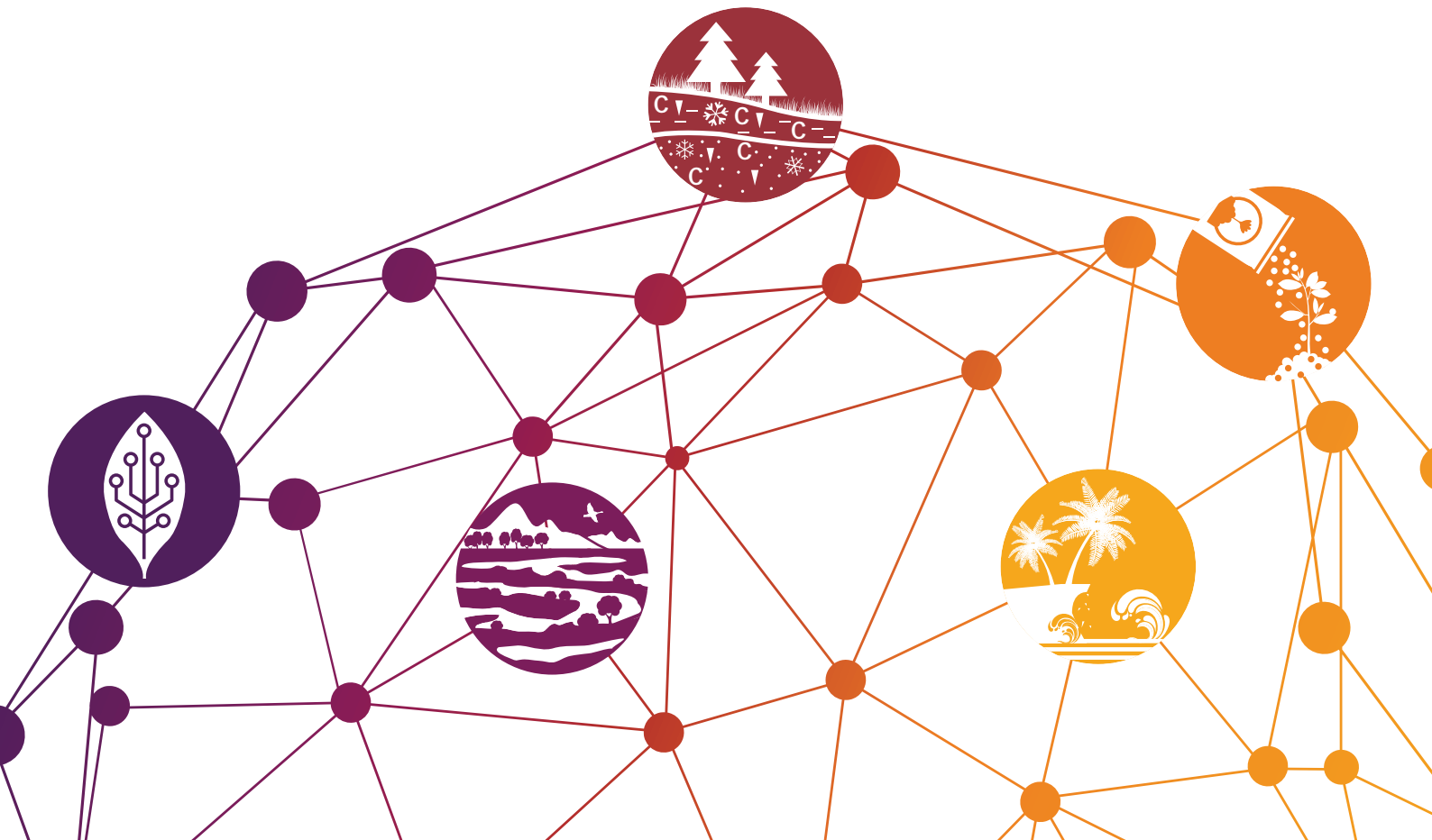


FRONTIERS 2018/19

Emerging Issues of Environmental Concern



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Science Division
UN Environment
P.O. Box 30552
Nairobi, 00100, Kenya
Tel: (+254) 20 7621234
E-mail: publications@unenvironment.org
Web: www.unenvironment.org



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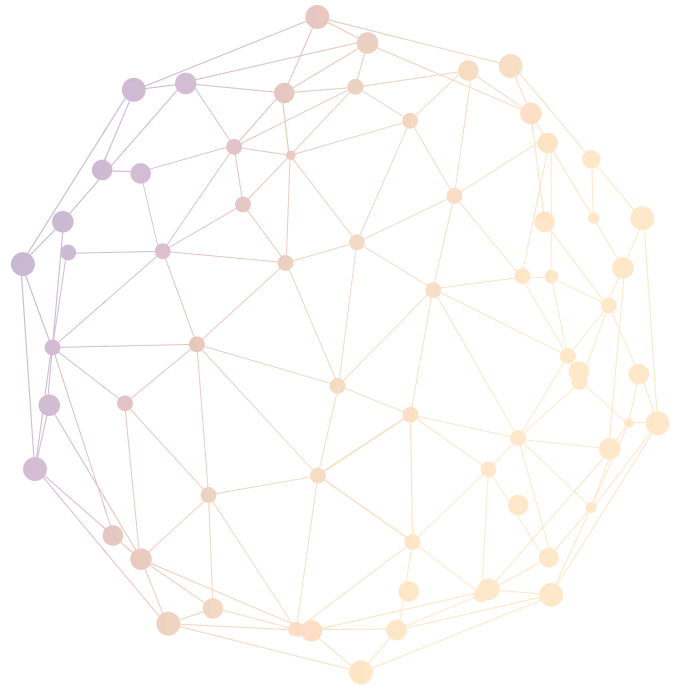




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Foreword



In the first decade of the 20th century, two German chemists – Fritz Haber and Carl Bosch – developed a way to produce synthetic nitrogen cheaply and on a large scale. Their invention spurred the mass production of nitrogen-based fertilizers, and thus transformed farming around the globe. It also marked the beginning of our long-term interference with the Earth's nitrogen balance. Every year, an estimated US\$200 billion worth of reactive nitrogen is now lost into the environment, where it degrades our soils, pollutes our air and triggers the spread of “dead zones” and toxic algal blooms in our waterways.

It's no wonder that many scientists are arguing that “the Anthropocene” should become the official name of the current geological era. In just a few decades, humankind has caused global temperatures to rise 170 times faster than the natural rate. We have also deliberately modified more than 75 per cent of the planet's land surface, and permanently altered the flow of more than 93 per cent of the world's

rivers. We are not only causing drastic changes to the biosphere, we are also now capable of rewriting – and even creating from scratch – the very building blocks of life.

Every year a network of scientists, experts and institutions across the world work with UN Environment to identify and analyze emerging issues that will have profound effects on our society, economy and environment. Some of these issues are linked to new technologies that have astonishing applications and uncertain risks, while others are perennial issues, such as the fragmentation of wild landscapes and the thawing of long-frozen soil. Another issue, nitrogen pollution, represents an unintended consequence of decades of human activity in the biosphere. While the final issue analyzed here, maladaptation to climate change, highlights our failure to adequately and appropriately adjust to the shifting world around us.

There is some good news to report. As you can read in the pages that follow, a holistic approach to the global challenge of nitrogen management is beginning to emerge. In China, India and the European Union, we are seeing promising new efforts to reduce losses and improve the efficiency of nitrogen fertilizers. Ultimately, the recovery and recycling of nitrogen, as well as other valuable nutrients and materials, can help us to farm cleanly and sustainably, a hallmark of a truly circular economy.

The issues examined in *Frontiers* should serve as a reminder that, whenever we interfere with nature – whether at the global scale or the molecular level – we risk creating long-lasting impacts on our planetary home. But by acting with foresight and by working together, we can stay ahead of these issues and craft solutions that will serve us all, for generations to come.

A handwritten signature in blue ink, appearing to read 'J. Msuya', with a stylized flourish underneath.

Joyce Msuya
Acting Executive Director
United Nations Environment Programme

Acknowledgements

Synthetic Biology: Re-engineering the environment

Lead Authors

Bartłomiej Kolodziejczyk, H2SG Energy Pte. Ltd., Singapore
Natalie Kofler, Yale Institute for Biospheric Studies, Yale University, Connecticut, United States

Contributors and Reviewers

Marianela Araya, Convention on Biological Diversity, Montreal, Canada
James Bull, College of Natural Sciences, University of Texas at Austin, Texas, United States
Jackson Chamber, Department of Biological Statistics and Computational Biology, Cornell University, New York, United States
Chen Liu, Department of Biological Statistics and Computational Biology, Cornell University, New York, United States
Yongyuth Yuthavong, National Science and Technology Development Agency of Thailand, Pathumthani, Thailand

Ecological Connectivity: A bridge to preserving Biodiversity

Lead Author

Gary Tabor, Center for Large Landscape Conservation, Montana, United States

Contributors and Reviewers

Maya Bankova-Todorova, The Mohamed bin Zayed Species Conservation Fund, Abu Dhabi, United Arab Emirates
Camilo Andrés Correa Ayram, Alexander von Humboldt Biological Resources Research Institute, Bogotá, Colombia
Letícia Couto Garcia, Federal University of Mato Grosso do Sul, Campo Grande, Brazil
Valerie Kapos, UN Environment – World conservation Monitoring Centre, Cambridge, United Kingdom
Andrew Olds, School of Science and Engineering, University of the Sunshine Coast, Maroochydore, Australia
Ileana Stupariu, Faculty of Geography, University of Bucharest, Romania

Permafrost Peatlands: Losing ground in a warming world

Lead Author

Hans Joosten, Greifswald University/Greifswald Mire Centre, Greifswald, Germany

Contributors and Reviewers

Dianna Kopansky, UN Environment, Nairobi, Kenya
David Olefeldt, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, Canada
Dmitry Streletskiy, Department of Geography, The George Washington University, Washington DC, United States

The Nitrogen Fix: From nitrogen cycle pollution to nitrogen circular economy

Lead Authors

Mark Sutton, Centre for Ecology & Hydrology, Edinburgh, United Kingdom
Nandula Raghuram, Guru Gobind Singh Indraprastha University, New Delhi, India
Tapan Kumar Adhya, Kalinga Institute of Industrial Technology Bhubaneswar, Odisha, India

Contributors and Reviewers

Jill Baron, U.S. Geological Survey, Colorado, United States
Christopher Cox, UN Environment, Nairobi, Kenya
Wim de Vries, Wageningen University and Research, Wageningen, The Netherlands
Kevin Hicks, Stockholm Environment Institute, York, United Kingdom
Clare Howard, Centre for Ecology & Hydrology, Edinburgh, United Kingdom
Xiaotang Ju, College of Agricultural Resources and Environmental Science, China Agricultural University, Beijing, China
David Kanter, College of Arts and Science, New York University, New York, United States
Cargele Masso, International Institute of Tropical Agriculture, Ibadan, Nigeria

Jean Pierre Ometto, National Institute for Space Research, São José dos Campos, Brazil
Ramesh Ramachandran, National Centre for Sustainable Coastal Management, Ministry of Environment, Forest and Climate Change, Chennai, India
Hans Van Grinsven, PBL Netherlands Environmental Assessment Agency, The Hague, The Netherlands
Wilfried Winiwarter, International Institute of Applied Systems Analysis, Laxenburg, Austria

Maladaptation to Climate Change: Avoiding pitfalls on the evolvability pathway

Lead Author

Catherine McMullen, Stockholm Environment Institute, Bangkok, Thailand

Contributors and Reviewers

Thomas Downing, Global Climate Adaptation Partnership, Oxford, United Kingdom

Anthony Patt, Institute for Environmental Decisions, ETH Zürich, Zürich, Switzerland

Bernadette Resurrección, Stockholm Environment Institute, Bangkok, Thailand

Jessica Troni, UN Environment, Nairobi, Kenya

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Alexandra Barthelmes and Cosima Tegetmeyer, Greifswald Mire Centre, Germany; Marin Klinger, National Snow and Ice Data Center, Colorado, United States; Salome Chamanje, David Cole, Nicolien Delange, Angeline Djampou, Philip Drost, Virginia Gitari, Jian Liu, Ariana Magini, Nada Matta, Pauline Mugo, Susan Mutebi-Richards, Shari Nijman, Andreas Obrecht, Samuel Opiyo, Meses Osani, Roxanna Samii, Rajinder Sian, Nandita Surendran and Josephine Wambua, UN Environment

Production advisers

Maarten Kappelle and Edoardo Zandri, UN Environment

Production team

Editor-in-chief: Pinya Sarasas, UN Environment
Technical support: Allan Lelei, UN Environment
Copy editor: Alexandra Horton, United Kingdom

Graphics, design and layout

Graphic designer: Audrey Ringler, UN Environment
Cartographer: Jane Muriithi, UN Environment

Printing

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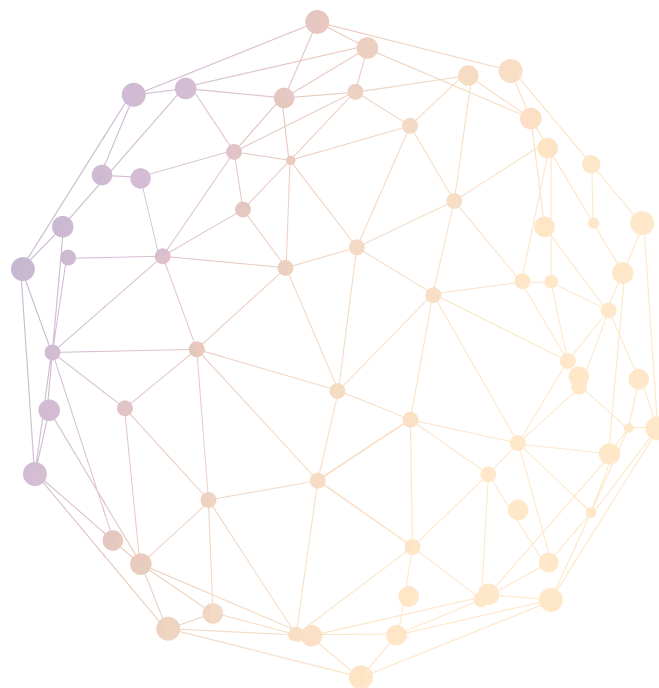




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Synthetic Biology: Re-engineering the environment

Opportunities and challenges

The world is facing unprecedented challenges to a healthy and sustainable future. Habitat destruction, invasive species, and overexploitation are contributing to immense biodiversity loss.¹ Unsustainable, extractive industry practices further burden the environment, and by extension, human welfare. Vector-borne infectious diseases pose a major threat to global health.² Rapid climate change is likely to expand the geographical range of tropical diseases and further stress already taxed species and ecosystems.³

A number of approaches devised to meet these challenges – some proposed and others already implemented – share a common strategy. That is, they depend upon the genetic manipulation of living organisms to acquire new functions

that otherwise do not exist in nature, in order to serve human needs. Scientists can modify microorganisms like *E. coli* by rewriting their genetic code to turn them into tiny living factories that produce biofuel.⁴ Both baker's yeast and *E. coli* can be engineered to produce adipic acid – a petroleum-derived chemical key to the fabrication of nylon – thus offering an alternative to petroleum-dependent production.^{5,6} Baker's yeast can also be reprogrammed to derive an antimalarial drug called artemisinin, which is normally sourced from the sweet wormwood plant.⁷ These are all examples of products made possible by the advanced genetic-engineering technology known as synthetic biology.

The majority of commercially available synthetic biology products have been developed to provide alternatives to existing high-value commodities, especially those dependent



Succinic acid is a high-value chemical used in the food, pharmaceutical and chemical industries. *Basfia succiniciproducens* as shown above is a natural succinic acid producing bacterium found in bovine rumen. To achieve the industrial-scale production, it is genetically engineered for improved productivity. 4,000x magnification.

Photo credit: BASF

on the petroleum supply chain and non-renewable resources.⁸ Moreover, synthetic alternatives and replacements for substances conventionally derived from nature are also gaining ground in research and market spaces.⁹⁻¹² Modern Meadow, a company behind the invention of a collagen-producing yeast, aims to deliver a sustainable leather alternative with properties and texture similar to animal-derived leather.¹¹ Synthetic biology has also opened up a new landscape for advanced materials with novel functionalities and performance, such as materials that can self-assemble or self-repair.¹³

The recent emergence of CRISPR (pronounced *crisper* and short for *clustered regularly interspaced short palindromic repeats*) as a gene-editing tool has enabled even more precise and inexpensive methods of engineering individual organisms, biological systems, and entire genomes.^{14,15} Applications of synthetic biology are advancing beyond the manipulation of microbes in the laboratory to engineering the propagation of species outside controlled settings, for specific ends. Strategies to release genetically engineered organisms into the environment to permanently alter entire populations of target species have been proposed as a means to eradicate vectors of disease, eliminate invasive species, and lend resilience to threatened plants and animals.¹⁶



The Convention on Biological Diversity considers that the following operational definition is useful as a starting point for the purpose of facilitating scientific and technical deliberations under the Convention and its Protocols.

“Synthetic biology is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems.”²⁰

The intentional or accidental release of genetically engineered organisms into the environment could have significant negative impacts on both human and environmental health. Misuse of these technologies and a failure to account for unintended consequences could cause irreversible environmental damage and pose significant geopolitical threats.¹⁷ The potential far-reaching impacts of synthetic biology demand governance methods and research guidelines that promote its ethical and responsible use.^{18,19}



The filamentous fungus, *Aspergillus niger*, can naturally produce enzymes that are commercially important in the food and animal feed industries. The microorganism is genetically modified to enable the large-scale enzyme production. 180x magnification.

Photo credit: BASF

Rewriting the code of life

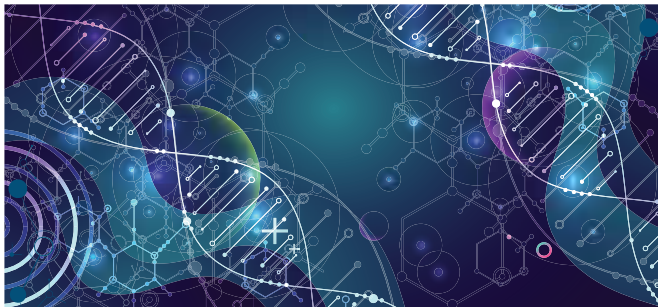
The development of recombinant DNA technology in the 1970s marked a major shift in how humans control genomes.²¹ Genetic sequencing technologies allowed for tracts of DNA to be read and understood, providing the blueprint to engineer genomes for new gene expressions. DNA sequences can be completely rewritten by deleting, adding or replacing segments. Entire portions of DNA can now be chemically synthesized and assembled, which has led to the creation of synthetic life.²²

The latest gene editing tool, CRISPR-Cas9, has garnered significant excitement in the scientific community and general public alike. First described in 2012, CRISPR is faster, cheaper, more accurate, and more efficient than any of its gene-editing predecessors.^{23,24} It has speeded up the editing process from several months to just a few days.^{25,26}

The CRISPR-Cas9 gene-editing technique was inspired by a naturally occurring defence system of certain bacteria against viral invasion.^{27,28} In nature, a bacterium can deploy the Cas9 enzyme to cut invasive genetic material inserted by a virus, effectively disabling the attack. Researchers have adapted this mechanism to cut DNA at any specific location. In CRISPR-Cas9 gene editing, scientists use a guide RNA to direct the Cas9 enzyme to a precise portion of DNA.

The Cas9 enzyme then acts as a pair of molecular scissors, cutting or deleting the targeted segment. By exploiting the natural DNA repair process, researchers can also insert a customized DNA segment into the disrupted strand.²⁹

 **Video: Synthetic biology explained**



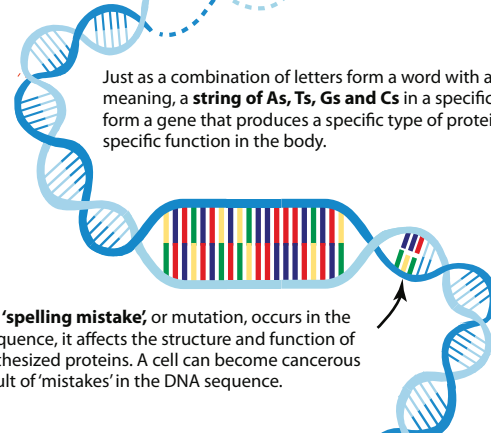
Video link: <https://www.youtube.com/watch?v=rDSuNAMbDaQ>
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DNA is in every living organism's blueprint. It guides the production of proteins needed for an organism to function.

DNA, or deoxyribonucleic acid, is made up of four nucleotide bases bonding in pairs.

Adenine pairs with **Thymine**
Cytosine pairs with **Guanine**



Just as a combination of letters form a word with a certain meaning, a **string of As, Ts, Gs and Cs** in a specific order form a gene that produces a specific type of protein for a specific function in the body.

When a '**spelling mistake**', or mutation, occurs in the DNA sequence, it affects the structure and function of the synthesized proteins. A cell can become cancerous as a result of 'mistakes' in the DNA sequence.

Scientists can determine the precise order of the letters through **DNA sequencing**. The complete set of human DNA, or the human genome, has 3 billion combinations or base pairs.



2.7 billion base pairs



651 million base pairs



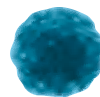
12 million base pairs baker's yeast



278 million base pairs

Genetic engineering techniques have been used for decades to modify organisms by altering the location of genetic materials, for example in genetically modified organisms (GMOs), where a gene from one species is isolated and transferred to an unrelated species in order to achieve the desired characteristic in the target organism.

Synthetic biology is the next level of genetic engineering: the research is no longer confined to manipulating natural genetic materials, but involves the programming and construction of new biological systems using artificially synthesized DNA.



In 2010, scientists announced their success in creating the world's first synthetic bacterial cell after a decade of learning to design, synthesize and assemble a DNA sequence from scratch.



Using the natural baker's yeast genome as a blueprint, a consortium of scientists are now working to construct a yeast cell made out of entirely synthetic DNA.



The spherical spores produced by fungus *Emericella nidulans* are coated in a layer of the protein hydrophobin which repels water. The gene responsible for hydrophobin production has been introduced into *E. coli* bacteria to manufacture the protein with commercial applications. 400x magnification
Photo credit: BASF

This editing process can be likened to locating and precisely cutting a specific word or a sentence out of a document, and if desired, replacing it with new wording. CRISPR is now being used to repair disease-causing mutations in humans, achieve new traits in crops, and synthesize novel microorganisms.¹⁴ More recent developments include the use of CRISPR-Cas9 to edit RNA instead of DNA.³⁰

CRISPR gene editing is being used in research aiming to engineer wild organisms outside human-controlled environments. *Gene drives* are a synthetic biology application that depends on CRISPR gene editing to ensure the expression of desired gene edits in future generations of a wild species.³¹ The process involves an organism being engineered in a laboratory to encode a CRISPR-based gene drive and a desired gene edit. This organism is then released to mate with the normal population in the wild, forcing the inheritance of the desired gene edit along with the gene drive system in its offspring. The gene drive is a self-perpetuating process that repeats whenever the offspring mates with the wild population. And over time, the entire population of that species will all carry both the desired gene edit and the gene drive system. CRISPR-based gene drives can also ensure the inheritance of traits that disrupt reproduction, such as sterility, which could spread in a population and potentially lead to extinction. The application of CRISPR-based gene drives is most suited to sexually-reproducing species with short generation times, like most insects and some rodents.³²

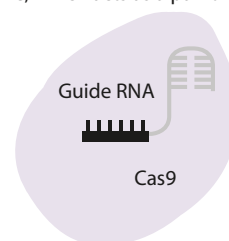
CRISPR-Cas9 genome editing technique

In nature, CRISPR-Cas9 is the bacteria's defense and immunity strategy against viral attacks, utilizing the system to precisely identify and cut the DNA of an invading virus, thus disabling the attack. Scientists have adapted the CRISPR-Cas9 mechanism for genome editing as it offers a more precise, relatively cheaper and faster way to modify a genome.

1
Scientists identify a section of DNA they want to modify.



2
Scientists then create a genetic sequence, called a guide RNA, that matches the targeted DNA section, and bind the guide RNA to the Cas9 enzyme, which acts as a pair of molecular scissors.



3
Guide RNA locates the targeted section and tells Cas9 where to cut.

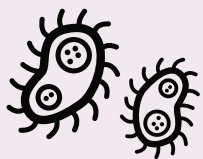


4
A new piece of DNA can be inserted at the site to replace the cut section.



Synthetic Biology

Sustainability applications



Many industries have made use of synthetic biology. Microorganisms, from bacteria to yeasts, are genetically engineered to become tiny factories producing more sustainable ingredients for medicines, vaccines, biofuels, green chemicals and new materials.

Pharmaceutical products



E. coli is altered to manufacture a **vaccine** against chlamydia, which is becoming more resistant to conventional antibiotics



Green and bio-based chemicals

A variety of chemicals in everyday products are derived from petroleum. Synthetic biology enables the production of substances that can replace petroleum-based chemicals.

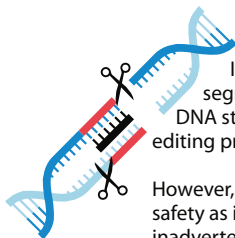
Alternatives to chemicals derived from unsustainable sources

The **blood of horseshoe crabs** is a major biomedical commodity used in pharmaceutical testing for bacterial contamination. A synbio substitute could reduce or replace the need for harvesting the nearly extinct species from the oceans.



Lactic acid, succinic acid and propanediol are among chemicals made by genetically engineered microbes that are commercially available in the global market

CRISPR-Cas9 genome editing technique



The discovery of CRISPR-Cas9 has changed the entire outlook of synthetic biology research. It enables scientists to cut out a particular DNA segment of a desired sequence or replace it with a new DNA strand. Many fields of medical research require such editing precision to revolutionize treatments.

However, the technique is also subject to scrutiny for its safety as it involves a potential off-target effect, whereby it inadvertently cuts out DNA that has a similar sequence to the targeted strand, potentially triggering cancer in edited cells.

Market and investment

US\$13.9 billion

Projected global market value of synthetic biology applications by 2022



US\$1.9 billion

2018 Global investment in synthetic biology startups



Do-It-Yourself Biology or DIY Bio

The movement of so-called 'citizen scientists' interested in performing synthetic biology experiments has gained significant traction globally. Biology enthusiasts – many without scientific background – meet in garage labs to conduct experiments using specialised DIY kits and simple protocols available online.

Some of the group have specialised equipment and hire professional staff to help citizen scientists, biohackers and biology enthusiasts in developing their projects.

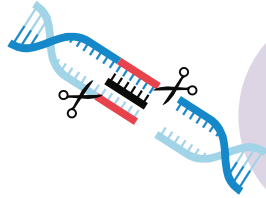
Risks and policy considerations

There are concerns that synthetic biology could be used to re-engineer existing pathogenic viruses, making them more dangerous or produce biochemicals with only modest resources and organizational footprint.

Synthetic biology presents new challenges that need to be addressed through the consolidated action of governmental and international bodies. Development of effective methods to better manage emerging risks is essential in ensuring technological safety.

Applications for conservation and public health

CRISPR-based gene drives may be key to addressing some global challenges, such as vector-borne diseases or invasive species, but they require multifaceted societal debate because of their power to modify, suppress or replace the entire population of the target species, bypassing the fundamental principles of evolution



Gene drives have been made possible by the development of CRISPR-Cas9 technology

Gene drives with suppression intent can force the inheritance of detrimental genetic alterations, such as sterility, potentially reducing the target population to zero. The suppression drive is intended to control the populations of malaria-carrying mosquitoes in the environment.



American chestnut trees are near extinction due to chestnut blight, a fungal disease native to Asia. Pending regulatory approval, the American chestnut can be engineered to be blight-resistant and spread in the wild.

The release of only a few gene-drive-bearing organisms into the environment can transform an entire species population and potentially the whole ecosystem

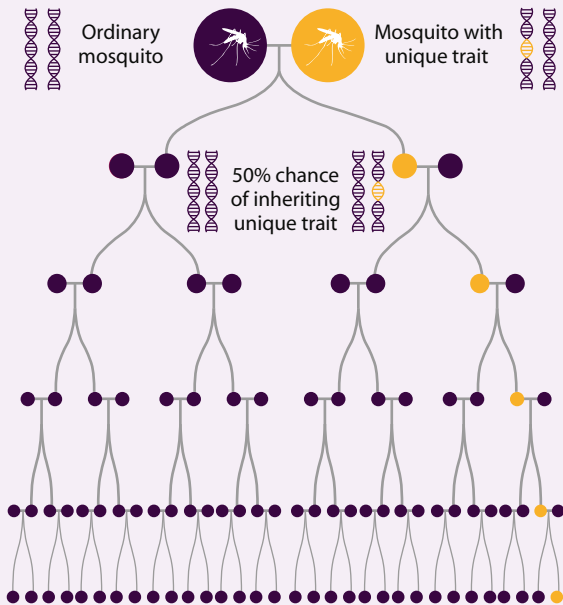


Genetic cross-contamination between species and unintended ecological damage are some of the legitimate concerns that have not yet been resolved

CRISPR-based gene drives: Manipulating the wild populations of plants and animals

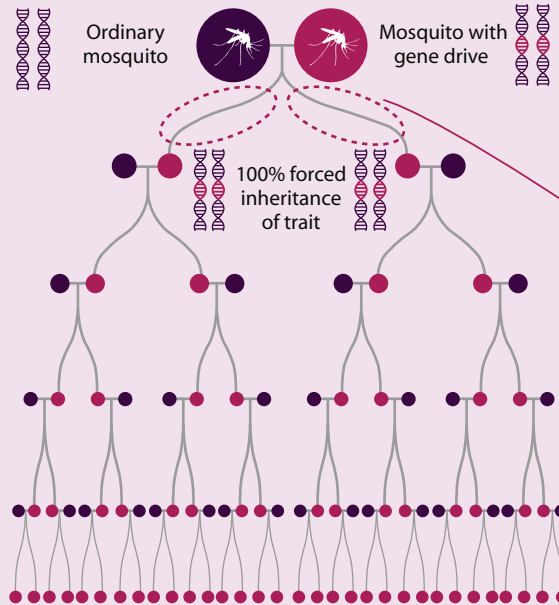
Normal inheritance

In sexual reproduction, each parent passes half its DNA to its offspring. A parent's unique genetic trait has a 50-50 chance of being inherited by the next generation. Over many generations the unique genetic character still remains in the population but at low frequency. The normal inheritance also applies to the case of an offspring produced by a normal parent and a classic GMO parent.



Gene drive inheritance

A synthetic gene drive circumvents the rules of normal genetic inheritance. This self-perpetuating mechanism is designed to ensure preferential inheritance of a modified genetic trait in future generations. Over time the entire population inherits the preferred engineered trait.



During fertilization, the offspring inherits one set of DNA from the ordinary parent and one containing the CRISPR-equipped gene drive from the genetically engineered parent. CRISPR-Cas9 looks for the target site in the ordinary DNA and cuts it.

When the cut DNA attempts to repair the damage, it copies the engineered strand containing the gene drive.

The offspring ends up having two copies of the genetically engineered DNA with gene-drive capability to pass on to future generations.

Applications redefined: From laboratory to ecosystem

Synthetic biology could indirectly benefit conservation efforts by allowing the development of artificial alternatives to commercial products normally sourced from the wild. For example, the blood of the horseshoe crab is a major biomedical commodity used to test pharmaceuticals for bacterial contamination. Unsustainable harvesting is pushing the species towards global extinction.³³ A synthetic substitute has been developed that could reduce or replace the need to harvest the endangered crabs.^{34,35} Likewise, engineered microbes and microalgae capable of producing alternatives to omega-3 oils could lessen pressure on declining wild fish stocks.³⁶

Conservation measures that propose a more direct application of the technology on target species have recently emerged. Releasing genetically engineered organisms into the environment could restore the health or enhance the resilience of damaged populations. For example, using an approach that predates CRISPR, scientists have synthesized the oxylate oxidase gene normally expressed by wheat, and forced its expression in the American chestnut tree. This gene can neutralize the toxin secreted by the blight that has driven the tree functionally extinct.^{37,38} Pending regulatory approval, blight-resistant chestnuts could be planted to re-establish this once-dominant species in eastern U.S. forests. Unlike genetically modified crops, where safety concerns largely centre around containment, the engineered American chestnut is deliberately designed to spread and flourish in the wider environment.

As climate change is predicted to increase rates of species extinction worldwide, CRISPR's availability is likely to hasten applications for ecosystem restoration.³⁹ Scientists have proposed using CRISPR for threatened species, such as corals that are under immense stress from increased ocean temperatures, acidification and pollution. Proof-of-concept CRISPR research is underway to rewrite coral genomes to express mutations that endow resilience.^{40,41} However, frameworks for field implementation of this research have yet to be developed.

 Video: Genetically modified mosquitoes



Video Link: <https://www.youtube.com/watch?v=zISTGkDyEEM>

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CRISPR-based strategies could also remove invasive species from threatened ecosystems. On many Pacific islands, for example, invasive rodents are decimating native bird populations.⁴² Through international collaboration, the Genetic Biocontrol of Invasive Rodents programme is developing CRISPR-based gene drives that would spread sterility.^{43,44} In New Zealand, CRISPR-based gene drives are being considered to help achieve the elimination of all invasive predators by 2050.⁴⁵ In Hawaii, gene drives have been proposed to reduce avian malaria spread by house mosquitoes that has caused serious declines in rare bird populations.^{46,47} However, recent research indicates that gene drives may face resistance and limited efficacy in wild mosquito populations.^{48,49}

It has even been suggested that extinct species could be resurrected for their ecological benefits, such as reviving a woolly-mammoth-like animal by gene editing the DNA of its closest living relative, the Asian elephant.^{50,51} Proposals for de-extinction of species are not only highly debatable, but also re-emphasize the importance of addressing the root cause of extinctions. Such possible genetic interventions, even if unrealized, encourage a valid debate on how biotechnology can support, coexist with, or undermine the goals of conservation.⁵²

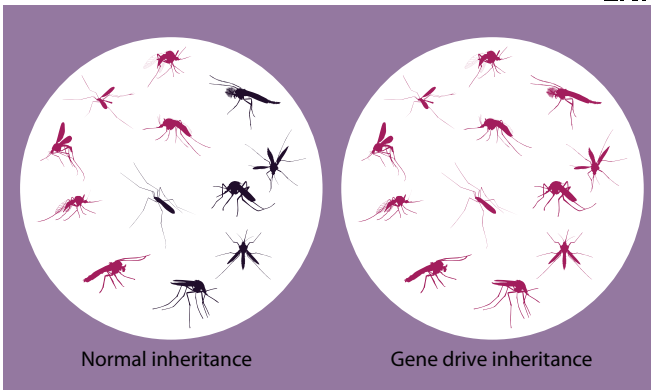


De-extinction

Attempts to revive species that have recently become extinct or are close to extinction have been made to date using back-breeding and cloning techniques.⁵⁸⁻⁶⁰ These approaches depend on the availability of tissues from extinct animals to clone, and extant species for crossbreeding or to serve as a surrogate.^{61,62} None of the de-extinction efforts have succeeded so far. Bringing back species that have long disappeared from the planet and left very little trace of their DNA is only remotely plausible. It would require the reconstruction of the entire genome and the existence of a closely related species for viable surrogacy. Even if the technological difficulties can one day be overcome, significant challenges remain in relation to how the de-extinct species would function in today's environment. Fundamental ecological concerns include the uncertainty of species competition and interaction; the susceptibility of de-extinct species to diseases and parasites; the possibilities of serving as a disease vector or becoming invasive species themselves; and the probability of establishing and sustaining a healthy population from individuals with low genetic diversity.⁶¹



Video: What is a gene drive?



Video Link: <https://www.youtube.com/watch?v=75IP50LEHrU>

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Video: Why horseshoe crab blood is so expensive



Video link: <https://www.youtube.com/watch?v=LgQZWSILBnA>
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To reduce the global disease burden, various synthetic biology strategies aim directly at suppressing populations of disease vectors. A company called Oxitec has genetically engineered mosquitoes to express a synthetic lethal gene and has released them in South America, South-East Asia, and several Caribbean nations to suppress the vector for Dengue fever, Zika virus, yellow fever, and chinkengunya.^{53,54} These so-called 'self-limiting' mosquitoes pass a lethal gene to their offspring, preventing them from surviving to adulthood. This method of suppression is, however, reversible without continual releases to sustain the engineered mosquito population in the wild. To circumvent this issue, Target Malaria, an international consortium funded by the Bill and Melinda Gates Foundation, is developing CRISPR-based gene drives to permanently control the malaria vector in sub-Saharan Africa.⁵⁵ CRISPR-based gene drives are highly invasive as, in theory, a one-time release of a few gene-drive-bearing organisms could completely suppress an entire wild population. Another strategy is to use gene drives that do not suppress the population, but instead limit the ability of mosquitoes to transmit pathogens.⁵⁶ CRISPR-based gene drives have also been devised to permanently immunize white-footed mice against Lyme disease on islands in Massachusetts, USA.⁵⁷

Innovating with wisdom

The release of genetically engineered organisms accidentally or intentionally into the environment has raised valid concerns about biosafety and unpredictable consequences. For organisms engineered in closed research or industrial facilities, containment procedures and enforced regulations on waste disposal help to avoid an escape, although this is never fail-proof.⁶³ In the case of intentional release, concerns over potential genetic cross-contamination between species, ecological interactions and impacts on ecosystems and their services remain largely unresolved.⁶⁴ Altering a disease carrier genetically could potentially cause a pathogen to evolve and become more virulent, or to be carried by a new vector.⁶⁵

To date, CRISPR-based gene drives have been tested only on small populations in controlled settings, with one recent experiment successfully collapsing the entire malaria-carrying mosquito population in the laboratory.⁶⁶ As a first step towards wider trials, Target Malaria has recently gained permission to release 10,000 modified mosquitoes in Burkina Faso. These specimens will be genetically engineered to be sterile, but with no gene drives, to test how well they compete with wild males.⁶⁷ However, such field trials to evaluate the efficacy of the gene-drive system could pose inherent risks.^{68,69}

Under the precautionary principle, stringent risk assessment and the inclusion of diverse stakeholder perspectives should be applied in the development and handling of innovative synthetic biology applications and products.^{19,70,71} The precautionary principle states that when human activities may lead to unacceptable harm that is scientifically plausible but uncertain, action should be taken to avoid or diminish that harm.⁷² A concept of substantial equivalence – that a genetically modified organism is as safe as its traditional counterpart – is often mentioned in conjunction with the precautionary principle.⁷³ Some countries have extensive policy and regulations in place concerning genetic engineering and research, while for others, non-functional regulatory systems, policy gaps and risk-assessment capacity are major challenges.⁷⁴⁻⁷⁷

Attempts have been made to identify, evaluate and address the ethical and biosafety concerns of synthetic biology. The U.S. National Academies of Science, Engineering,

and Medicine published a report on gene drives in 2016 highlighting the need for stringent environmental risk assessments and deliberation that charters human values and necessitates rigorous public engagement.¹⁹

In December 2017, the ad-hoc technical expert group on synthetic biology, established by the Parties to the Convention on Biological Diversity, concluded that organisms – developed or being developed through current methods of synthetic biology, including those containing gene drives – fall under the description of living modified organisms (LMOs), which are regulated under the legally-binding Cartagena Protocol.⁷⁸ With 171 Party nations, the Protocol applies the precautionary approach and requires that each Party take all necessary measures to ensure the safe handling, transport and use of the resulting LMOs.⁷⁹

SYNBIOSAFE, an EU-funded research project, was established to identify key issues in safety, security, risk management ethics and, importantly, the science–society interface, which emphasizes public education and dialogue among scientists, businesses, government, and ethicists.^{80,81} Some gene-drive developers have also proposed ethical research guidelines that emphasize the need for meaningful public engagement.⁸²



Video: Why is this African village letting mosquitoes in?



Video Link: <https://www.youtube.com/watch?v=ooYShrGtKUQ>
Photo credit: Dmitry Trashchenko / Shutterstock.com

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▶ Video: Could genetically engineered mice reduce Lyme disease?



Video Link: <https://www.youtube.com/watch?v=FOCNixYPsf4>
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Nevertheless, the intentional release of modified organisms and their potential to permanently transform wild species and cross international borders will likely test the limits of current policy, leading some environmental groups to call for a moratorium on all gene-drive research.⁸³ Other regulatory concerns focus on the potential use of synthetic biology for military offensive purposes.^{84,85}

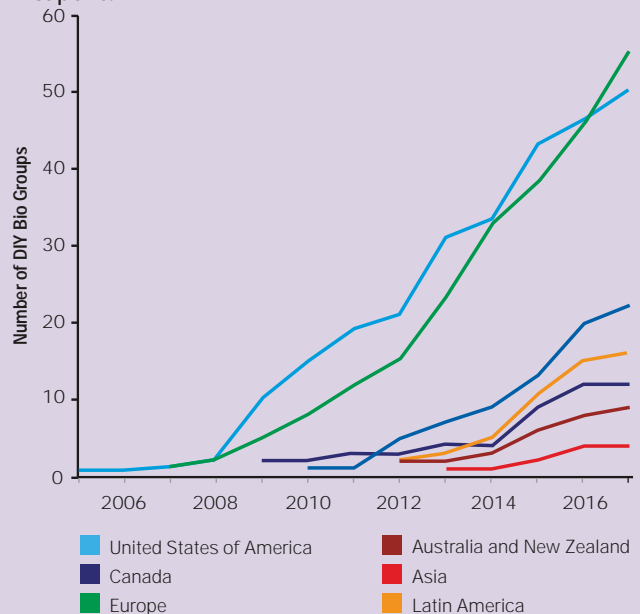
Current ethical frameworks may not be able to keep pace with the rapid progress of synthetic biology and its inherent complexity, especially concerning wild species.⁸⁶ Decisions to release engineered organisms into the wild will be shaped by the prevailing environmental ethic, or how a majority of citizens relate to non-human nature.⁸⁷ Altering the genetic code of wildlife is seen by some as a gross overstep by humans, echoing concerns about genetically modified crops. Others may feel that there is a moral responsibility to use a technology that could save lives or restore damaged ecosystems.⁸⁷ These contrasting value systems require responsible decision-making for resolution.⁸⁹ Synthetic biology applications also raise questions of who has ownership of an LMO and its genome, what protection is available for vulnerable communities, and how to ensure those most impacted have a voice. It is crucial that balanced and inclusive deliberative forums steer the field of synthetic biology and ensure that its environmental applications are used to the benefit of all on our shared planet.



Citizen scientists, biohackers and garage labs

Synthetic biology and genome editing have attracted interest not only from companies, but also regular citizens. Do-It-Yourself Biology, also known as “DIY Bio”, the movement of “citizen scientists” interested in synthetic biology experiments has become an international phenomenon over the last decade. Often with little prior knowledge of the field, enthusiasts meet in makeshift labs to take crash courses in biotechnology and conduct hands-on experiments.^{90,91} Simple protocols found online and specialized kits costing US\$150–1,600 have driven the movement’s rapid expansion.

DIY Bio labs can be found in most major cities and by 2017 there were about 168 groups worldwide.^{92,93} Regulating the use of easily accessible and low-cost technologies like CRISPR and gene editing kits will likely be a challenge for authorities. There is also growing concern that the technology could be misused by terrorists to destroy agricultural crops or turn harmless microbes into biological weapons.⁹⁴



Source: The Brookings Institute⁹³

References

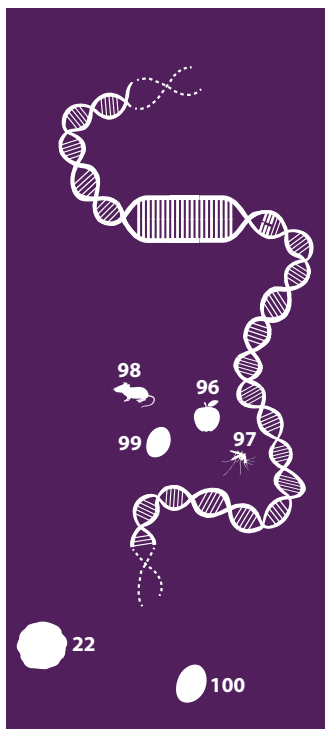
- International Union for Conservation of Nature (2018). The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/>
- World Health Organization (2017). *Global vector control response 2017-2030*. Geneva. <http://www.who.int/vector-control/publications/global-control-response/en/>
- Scheffers, B.R., De Meester, L., Bridge, T.C., Hoffmann, A.A., Pandolfi, J.M., Corlett, R.T., *et al.* (2016). The broad footprint of climate change from genes to biomes to people. *Science* 354(6313), aaf7671. <https://doi.org/10.1126/science.aaf7671>
- Heo, M.J., Jung, H.M., Um, J., Lee, S.W. and Oh, M.K. (2017). Controlling citrate synthase expression by CRISPR/Cas9 genome editing for n-butanol production in *Escherichia coli*. *ACS Synthetic Biology* 6(2), 182-189. <https://doi.org/10.1021/acssynbio.6b00134>
- Raj, K., Partow, S., Correia, K., Khusnutdinova, A.N., Yakunin, A.F. and Mahadevan, R. (2018). Biocatalytic production of adipic acid from glucose using engineered *Saccharomyces cerevisiae*. *Metabolic Engineering Communications* 6, 28-32. <https://doi.org/10.1016/j.meten.2018.02.001>
- Averesch, N.J.H., Martínez, V.S., Nielsen, L.K. and Krömer, J.O. (2018). Toward synthetic biology strategies for adipic acid production: An *in silico* tool for combined thermodynamics and stoichiometric analysis of metabolic networks. *ACS Synthetic Biology* 7(2), 490-509. <https://doi.org/10.1021/acssynbio.7b00304>
- Peplow, M. (2016). Synthetic biology's first malaria drug meets market resistance. *Nature News*, 23 February. Doi: 10.1038/530390a. <https://www.nature.com/news/synthetic-biology-s-first-malaria-drug-meets-market-resistance-1.19426>
- Kelley, N.J., Whelan, D.J., Kerr, E., Apel, A., Beliveau, R. and Scanlon, R. (2014). Engineering biology to address global problems: Synthetic biology markets, needs, and applications. *Industrial Biotechnology* 10, 140-149. <https://www.liebertpub.com/doi/pdf/10.1089/ind.2014.1515>
- McEachran, R. (2015). Creators defend vanilla flavour made using synthetic biology. *The Guardian*, 28 May 2015. <https://www.theguardian.com/sustainable-business/2015/may/28/creators-defend-vanilla-flavour-made-using-synthetic-biology>
- Bhanawase, S.L. and Yadav, G.D. (2017). Novel silica-encapsulated Cu-Al hydrotalcite catalyst: oxidative decarboxylation of vanillyl mandelic acid to vanillin in water at atmospheric pressure. *Industrial & Engineering Chemistry Research* 56(45), 12899-12908. <https://pubs.acs.org/doi/10.1021/acs.iecr.6b04982>
- Purcell, B.P., Williamson, D.T., Marga, F.S., Shofer, S.J. and Cassingham, D.M. (2016). Method for making a biofabricated material containing collagen fibrils. International Patent Application No. PCT/US2017/017889, filed 15 February 2017. <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017142896&tab=PCTBIBLIO&maxRec=1000>
- Amyris (2018). *Amyris Aprinnova joint venture launches pharmaceutical grade Neosance Squalane USP — opens new market among FDA regulated products*. 8 February. <http://investors.amyris.com/news-releases/news-release-details/amyris-aprinnova-joint-venture-launches-pharmaceutical-grade>
- Le Feuvre, R.A. and Scrutton, N.S. (2018). A living foundry for synthetic biological materials: a synthetic biology roadmap to new advanced materials. *Synthetic and Systems Biotechnology*, 3, 105-112. <https://doi.org/10.1016/j.synbio.2018.04.002>
- Barrangou, R. and Doudna, J.A. (2016). Applications of CRISPR technologies in research and beyond. *Nat Biotechnol* 34, 933-941. <https://doi.org/10.1038/nbt.3659>
- Piaggio, A.J., Segelbacher, G., Seddon, P.J., Alphey, L., Bennett, E.L., Carlson, R.H. *et al.* (2017). Is it time for synthetic biodiversity conservation? *Trends in Ecology & Evolution* 32, 97-107. <https://doi.org/10.1016/j.tree.2016.10.016>
- Redford, K.H., Adams, W., Carlson, R., Mace, G.M. and Ceccarelli, B. (2014). Synthetic biology and the conservation of biodiversity. *Oryx* 48, 330-336. <https://doi.org/10.1017/S0030605314000040>
- Esvelt, K.M. and Gemmell, N.J. (2017). Conservation demands safe gene drive. *PLOS Biology* 15, e2003850. <https://doi.org/10.1371/journal.pbio.2003850>
- Nuffield Council on Bioethics (2012). *Emerging biotechnologies: technology, choice and the public good*. London. http://nuffieldbioethics.org/wp-content/uploads/2014/07/Emerging_biotechnologies_full_report_web_0.pdf
- National Academies of Sciences, Engineering, and Medicine (2016). *Gene drives on the horizon: Advancing science, navigating uncertainty, and aligning research with public values*. Washington DC: The National Academies Press. <https://doi.org/10.17226/23405>
- Convention on Biological Diversity (2016). Decision adopted by the Conference of the Parties to the Convention on Biological Diversity XIII/17 Synthetic biology. 16 December. CBD/COP/DEC/XIII/17. <https://www.cbd.int/doc/decisions/cop-13/cop-13-dec-17-en.pdf>
- Cohen, S.N., Chang, A.C.Y., Boyer, H.W. and Helling, R.B. (1973) *Construction of biologically functional bacterial plasmids in vitro*. Proceedings of the National Academy of Sciences of the United States of America 70, 3240-3244
- Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., Algire, M.A. *et al.* (2010). Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329(5987), 52-56. Doi: 10.1126/science.1190719. <http://science.sciencemag.org/content/329/5987/52>
- Sternberg, S.H. and Doudna, J.A. (2015). Expanding the biologist's toolkit with CRISPR-Cas9. *Molecular Cell* 58(4), 568-574. <https://doi.org/10.1016/j.molcel.2015.02.032>
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096), 816-821. <https://doi.org/10.1126/science.1225829>
- Kim, Y.G., Cha, J. and Chandrasegaran, S. (1996). *Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain*. Proceedings of the National Academy of Sciences 93, 1156-1160. <http://www.pnas.org/content/93/3/1156>
- Wei, C., Liu, J., Yu, Z., Zhang, B., Gao, G. and Jiao, R. (2013). TALEN or Cas9 - rapid, efficient and specific choices for genomic modifications. *Journal of Genetics and Genomics* 40, 281-289. <https://doi.org/10.1016/j.jgg.2013.03.013>
- Horvath, P. and Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. *Science* 327(5962), 167-170. <https://doi.org/10.1126/science.1179555>
- Rath, D., Amlinger, L., Rath, A. and Lundgren, M. (2015). The CRISPR-Cas immune system: Biology, mechanisms and applications. *Biochimie* 117, 119-128. <https://doi.org/10.1016/j.biochi.2015.03.025>

29. Hsu, P.D., Lander, E.S. and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 157(6), 1262-1278. <https://doi.org/10.1016/j.cell.2014.05.010>
30. Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J. *et al.* (2017). RNA editing with CRISPR-Cas13. *Science* 358(6366), 1019-1027. <https://doi.org/10.1126/science.aqa0180>
31. Esvelt, K.M., Smidler, A.L., Catteruccia, F. and Church, G.M. (2014). Concerning RNA-guided gene drives for the alteration of wild populations. *eLife* 3, e03401. <https://doi.org/10.7554/eLife.03401>
32. Champer, J., Buchman, A. and Akbari, O.S. (2016). Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nature Reviews Genetics* 17(3), 146-159. <https://doi.org/10.1038/nrg.2015.34>
33. Smith, D.R., Brockmann, H.J., Beekey, M.A., King, T.L., Millard, M.J. and Zaldivar-Rae, J. (2017). Conservation status of the American horseshoe crab (*Limulus polyphemus*): a regional assessment. *Reviews in Fish Biology and Fisheries* 27(1), 135-175. <https://doi.org/10.1007/s11160-016-9461-y>
34. Ding, J.L. and Ho, B. (2010). Endotoxin detection - from *Limulus* amoebocyte lysate to recombinant factor C. *Subcell Biochem* 53, 187-208. https://doi.org/10.1007/978-90-481-9078-2_9
35. Zhang, S. (2018). *The last days of the blue-blood harvest*. The Atlantic, May 9. <https://www.theatlantic.com/science/archive/2018/05/blood-in-the-water/559229/>
36. Sprague, M., Betancor, M.B. and Tocher, D.R. (2017). Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. *Biotechnology Letters* 39(11), 1599-1609. <https://doi.org/10.1007/s10529-017-2402-6>
37. Newhouse, A.E., Polin-McGuigan, L.D., Baier, K.A., Valletta, K.E.R., Rottmann, W.H., Tschaplinski, T.J. *et al.* (2014). Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. *Plant Science* 228, 88-97. <https://doi.org/10.1016/j.plantsci.2014.04.004>
38. Steiner, K.C., Westbrook, J.W., Hebard, F.V., Georgi, L.L., Powell, W.A. and Fitzsimmons, S.F. (2017). Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. *New Forests* 48, 317-336. <https://www.sciencedirect.com/science/article/pii/S016894521400079X>
39. Urban, M.C. (2015). Accelerating extinction risk from climate change. *Science* 348, 571-573. <https://doi.org/10.1126/science.aaa4984>
40. Van Oppen, M.J.H., Oliver, J.K., Putnam, H.M. and Gates, R.D. (2015). *Building coral reef resilience through assisted evolution*. *Proceedings of the National Academy of Sciences* 112, 2307-2313. <https://doi.org/10.1073/pnas.1422301112>
41. Cleves, P.A., Strader, M.E., Bay, L.K., Pringle, J.R. and Matz, M.V. (2018). *CRISPR/Cas9-mediated genome editing in a reef-building coral*. *Proceedings of the National Academy of Sciences*. <https://doi.org/10.1073/pnas.1722151115>
42. Harper, G.A. and Bunbury, N. (2015). Invasive rats on tropical islands: Their population biology and impacts on native species. *Global Ecology and Conservation*, 3, 607-6027. <https://doi.org/10.1016/j.gecco.2015.02.010>
43. Leitschuh, C.M., Kanavy, D., Backus, G.A., Valdez, R.X., Serr, M., Pitts, E.A. *et al.* (2018). Developing gene drive technologies to eradicate invasive rodents from islands. *Journal of Responsible Innovation* 5, 121-138. <https://doi.org/10.1080/232299460.2017.1365232>
44. The Genetic Biocontrol of Invasive Rodents (2018). GBIRD program. <http://www.geneticbiocontrol.org>
45. Predator free New Zealand (2018). Predator free NZ. <https://predatorfree.nz.org>
46. Paxton, E.H., Camp, R.J., Gorresen, P.M., Crampton, L.H., Leonard, D.L. Jr. and VanderWerf, E.A. (2016). Collapsing avian community on a Hawaiian island. *Science Advances* 2(9), e1600029. <http://advances.sciencemag.org/content/2/9/e1600029>
47. Regalado, A. (2016). The plan to rescue Hawaii's birds with genetic engineering. *MIT Technology Review*, 11 May. <https://www.technologyreview.com/s/601383/the-plan-to-rescue-hawaiis-birds-with-genetic-engineering/>
48. Hammond, A.M., Kyrou, K., Bruttini, M., North, A., Galizi, R., Karlsson, X. *et al.* (2017). The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito. *PLoS Genet* 13(10), e1007039. <https://doi.org/10.1371/journal.pgen.1007039>
49. Shaw, W.R. and Catteruccia, F. (2018). Vector biology meets disease control: using basic research to fight vector-borne diseases. *Nature Microbiology*. <https://doi.org/10.1038/s41564-018-0214-7>
50. Zimov, S.A., Zimov, N.S., Tikhonov, A.N. and Chapin, F.S. (2012). Mammoth steppe: a high-productivity phenomenon. *Quaternary Science Reviews* 57, 26-45. <https://doi.org/10.1016/j.quascirev.2012.10.005>
51. Shapiro, B. (2015). Mammoth 2.0: will genome engineering resurrect extinct species? *Genome Biology* 16, 228. <https://doi.org/10.1186/s13059-015-0800-4>
52. Kaebnick, G.E. and Jennings, G. (2017). De-extinction and conservation. *Hastings Center Report* 47(4), S2-S3. <https://doi.org/10.1002/hast.744>
53. Phuc, H.K., Andreasen, M.H., Burton, R.S., Vass, C., Epton, M.J., Pape, G. *et al.* (2007). Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol* 5, 11. <https://doi.org/10.1186/1741-7007-5-11>
54. Harris, A.F., McKemey, A.R., Nimmo, D., Curtis, Z., Black, I., Morgan, S.A. *et al.* (2012). Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat Biotechnol* 30, 828-830. <https://doi.org/10.1038/nbt.2350>
55. Target Malaria (2017). Our work. <http://targetmalaria.org/our-work/>
56. Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F. *et al.* (2011). Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476, 454-457. <https://doi.org/10.1038/nature10356>
57. MIT Media Lab (2017). Preventing tick-borne disease by permanently immunizing mice. <https://www.media.mit.edu/projects/preventing-tick-borne-disease-by-permanently-immunizing-mice/overview/>
58. Folch, J., Cocero, M.J., Chesné, P., Alabart, J.L., Domínguez, V., Coglié, Y. *et al.* (2009). First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. *Theriogenology*, 71(6), 1026-1034. <https://doi.org/10.1016/j.theriogenology.2008.11.005>
59. Shapiro, B. (2016). Pathways to de-extinction: how close can we get to resurrection of an extinct species? *Functional Ecology*. <http://dx.doi.org/10.1111/1365-2435.12705>
60. Stokstad, E. (2015). Bringing back the aurochs. *Science*, 350, 1144-1147. <https://doi.org/10.1126/science.350.6265.1144>
61. Richmond, D.J., Sinding, M.H.S. and Gilbert, M.T.P. (2016). The potential and pitfalls of de-extinction. *Zoologica Scripta*, 45, 22-36. <https://doi.org/10.1111/zsc.12212>

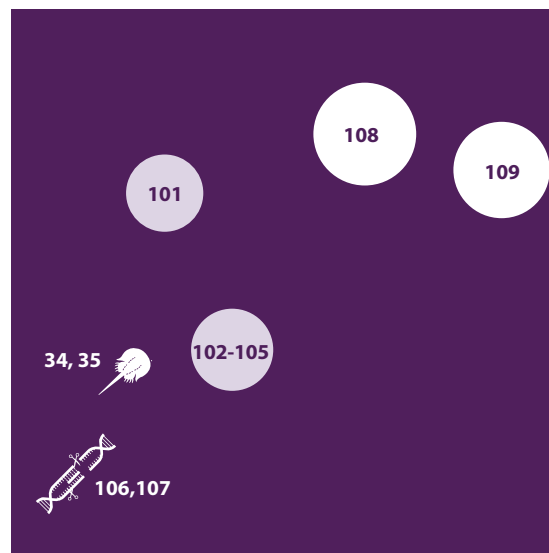
62. Sherkow, J.S. and Greely, H.T. (2013). What if extinction is not forever? *Science* 340(6128), 32-33. <https://doi.org/10.1126/science.1236965>
63. Moe-Behrens, G.H.G., Davis, R. and Haynes, K.A. (2013). Preparing synthetic biology for the world. *Front Microbiol* 4, 5. <https://doi.org/10.3389/fmicb.2013.00005>
64. Hayes, K.R., Hosack, G.R., Dana, G.V., Foster, S.D., Ford, J.H., Thresher, R. et al. (2018). Identifying and detecting potentially adverse ecological outcomes associated with the release of gene-drive modified organisms. *Journal of Responsible Innovation* 5(51), 5139–5158. <https://doi.org/10.1080/23299460.2017.1415585>
65. David, A.S., Kaser, J.M., Morey, A.C., Roth, A.M. and Andow, D.A. (2013). Release of genetically engineered insects: a framework to identify potential ecological effects. *Ecology and Evolution* 3(11), 4000-4015. <https://doi.org/10.1002/ece3.737>
66. Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K. et al. (2018). A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nature Biotechnology*, 36, 1062-1066. <http://dx.doi.org/10.1038/nbt.4245>
67. Alliance for Science (2018). African scientists confident GMO mosquitoes will be game changer in fight to control malaria, September 13. <https://alliancefor-science.cornell.edu/blog/2018/09/african-scientists-confident-gmo-mosquitoes-will-game-changer-fight-control-malaria/>
68. Akbari, O.S., Bellen, H.J., Bier, E., Bullock, S.L., Burt, A., Church, G.M. et al. (2015). Safeguarding gene drive experiments in the laboratory. *Science* 349(6251), 927. <https://doi.org/10.1126/science.aac7932>
69. James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., Godfray, H.C.J., Gottlieb, M. et al. (2018). Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: Recommendations of a Scientific Working Group. *The American Journal of Tropical Medicine and Hygiene* 98(6_Suppl), 1-49. <https://doi.org/10.4269/ajtmh.18-0083>
70. Kwok, R. (2010) Five hard truths for synthetic biology. *Nature* 463, 288-290. <https://doi.org/10.1038/463288a>
71. Kaebnick, G.E., Heitman, E., Collins, J.P., Delborne, J.A., Landis, W.G., Sawyer, K. et al. (2016) Precaution and governance of emerging technologies. *Science* 354, 710-711. <http://dx.doi.org/10.1126/science.aah5125>
72. Kriebel, D., Tickner, J., Epstein, P., Lemons, J., Levins, R., Loechler, E.L. et al. (2001). The precautionary principle in environmental science. *Environmental Health Perspectives* 109, 871-876. <https://ehp.niehs.nih.gov/doi/10.1289/ehp.01109871>
73. Organisation for Economic Co-operation and Development (1993) *Safety evaluation of foods derived by modern biotechnology: concepts and principles*. Paris: OECD.
74. Oye, K.A., Esvelt, K., Appleton, E., Catteruccia, F., Church, G., Kuiken, T. et al. (2014) Regulating gene drives. *Science* 345, 626-628. <https://doi.org/10.1126/science.1254287>
75. Douglas, C.M.W. and Stemerding, D. (2014) Challenges for the European governance of synthetic biology for human health. *Life Sciences, Society and Policy* 10, 6. <https://doi.org/10.1186/s40504-014-0006-7>
76. Trump, B.D. (2017). Synthetic biology regulation and governance: Lessons from TAPIC for the United States, European Union, and Singapore. *Health Policy* 121, 1139-1146. <https://doi.org/10.1016/j.healthpol.2017.07.010>
77. Glover, B., Akinbo, O., Savadogo, M., Timpo, S., Lemgo, G., Sinebo, W. et al. (2018). Strengthening regulatory capacity for gene drives in Africa: leveraging NEPAD's experience in establishing regulatory systems for medicines and GM crops in Africa. *BMC Proc.* 12(8). <https://doi.org/10.1186/s12919-018-0108-y>
78. Convention on Biological Diversity (2017). *Report of the ad hoc technical expert group on synthetic biology*. Montreal, Canada, 5-8 December 2017. CBD/SYNBIO/AHTEG/2017/1/3. <https://www.cbd.int/doc/c/aa10/9160/6c3fcedf265db/ee686715016/synbio-ahteg-2017-01-03-en.pdf>
79. Convention on Biological Diversity (2018). The Cartagena Protocol on Biosafety. Convention on Biological Diversity, Montreal. <http://bch.cbd.int/protocol>
80. Schmidt, M., Torgesen, H., Ganguli-Mitra, A., Kelle, A., Deplazes, A. and Biller-Andorno, N. (2008). SYNBIOSAFE e-conference: online community discussion on the societal aspects of synthetic biology. *Systems and Synthetic Biology* 2, 7-17. <https://doi.org/10.1007/s11693-008-9019-y>
81. Schmidt, M., Kelle, A., Ganguli-Mitra, A. and de Vriend, H. (2009). *Synthetic Biology: the technoscience and its societal consequences*. Springer, Netherlands. <https://doi.org/10.1007/978-90-481-2678-1>
82. Emerson, C., James, S., Littler, K. and Randazzo, F. (2017). Principles for gene drive research. *Science*, 358, 1135-1136. <https://doi.org/10.1126/science.aap9026>
83. ETC Group. (2016). Reckless driving: gene drives and the end of nature, 1 September. <http://www.etcgroup.org/content/reckless-driving-gene-drives-and-end-nature>
84. Callaway, E. (2017). US defence agencies grapple with gene drives. *Nature News*, 21 July. <https://doi.org/10.1038/nature.2017.22345>
85. Defense Advanced Research Projects Agency (2018). Safe Genes program, DARPA. <https://www.darpa.mil/program/safe-genes>
86. Kaebnick, G.E., Gusmano, M.K. and Murray, T.H. (2014). The ethics of synthetic biology: next steps and prior questions. *Hastings Center Report* 44, 54-526. <https://doi.org/10.1002/hast.392>
87. Batavia, C. and Nelson, M.P. (2017). For goodness sake! What is intrinsic value and why should we care? *Biological Conservation* 209, 366-376. <http://dx.doi.org/10.1016/j.biocon.2017.03.003>
88. Kaebnick, G.E. (2017). The spectacular garden: where might de-extinction lead? *Hastings Center Report* 47, S60-S64. <https://doi.org/10.1002/hast.754>
89. Kofler, N., Collins, J.P., Kuzma, J., Marris, E., Esvelt, K., Nelson, M.P. et al. (2018). Editing nature: Local roots of global governance. *Science* 362(6414), 527-529. <https://doi.org/10.1126/science.aat4612>
90. Ledford, H. (2010). Garage biotech: Life hackers. *Nature* 467, 650-652. <https://doi.org/10.1038/467650a>
91. Regalado, A. (2017). One man's quest to hack his own genes. *MIT Technology Review*, January 10. <https://www.technologyreview.com/s/603217/one-mans-quest-to-hack-his-own-genes/>
92. Ochoa Cruz, E.A., de la Barrera Benavidez, O.J., Giménez, M., Chavez, M. and Van Sluys, M-A. (2016). The biohacking landscape in Latin America. *BioCoder* 10, 5-12. <https://www.oreilly.com/ideas/biohacking-latin-america>.
93. Kolodziejczyk, B. (2017). Do-it-yourself biology shows safety risks of an open innovation movement. Brookings Institution, October 9. <https://www.brookings.edu/blog/techtank/2017/10/09/do-it-yourself-biology-shows-safety-risks-of-an-open-innovation-movement>

94. United Nations (2018). Terrorists potentially target millions in makeshift biological weapons 'laboratories', UN forum hears. UN News, 17 August 2018. United Nations, New York. <https://news.un.org/en/story/2018/08/1017352>
95. National Human Genome Research Institute (NHGRI). (2002). International Team of Researchers Assembles Draft Sequence of Mouse Genome. <https://www.genome.gov/10002983/2002-release-draft-sequence-of-mouse-genome>

Graphic references



96. Daccord, N., Celton, J., Linsmith, G., Becker, C., Choise, N., Schijlen, E., van de Geest, H., et al. (2017). High-quality *de novo* assembly of the apple genome and methylome dynamics of early fruit development. *Nature Genetics*, 49(7), 1099-1106. <https://doi.org/10.1038/ng.3886>
97. Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., et al. (2002). The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science*, 298(5591), 129-149. <https://doi.org/10.1126/science.1076181>
98. Cooper, G. (2000). *The Cell: A Molecular Approach*. 2nd ed. Sunderland, MA: Sinauer Associates.
99. Annaluru, N., Muller, H., Mitchell, L., Ramalingam, S., Stracquadanio, G., Richardson, S., Dymond, J., et al. (2014). Total Synthesis of a Functional Designer Eukaryotic Chromosome. *Science*, 344(6179), 55-58. <https://doi.org/10.1126/science.1249252>



100. SAVI (2019). Synthetic yeast 2.0. The Science Across Virtual Institutes program. <http://syntheticyeast.org>
101. He, W., Felderman, M., Evans, A., Geng, J., Homan, D., Bourguet, F., Fischer, N., et al. (2017). Cell-free production of a functional oligomeric form of a Chlamydia major outer-membrane protein (MOMP) for vaccine development. *Journal of Biological Chemistry*, 292(36), 15121-15132. <https://doi.org/10.1074/jbc.M117.784561>
102. Woodrow Wilson Center (2019). Synthetic biology project. <http://www.synbio-project.org/cpi/applications/>
103. Reverdia (2019). Biosuccinium® sustainable succinic acid. <https://reverdia.com/biosuccinium-menu/biosuccinium/>
104. GC Innovation America (2019). Biotechnology Research & Development. <https://www.gcinnovationamerica.com/biocatalyst-rd/>
105. DuPont Tate & Lyle Bio Products Company (2019). Susterra® Propanediol. <http://duponttateandlyle.com/susterra>
106. Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K., Kommineni, S., et al. (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nature Medicine*, 24, 939-946. <https://doi.org/10.1038/s41591-018-0050-6>
107. Haapaniemi, E., Botla, S., Persson, J., Schmierer, B. and Taipale, J. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nature Medicine*, 24, 927-930. <https://doi.org/10.1038/s41591-018-0049-z>
108. BCC Research (2018). Synthetic Biology Global Markets to Reach \$13.9 Billion by 2022. [https://www.bccresearch.com/pressroom/bio/synthetic-biology-global-markets-to-reach-\\$139-billion-by-2022](https://www.bccresearch.com/pressroom/bio/synthetic-biology-global-markets-to-reach-$139-billion-by-2022)
109. Cumbers, J. and Bünger, M. (2019). Synthetic Biology Annual Investment Report (2018) - SynBioBeta. SynBioBeta.com. <https://synbiobeta.com/synthetic-biology-industry-strategy-reports/investment-report-2018>