# Synthetic Biology: Considerations for Biosafety Professionals

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# Statement

- The views included in this presentation represent the views of the author, and do not necessarily represent the views of the author's employer.
- Please direct any questions or comments regarding this presentation to David.Gillum@asu.edu.

# Outline

- Engineers, biologists, chemists, oh my!
- A few related quotes
- Definition of synthetic biology
- Global spending on synthetic biology
- Biosafety, biosecurity, and ethical concerns
- Parting thoughts
- Acknowledgements

# Engineering The World

- “A Scientist discovers that which exists; an Engineer creates that which never was.”

  - Theodore von Karmen

# Experts and DIY

- Individuals working in the field of synthetic biology:
  - "Experts"
    - Engineers, biologists, chemists, mathematicians, programmers, etc.
  - Citizen scientists
  - Do-It-Yourself (DIY) practitioners. (1)

(1) Grushkin, D, Kuiken, T, and P Millet. "Seven Myths & Realities about Do-It-Yourself Biology." Synthetic Biology Project; 5 Nov 2013. http://www.synbioproject.org/publications/6676/

*"Home-brewed heroin may soon be in the works"*

*–The Economist. May 23, 2015*

(1) http://www.economist.com/node/21651571/print

(2) http://www.nature.com/nchembio/journal/vaop/ncurrent/full/nchembio.1816.html

*"Gene-editing record smashed in pigs"*

*– Scientific American, October 7, 2015*

(5) http://www.scientificamerican.com/article/gene-editing-record-smashed-in-pigs/

*"Diffusion of synthetic biology: a challenge to biosafety"*

*–Markus Schmidt. July 9, 2008*

(3) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2671588/

*"Synthetic biology needs safety mechanisms"*

*– Science 2.0. September 17, 2015*

(6) http://www.science20.com/news_articles/synthetic_biology_needs_safety_mechanisms-157182

*"Synthetic biology is not just good, it's good for you"*

*TechCrunch, September 28, 2015*

(4) http://techcrunch.com/2015/09/28/synthetic-biology-is-not-just-good-its-good-for-you/

*"First self-replicating synthetic bacterial cell"*

*– J. Craig Venter Institute. May 20, 2010*

(7) J. Craig Venter Institute. http://www.jcvi.org/cms/press/press-releases/full-text/article/first-self-replicating-synthetic-bacterial-cell-constructed-by-j-craig-venter-institute-researcher/

*"How scientists are creating synthetic life from scratch"*

*Vox, June 20 2014*

(8) http://www.vox.com/2014/6/20/5815582/synthetic-biology-genetic-engineering-explainer

# Definition

"Synthetic Biology is:

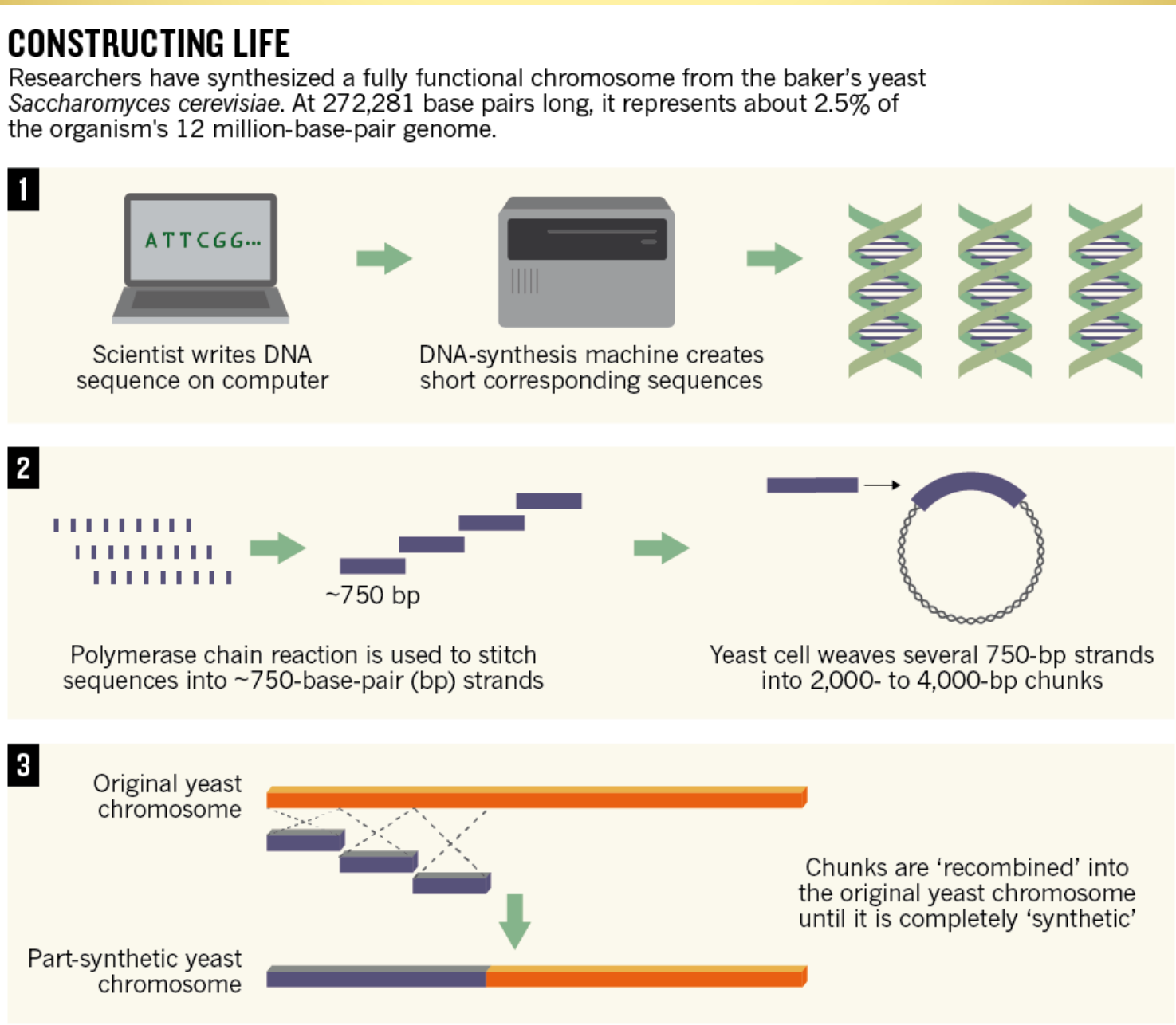
- A) the design and construction of new biological parts, devices, and systems, and
- B) the re-design of existing, natural biological systems for useful purposes." (9)

(9) www.SyntheticBiology.org

# GE vs. SB

- Genetic Engineering vs. Synthetic Biology [10]
  - Adding or modifying a single gene using conventional genetic engineering techniques is generally NOT considered synthetic biology.
  - Adding a whole suite of genes or creating an entirely new genetic code that doesn't exist in nature is synthetic biology.
  - Using synthetically-created nucleic acids, parts, and devices is also considered synthetic biology.

(10) How scientists are creating synthetic life from scratch. Vox, June 20 2014. http://www.vox.com/2014/6/20/5815582/synthetic-biology-genetic-engineering-explainer




(11) First synthetic yeast chromosome revealed. *Nature News.* http://www.nature.com/news/first-synthetic-yeast-chromosome-revealed-1.14941

# Global Value ($ Million)

| BCC Research, 2011 [12] | 2010 | 2011 | 2016 | CAGR* |
|---|---|---|---|---|
| **Diagnostics / pharmaceuticals** | $902.1 | $1,314.7 | $5,373.3 | 32.5% |
| **Chemicals** | $125.4 | $185.0 | $2,783.9 | 72% |
| **Research and Development** | $73.1 | $82.8 | $265.4 | 26.2% |
| **Agriculture** | $26.7 | $36.1 | $307.9 | 53.5% |
| **Energy** | $19.6 | $25.8 | $2,108.1 | 141.2% |
| **Total** | $1,146.9 | $1,644.4 | $10,838.6 | 25.8% |

* Compound Annual Growth Rate

(12) Synthetic Biology. Global Emerging Markets, BIO0bbB, BCC Research; ISBN: 1-59623-834-8, November 2011.

# Examples of Industry

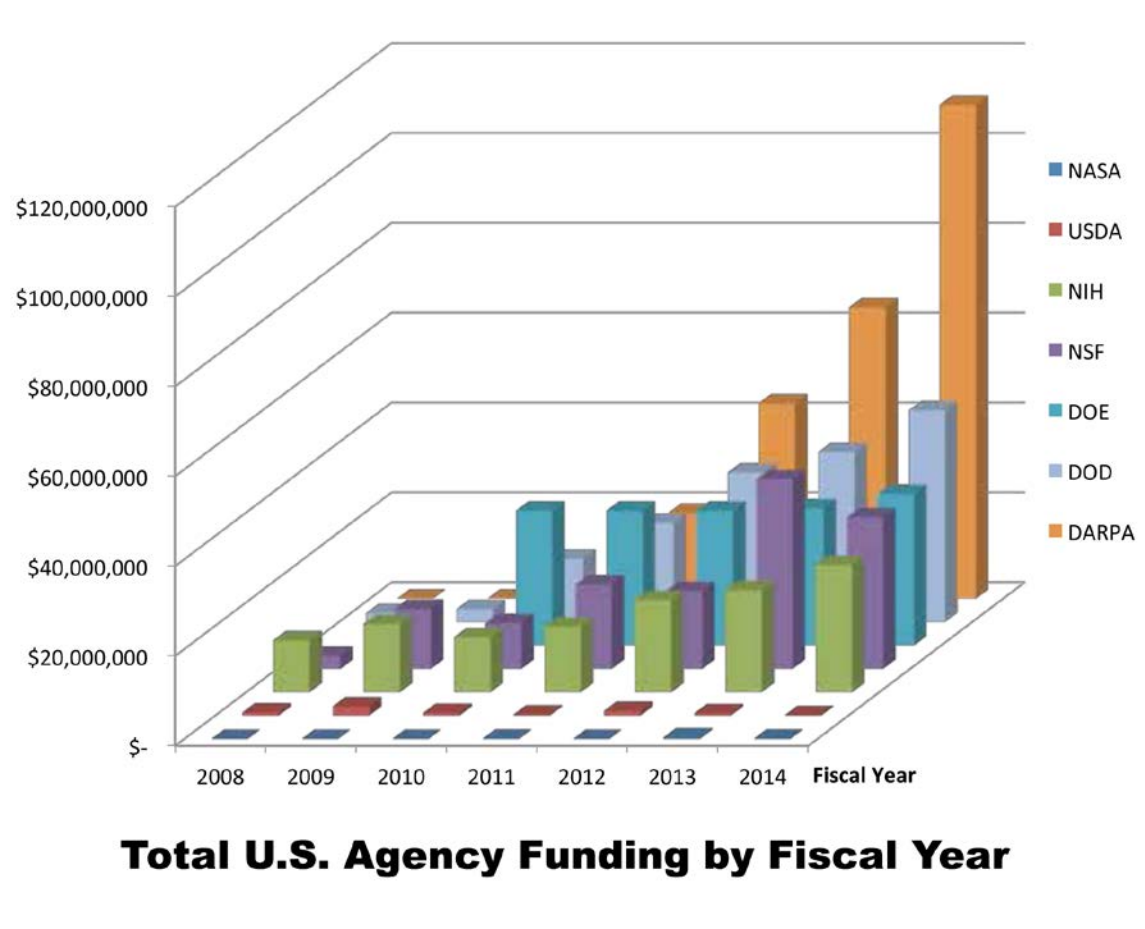

(13) Haynes, K. 2015. Arizona State University. http://haynes.lab.asu.edu.

# $$ SynBio Spending $$

- ~ $820 million dollars spent on synthetic biology research from 2008-2014 in U.S. (14)
- Defense Advanced Research Projects Agency (DARPA) has increased funding from…
  - $0 in 2010 to
  - >$100 million in 2014.

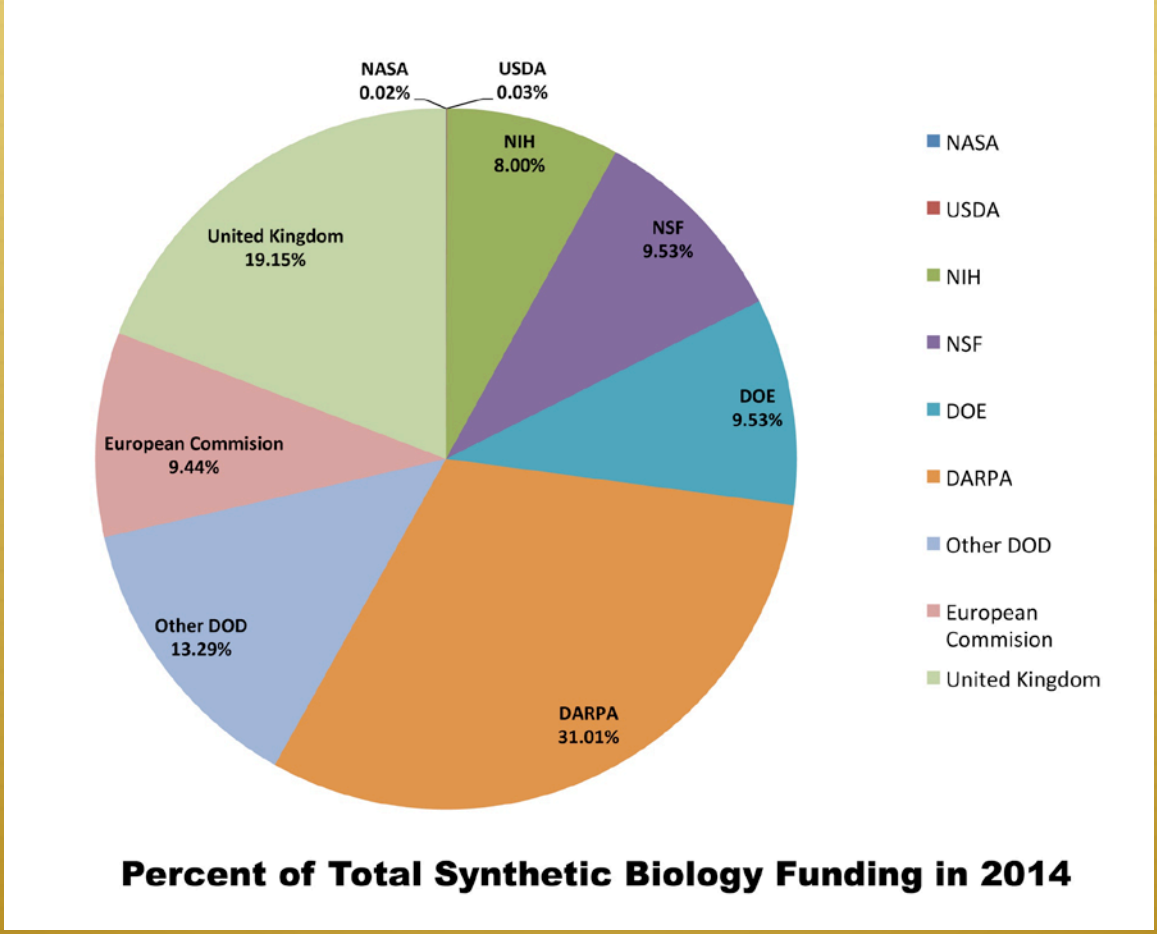
(14) Wilson Center. Synthetic Biology Project. U.S. Trends in Synthetic Biology Research Spending. http://www.synbioproject.org/site/assets/files/1386/final_web_print_sept2015.pdf.

# $$ SynBio Spending $$




(15) Wilson Center. Synthetic Biology Project. U.S. Trends in Synthetic Biology Research Spending. http://www.synbioproject.org/site/assets/files/1386/final_web_print_sept2015.pdf.

# $$ SynBio Spending $$




(16) Wilson Center. Synthetic Biology Project. U.S. Trends in Synthetic Biology Research Spending. http://www.synbioproject.org/site/assets/files/1386/final_web_print_sept2015.pdf.

# Funding Concerns

- <1% of funding in synthetic biology is being spent on 'risk research' – to examine the impact of synthetic biology on the environment or on humans. (17)
- <1% is being spent on studying:
  - Moral aspects
  - Legal concerns
  - Ethical issues

(17) Basulto D. The big trends in synthetic biology you need to know. The Washington Post. https://www.washingtonpost.com/news/innovations/wp/2015/10/08/the-big-trends-in-synthetic-biology-you-need-to-know/

# SynBio and Biosafety

- Synthetic biology research is currently focused on work with microorganisms (e.g., bacteria, viruses, fungus) and there is concern that novel pathogens could be created or existing pathogens made more virulent. (18)
- Added concern for the potential for altered or synthetic genetic material to escape and contaminate the environment and indigenous organisms. (19)

(18) Wei et al. Biosafety Considerations of Synthetic Biology: Lessons Learned from Transgenic Technology. Curr Synthetic Sys Biol; 2014, 2:3.http://www.omicsonline.org/open-access/biosafety-considerations-of-synthetic-biology-lessons-learned-from-transgenic-technology-2332-0737-2-1000115.pdf

(19) Wright et al. Building-in biosafety for synthetic biology. Microbiology; 2013 (159):1221–1235. http://mic.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.066308-0.

# SynBio and Biosafety

- "Synthetic biology is
  - not constrained by the requirement of using existing genetic material and
  - thus has great potential to be used to generate organisms
  - both currently existing and novel
  - including pathogens that could threaten public health, agriculture, plants, animals, the environment, or material." (20)

(20) Department of Health and Human Services. Fact Sheet on the Screening Framework Guidance, 2010. http://www.phe.gov/Preparedness/legal/guidance/syndna/Pages/factsheet.aspx.

# Regulations / Guidance

- The NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* requires a risk-based assessment of physical containment to protect researchers and to prevent releases into the environment. (21)
- *NIH Guidelines* address contained research (U.S. government funded only).

(21) NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

# Regulations / Guidance

- Other regulatory agencies provide oversight of field trials, open ponds, etc.: [22]
  - Animal and Plant Health Inspection Service (APHIS)
  - Environmental Protection Agency (EPA)
  - Food and Drug Administration (FDA)

(22) Coordinated Framework for Regulation of Biotechnology. U.S. Office of Science Policy. https://www.aphis.usda.gov/brs/fedregister/coordinated_framework.pdf.

# Coordinated Framework

- Published in 1986 – "existing federal laws appeared adequate for the regulation of products made with biotechnology." (23)

(23) Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options. May, 2014. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf.

# APHIS

- Regulates field trials of genetically engineered crops and plants under general authority to regulate plant pests.
- Reviews requests to "deregulate" the crop or plant in order for it to be grown without a permit at a commercial scale. (24)

(24) Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options. May, 2014. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf

# “Glowing Plants”

- Glowing plants?
  - Performed without any regulatory oversight in the United States. [25]

(25) Kickstarter. Glowing Plants: Natural Lighting with no Electricity. https://www.kickstarter.com/profile/antonyevans.

# EPA

- Regulates genetically engineered microbes as "new chemical substances" under the Toxic Substances Control Act (TSCA).
- Regulates genetically engineered pesticides (including biopesticides and pesticides incorporated into plants) under its authority to regulate pesticides. (26)

(26) Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options. May, 2014.http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf.

# FDA

- Regulates food, food additives, human and animal drugs, and certain other products, including those that have been produced through genetic engineering." (27)

(27) Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf.

| Product type | With this intended use or characteristic | Meets this definition (under given statute) | Main focus for decision making under applicable statute | Authority to consider potential risks outside of main focus for decision making | Authority to test and assess potential risks (pre-market) | Authority to restrict use or marketing based on potential risk | Authority to address concerns that arise after the product is marketed |
|---|---|---|---|---|---|---|---|
| Any product, including modified plants, animals, and microbes | That will be used as a pesticide | Pesticide or Plant-incorporated protectant (EPA/FIFRA) | Human, animal & ecosystem health | ● | | | |
| | That will be used as a drug | Drug or Animal Drug (FDA/FDCA) | Human & animal health | ◉ | | | |
| | That will produce a drug | Drug Manufacturing Facility (FDA/FDCA) | Human & animal health | ◉ | Early trials may lack oversight | | |
| | That will be added to food and is not generally recognized as safe | Food additive (FDA/FDCA) | Human & animal health | ◉ | | | |
| | That will be used as or will produce a dietary supplement | Dietary Supplement (FDA/FDCA) | Human & animal health | ○ | | | |
| | That will be used as or will produce a cosmetic | Cosmetic (FDA/FDCA) | Human & animal health | ○ | | | |
| | That is a plant pest, uses a plant pest in its creation, or incorporates its DNA | Plant Pest or Regulated Article (USDA-APHIS/PPA) | Plant health | ◉ | | | |
| Any intergeneric microorganism | That will be used for any commercial purpose not listed above | Intergeneric microorganism (EPA/TSCA) | Human, animal & ecosystem health | ● | | See text | |
| Any gene(s) inserted into an animal | That will be used for any purpose | Animal drug (FDA/FDCA) | Human & animal health | ◉ | | | |
| Any modified organism | That will be used as a food | "Substantially equivalent" (FDA/FDCA) | Human & animal health | ○ | Voluntary process | | |

○ No authority; no assessment ◉ No authority; assessment under NEPA only ● Full authority over any potential risks □ No authority ▒ Limited or uncertain authority ■ Demonstrated Authority

(28) Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf.

# Guidance

- CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL):
  - "The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent." (29)

(29) CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL), December 2009, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf.

# Prevention

- Understanding how to properly work with synthetic biology will help investigators prevent unintentional accidents, exposures, and releases.
- A **risk assessment** must be performed on a case-by-case basis to ensure that adequate biosafety and biosecurity procedures are in place to protect personnel, the community, and the environment.

# Biosafety Questions

- What synthetic parts are being used?
- Is the part from an organism? A pathogen? What is the risk group?
- What is the innate function of the part? Is it toxic or pathogenic to humans, plants, animals, or other organisms? Are high risk genes being used?
- Is a recipient organism used? What is the risk group?
- Is a vector used? What is the risk group?

# Biosafety Questions

- How will the synthetic organism / part be acquired:
  - Culture collection
  - Another lab
  - Isolation by PCR
  - Ordering from a DNA synthesis company
  - Other?
- Are additional safety precautions being taken with the synthetic organism / part (e.g. handling it in a separate lab area, wearing additional protective equipment)?

# Biosafety Questions

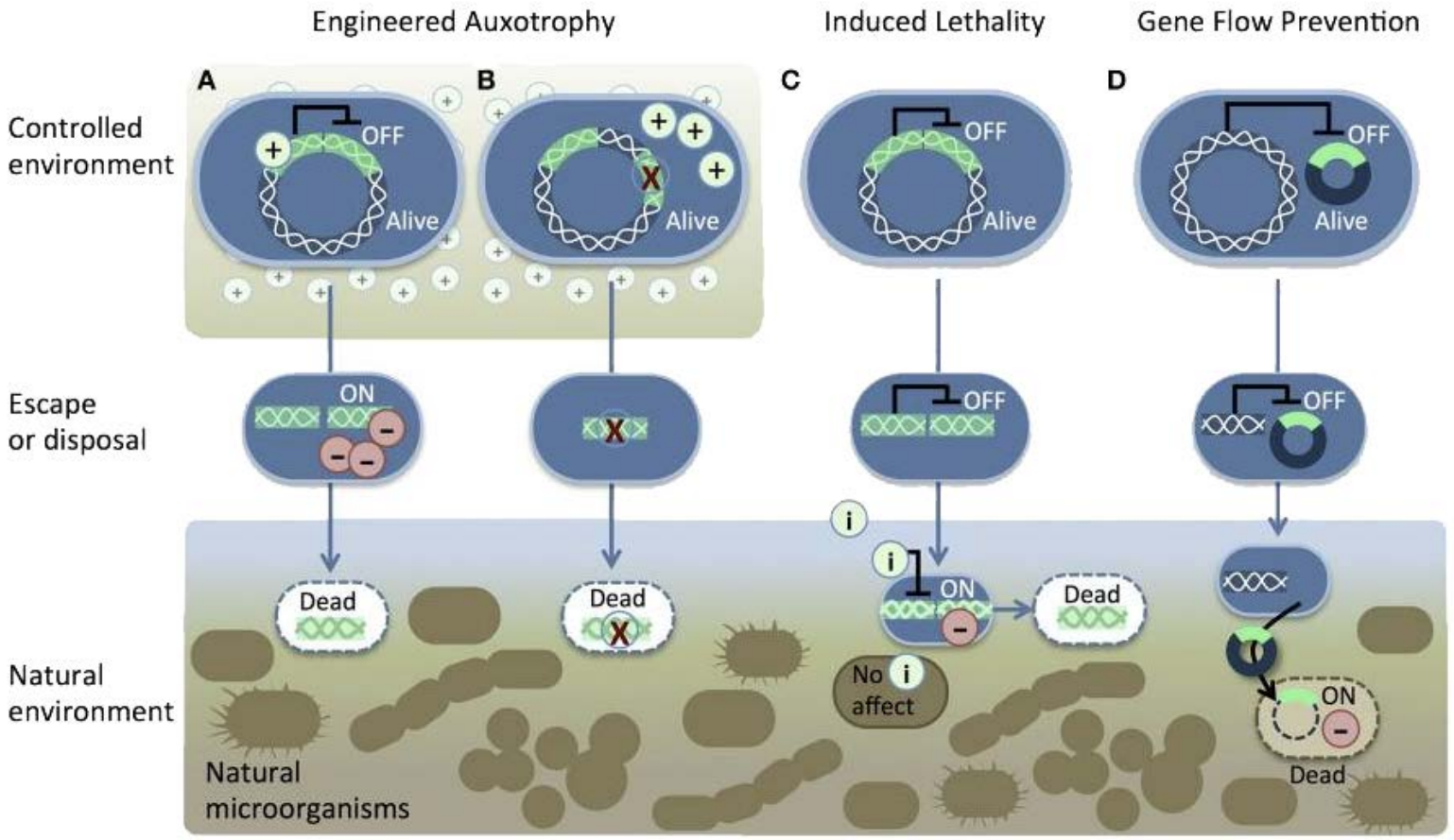
- How will the synthetic organism or part interact with natural ones?
- How might the synthetic organism evolve and adapt?
- What is the potential for gene transfer into unmodified organisms?
- What is the infectivity of the synthetic organism (e.g., virulence, infective dose, mode of transmission)?
- What is the availability and effectiveness of prophylactic or therapeutic measures?

# Genetic Safeguards

- Are engineered auxotrophic strains being used (dependent on a chemical not available in nature)?
- Is induced lethality an option for the organism (using a gene that is toxic to the cell)?
- Does the system incorporate gene flow prevention (toxic peptide encoded on a plasmid)?
  - Although genetically engineered safeguard systems offer technical solutions to restrict functioning of engineered cells, **none work perfectly**. (30)

(30) Wright O., Stan G.-B., Ellis T. (2013) Building-in biosafety for synthetic biology. Microbiology 159, 1221-1235.

# Genetic Control Strategies




(31) Moe-Behrens, G. H. G., Davis, R., & Haynes, K. A. (2013). Preparing synthetic biology for the world. *Frontiers in Microbiology*, *4*, 5. doi:10.3389/fmicb.2013.00005.

# Gene Drive Containment

**Potentially stringent confinement strategies for gene drive research**

Multiple stringent confinement strategies should be used whenever possible.

| TYPE | STRINGENT CONFINEMENT STRATEGY | EXAMPLES |
|---|---|---|
| Molecular | Separate components required for genetic drive | sgRNA and Cas9 in separate loci (*8*) |
| | Target synthetic sequences absent from wild organisms | Drive targets a sequence unique to laboratory organisms (*3*,*4*,*8*) |
| Ecological | Perform experiments outside the habitable range of the organism | *Anopheles* mosquitoes in Boston |
| | Perform experiments in areas without potential wild mates | *Anopheles* mosquitoes in Los Angeles |
| Reproductive | Use a laboratory strain that cannot reproduce with wild organisms | *Drosophila* with compound autosomes* |
| Barrier | Physical barriers between organisms and the environment | Triply nested containers, >3 doors (*6*) |
| | •Remove barriers only when organisms are inactive | Anesthetize before opening (*6*) |
| | •Impose environmental constraints | Low-temperature room, air-blast fans |
| | •Take precautions to minimize breaches due to human error | Keep careful records of organisms, one investigator performs all experiments (*6*) |

*An example of reproductive confinement would be *Drosophila* laboratory strains with a compound autosome, where both copies of a large autosome are conjoined at a single centromere. These strains are fertile when crossed inter se but are sterile when outcrossed to any normal or wild-type strain because all progeny are monosomic or trisomic and die early in development.

(32) Akbari et al. (2015). Safeguarding gene drive experiments in the laboratory. Science; 349(6251): 927-8. http://www.sciencemag.org.ezproxy1.lib.asu.edu/content/349/6251/927.full.pdf

# General Biosafety

- What is the type of work proposed (e.g. *in vitro, in vivo*, challenge studies, work with laboratory animals, non-standardized manipulations)?
- Is there a potential for aerosol generation or splashes?
- What concentration and volume will be used (e.g., cultures, supernatant)?
- What would happen in the event an exposure (e.g., needlestick, splash)? Is occupational health involved?

# Personnel Protection

- What biosafety level will be assigned?
- What administrative controls will be used?
- What engineering controls will be used?
- What personal protective equipment will be worn?

# Biosecurity

- Biosecurity involves:
  - Prevention of unauthorized possession, loss, theft, misuse or diversion of hazardous agents.
  - Misuse of scientific information to threaten elements of national security.

# Biosecurity

- A primary concern with synthetic biology is that insider threats, rogue states, and terrorist organizations will use the technology to re-engineer microorganisms, or living systems, with the intent to harm others.

# Biosecurity

- Ensure biosecurity in all aspects of the synthetic biology research, from the biological agents and chemicals being used in the laboratory, to the people performing the work, to the final product that is created.

# Ethical Concerns

- Ethics issues for synthetic biology include four main areas:
  - Blurring the lines between life and non-life;
  - Interacting / interfering with nature;
  - Expanding the gap between those who "have" and those who "have not"; and
  - Misusing the technology, intentionally or unintentionally, which could lead to serious threats to society and the environment. (33)

(33) European Commission. Preliminary Opinion on Synthetic Biology II - Risk assessment methodologies and safety aspects. http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_26_en.htm

# Parting Thoughts

- Building with Biology:
  - Participants interacted with scientists and other members of the SynBio community at 8 pilot events around the nation in 2015. (34)


(34) Building with Biology.
http://www.mos.org/buildingwithbiology

# Parting Thoughts

- SynBio is already in your world…for example:
  - The International Genetically Engineered Machine (iGEM) competition asks participants to attempt to build simple biological systems from standard, interchangeable parts and operate them in living cells. (35)
- iGEM Questions?
  - Kelly Drinkwater, *Biosafety Generalist*
  - kelly@igem.org


(35) International Genetically Engineered Machine (iGEM). http://igem.org/About.

# 2015 iGEM Competition

(36) International Genetically Engineered Machine (iGEM). http://www.igem.org/wiki/images/4/4d/IGEM_fromabove_2015.jpg.

# Example iGEM Questions

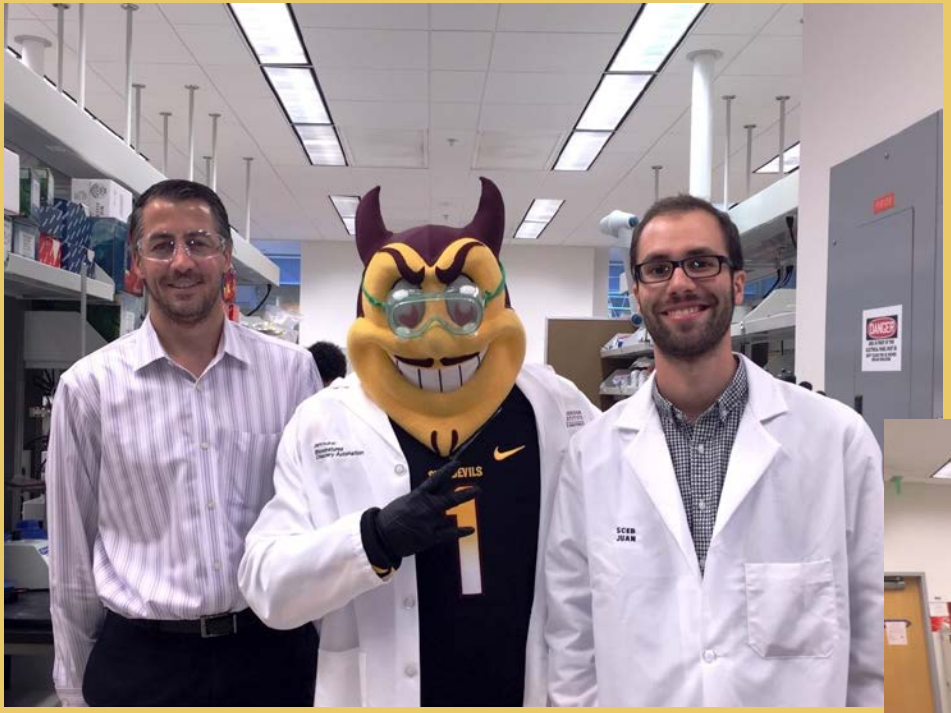
- **How <u>might</u> the project be used in the real world?** (37)
  - The project is foundational / no specific real-world application in mind (e.g., library of standardized promoters, system for communication between cells)
  - Only in the lab (e.g., reporter strain for measuring the strength of promoters)
  - In a factory (e.g., cells that make a flavor chemical for food, cells that make biofuel)
  - In a consumer product that ordinary people buy (e.g., cells that clean your clothes, bread made with engineered yeast)
  - In agriculture / on a farm (e.g., cells that guard against pests, engineered rice plants, cells that promote growth of crop plants)
  - In a small enclosed device (e.g., a bio-sensing strip with cells that detect arsenic)
  - In the natural environment (e.g., cells that remove pollution from lakes, engineered forest trees that can resist drought)
  - To be used in the human body, or in food (e.g., anti-cancer bacteria, bread made with engineered yeast, engineered rice plants)
  - Other (e.g., bacteria that live on Mars)

(37) International Genetically Engineered Machine (iGEM). http://2015.igem.org/Safety/About_Our_Project.

# Parting Thoughts

- “We are experiencing exponential changes in biology.”

  - George Church



# Thank you!


**David Gillum, Juan Maldonado, Giorgio Scarpellini, & Irene Mendoza**
**(and Dr. Emma Frow @ ASU and Kelly Drinkwater @ iGEM)**

# Contact Information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