids?

**Gene drive systems (Cambridge, MA)**

*Kevin Esvelt and George Church, Harvard University*

The potential scale, scope, and ease of access of hypothesized future gene drive systems moved participants from conversations about close analogues of present-day systems to thoughts far out along the technology horizon. The resulting questions searched for the limitations of present technologies and policies, as well as closely considered the nature of the ecological transitions that could take place. Additional questions addressed the ethical implications of such a product; those discussions are not included here.

**Stability of gene drive systems**

• How hard would it be for a gene drive to evolve to naturally carry along a different gene? To eliminate a non-targeted gene? Should the types of gene drives investigated be influenced by this factor?
• Acknowledging that a gene drive will only travel through sexually reproducing, interbreeding organisms, what is the risk of it moving to a related species? Could this be tested by checking potential for interbreeding across related species in the laboratory?
• How could you measure for stability or instability in the gene drive system?

**Characterization of the system**

• A risk assessment is difficult to effectively develop when biocontrol is included in the system, and even more so when the goal is to repeatedly reintroduce the application. How will this be handled?
• Would the widespread application of gene drives increase the risk of them being pushed to other organisms?

• Could a population become vulnerable to subsequent gene drives due to the propagation of a first such gene drive?

• Are the possibilities of what could go wrong already being considered in the development of the system, or is that an afterthought? Have system suppressors been identified or characterized?

Ecological interactions

• Can a putative “reversal” gene drive intended to undo the effects of an earlier drive propagate through the population at a sufficient speed to undo the cascading ecological effects? How does the timescale of release affect this? Should all labs constructing gene drives require a reversal drive to be built at the same time?

• If applied to an invasive species, how would the risk of gene flow back to the native population be mitigated? Is the only solution to “immunize” the wild type population?

• What are the implications from a “successful” application of this system? If an invasive species is removed from the population, how will the gap be filled?

• How will the system be monitored as it propagates through the population? Will a marker be included to increase ease of tracking? Can this marker be rendered stable, or will it be naturally discarded during the process of spread?

• How will interactions vary between aquatic and terrestrial systems?

Bio-mining and bioremediation (Emeryville, CA)

*Patrick Nee, Universal BioMining*

In addition to clarifications about organism structure and design, main topics of conversation included monitoring, baseline data, containment, and species characterization. Only those questions relevant to the research agenda are included below. A series of tests recommended by participants, and the acknowledged gaps of those tests, are also reported here.

Species characterization

• Are there naturally occurring organisms that already perform this activity sufficiently?

• Does the engineering introduce novel molecules, or does it introduce existing processes to novel environments?
If the organism were to attain an additional attribute, such as halotolerance, would that significantly change its fitness either inside or outside the mining environment?

Does mining efficiency depend on species distribution?

How does species diversity vary by region?

**Methods of deployment**

Have competition tests with natural organisms been planned for the future?

Is the need for continuous re-inoculation designed, or unintentional?

Are there means for balancing increasing copper extraction efficiency against decreasing acid runoff?

Will trait engineering need to shift based on area of application? For example, if applied in non-arid environments, will new concerns arise?

**Methods of containment**

Has monitoring been used to assess the degree to which the mining environment is a closed system from an organism perspective, not just a chemical perspective?

What happens to the system when the heap has been deemed fully extracted? Can natural organisms be reintroduced to outcompete the modified organism?

Are the introduced traits expected to confer significant evolutionary advantages to the engineered organisms? How are these hypotheses tested, and do they hold true across mining and non-mining environments?

If the goal is to design for instability and decreased fitness, is the potential for horizontal gene transfer increased? How will this be tested?

**Monitoring and surveillance**

How is species diversity tracked?

Is species composition tracked in runoff and flood zones?

Have baseline data been collected regarding community organisms?

Given the current lack of surveillance, how are other unintended consequences identified and tested for?
Tests for characterizing the application

- **Monitoring:** Sample distally from the deployment location and set up a titration; validate a standardized assay to implement in a transect until confident the organism or its byproducts is no longer present.

- **Demonstration of impact:** Distance from deployment location is less important than heterogeneity of environments encountered. Tests must be included for each of these areas. Compare these areas against federal and state lists of species and areas of concerns. Develop representative mesocosms, and then test inoculating those environments and verify that the organism does not survive. Perform competitive and physiological assays to understand the differences between the ancestral organism and the engineered application.

Glowing Plants and wide-scale transgenics distribution *(Emeryville, CA)*

**Antony Evans, Glowing Plants**

Many questions focused on the ethical and philosophical issues associated with the project, including matters of consenting public and responsible science practice. However, only questions relating to the research agenda, like those impacting regulatory consideration and potential ecological effects, are included here.

Ecological interactions

- How will the impact of the bioluminescence from the plants on wild organisms be tracked across seed destinations? If this can’t be tracked or known ahead of time, then how is the application ready for release?

- How have the interactions of insects with the glowing plant been characterized? Does this have the potential to disrupt pollinators?

- *Arabidopsis* is frequently used in laboratories specifically because it is easy to grow and is a weedy species. How does this align with comments made regarding the difficulty of growing the plants, and the extreme unlikelihood that the seeds would take root and grow if released outdoors?

- Were any types of biocontrol mechanisms employed or tested in the system? Why was sterility not introduced into the system when it could serve such a purpose?
Determining regulatory coverage

- The seed packets will not be regulated because the seeds were created using gene guns, while the DIY maker kits will be regulated because the system will rely on Agrobacterium. Neither can be shipped internationally. However, once these are distributed, how will use be monitored?

Habitats of relevance

- If the plants are expected to be sent to thousands of individual sites around the country, how are habitats of relevance being determined? Are all of these locations being tracked, characterized, and assessed for specific vulnerabilities in advance of product release? The USDA APHIS test framework is insufficient for this purpose.

- How could a model be developed to evaluate such a widely distributed application? What questions would need addressing in order to appropriately characterize the effort?

Tests for characterizing the application

- Demonstration of impact: Compare the altered plants to other mustards, and assess how well they grow. A series of greenhouse competition assays in a variety of environments would be a good start, and any identified differences could point to areas requiring further study. Also study known pathway interactions up- and downstream. Emphasize the study of the resulting phenotypes, not the genetic modifications.

Open technologies for plant synthetic biology (Emeryville, CA)

Jim Haseloff, Cambridge University

The OpenPlant initiative (www.openplant.org) is a collaboration between the University of Cambridge and the John Innes Centre funded by the UK government. The initiative has three primary goals: 1) promote interdisciplinary exchange between foundational technologies and applied plant sciences, 2) promote a two-tier intellectual property system that protects investments in applications while encouraging the sharing of DNA components at earlier stages, and 3) promote responsible innovation for sustainable agriculture and conservation. The new initiative has identified Marchantia polymorpha as one target model system for testing engineering efforts. The initiative should provide a valuable mechanism for advancing plant research in synthetic biology in ways that have been primarily dominated by bacterial research thus far.
The Synthetic Biology Project was established in August 2008 at the Woodrow Wilson International Center for Scholars. The Project aims to foster informed public and policy discourse concerning the advancement of synthetic biology—an emerging interdisciplinary field that uses advanced science and engineering to make or re-design living organisms, such as bacteria, so that they can carry out specific functions. Synthetic biology involves making new genetic code, also known as DNA, which does not already exist in nature.

Work of the Synthetic Biology Project is supported by a grant from the Alfred P. Sloan Foundation.

For more information about the Project visit: www.synbioproject.org