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Opportunities and Challenges for Engineered Biology in Bioremediation

Facilitated by – Kent Sorenson

Sixth International Symposium on Bioremediation and Sustainable Environmental Technologies
May 8-11, 2023| Austin, Texas

The Panel

- Dayal Saran, VP of Research with Allonnia
- Leveraged metabolic engineering and biotechnology to commercialize the novel solutions in the area of industrial proteins and enzymes, flavors/fragrances and other ingredients, insect control agents, live biotherapeutics, and biofuels
- Master's degree in chemistry and a Ph.D. in biochemistry

- Pavle Jeremic, CEO and Founder of Aether Biomachines, a company operating at the intersection of manufacturing, synthetic biology, and deep learning to design enzymes to manufacture complex novel molecular products
- Leading the buildout of Aether's first-of-its-kind platform, with the objective of triggering a new industrial revolution

The Panel

- Keith Matthews, Counsel with Wiley Rein LLP, focuses on regulation of chemical products and ag biotech, including genetically engineered organisms regulated by EPA and the U.S. Department of Agriculture
- Former staff attorney and Assistant General Counsel in the Office of General Counsel at the U.S. EPA. Served for four years as the Director of the Biopesticides and Pollution Prevention Division (BPPD) in EPA's Office of Pesticide Programs

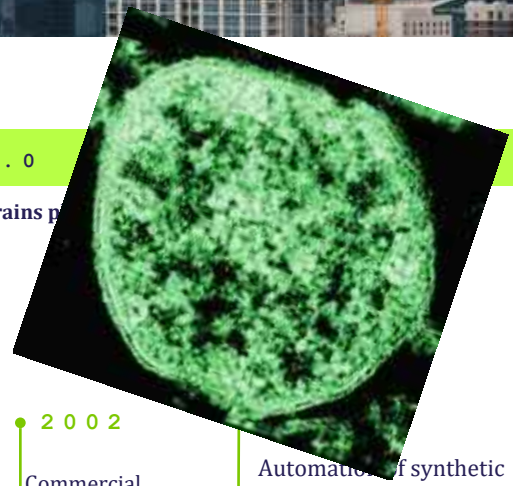
- Tammy Zimmer, Director of Regulatory at Ginkgo Bioworks, focused on Agriculture. Develops regulatory strategies for biological crop inputs.
- Chairs the Phytobiome Alliance Regulatory Working Group and works with the Biotechnology Innovation Organization (BIO), Biological Products Industry Alliance (BPIA), and other stakeholders to promote sustainable technologies in the agricultural industry.

Why Engineered Biology??

- Hard Problems!
  - PFAS, microplastics, naphthenic acids
  - Decarbonization
  - Sustainable mining
  - Upcycling plastics

WHY NOW?

A confluence of factors permit us to develop & deploy at scale p verful transformative biology solutions



BIOREMEDIATION 2.0

3.0

In Situ Soil and Groundwater Treatment (wild strains p

Rapid development of new strains for degradation, sequestration, upcycling and sensing

REMEDIATION

600BC

Romans rely c biological treatment of wastewater

1980

US Supreme Court grants patent for *P. putida*, the first for a genetically engineered organism

s putida engineered at GL to improve biodegradation rates

1987

Methane-enhanced bio-remediation of chlorinated solvents patented by DOE

1989

Bioremediation plays a major role in Exxon Valdez cleanup

2002

Commercial production of *Dehalococcoides* bioaugmentation culture

Automatio f synthetic biology enables rapid development of transformational biological solutions

2020

Allonnia harnesses synthetic biology to launch Bioremediation 3.0

AD
B

s

discover how
e copies of
connect two
DNA, & 3)
cut the DNA

1980s

Scientists discover how to monitor biological functions in microbes

1990s

DNA reading & writing commercialized. Yeast and E. coli sequenced revealing metabolic networks

2000–2010

Understanding of synthetic biology circuits deepened. Cost of DNA reading, and writing dropped significantly. Various SynBio start ups launched and targeted production of natural products via fermentation.

2010–PRESENT

Rise of Automation in Synthetic Biology: Microbial engineering was automated. Significant jump in the capability to engineer environmental microbes in parallel. Automation also reduced the cost of engineering

A confluence of factors permit us to develop & deploy at scale powerful transformative biology solutions

BIOREMEDIATION 1.0 — **Wastewater Treatment (wild strains)**

BIOREMEDIATION 2.0 — **In Situ Soil and Groundwater Treatment (wild strains plus P. putida)**

3.0 — **Rapid development of new strains for degradation, sequestration, upcycling and sensing**

REMEDIATION

600BC — Romans rely on biological treatment of wastewater

1914 — Invention of activated sludge for treatment of municipal wastewater

1968 — George Robinson uses bioremediation to clean up an oil spill in Santa Maria, CA

1971 — *Pseudomonas putida* genetically engineered at GE to improve biodegradation rates

1980 — US Supreme Court grants patent for *P. putida*, the first for a genetically engineered organism

1987 — Methane-enhanced bio-remediation of chlorinat solvents patented by DC

1989 — Bioremediation pl major role in Exxc Valdez cleanup

~2008–

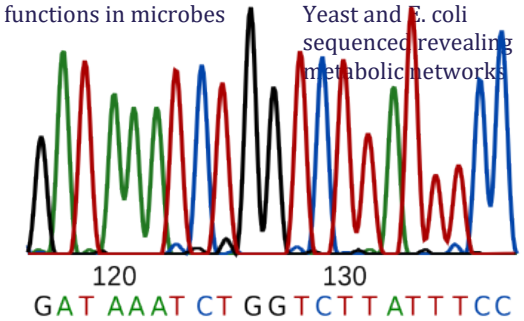
2020 — Allonnia harnesses synthetic biology to launch Bioremediation 3.0

ADVANCED BIOLOGY

1960s — Scientist discover microbes' ability to regulate cellular function

1970s — Scientist discover how to 1) make copies of DNA, 2) connect two pieces of DNA, & 3) precisely cut the DNA

1980s — Scientists discover how to monitor biological functions in microbes



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Thank you

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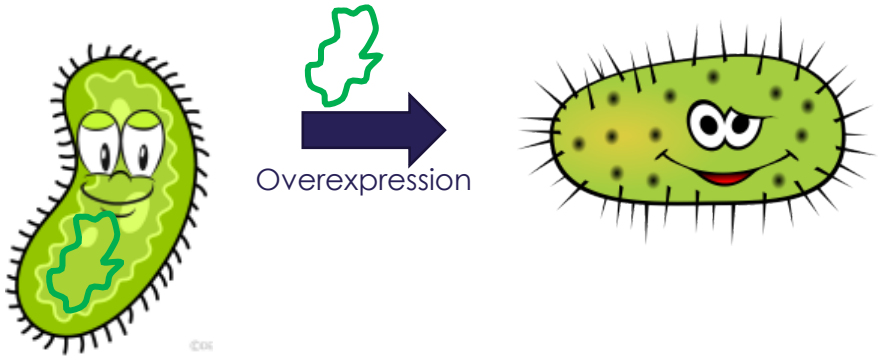
Engineered Biology –Whole cell engineering

May 2023

Genetic Engineering Vs Synthetic Biology

Genetic Engineering:

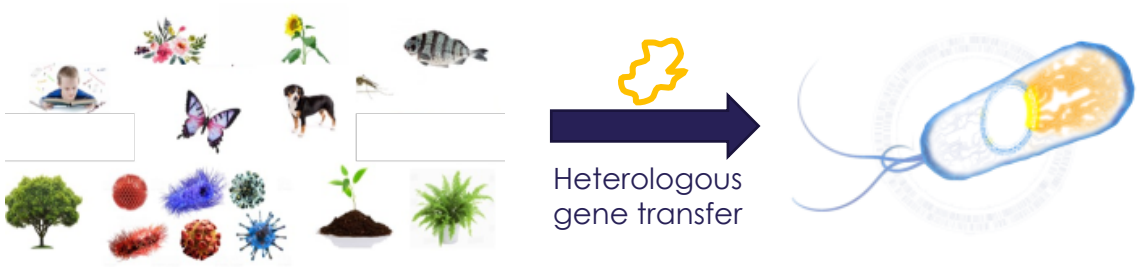
Genetic engineering is the process of manipulating an organism by modifying or deleting genes within an organism.



- Overexpression of native enzymes
- No synthetic gene required
- Limited to native enzyme
- No *de-novo* genes or interspecies gene transfer

Synthetic Biology:

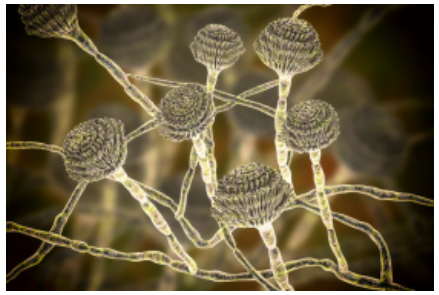
Synthetic biology broadly refers to the use of biological engineering to design and construct new synthetic biological parts, and systems that do not exist in nature or to redesign existing biological organisms.



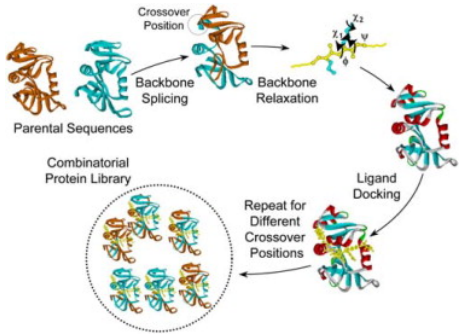
- Desired genetic traits from any species can be transferred
- Engineered enzymes and other genetic elements can be integrated to make the organism very efficient in performing a desired function under environmental conditions.

Synthetic Biology steps needed to further improve the efficacy of microbes

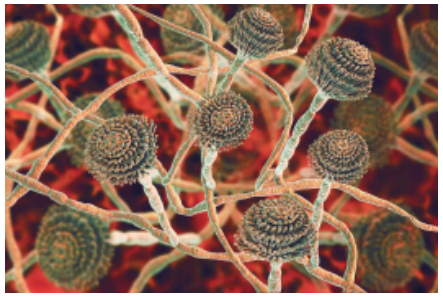
- Screen natural gene variants (diversity) of the target enzymes
- Engineer the best variant to further improve its activity and/or remove the undesirable traits
- Engineer/evolve host to function in non optimum conditions (low pH, mixed environment)
- Required access to custom software, sophisticated automation and HTP screening capabilities to achieve the points discussed above



Host selection: Screen and isolation of microbial host



Engineered protein: Engineer and optimize protein activity



Engineered Host: Optimize host to work under environmental conditions

A confluence of factors permit us to develop & deploy synthetic biology solutions

Two main factors:

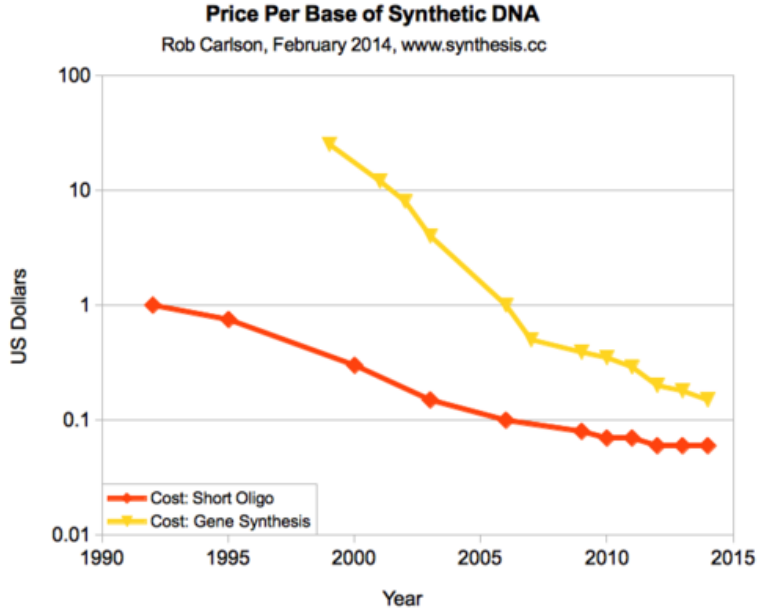
Sequencing

Slide from George Church presentation

Exponentially shrinking cost of reading & interpreting your genome

- The exploration of genetic diversity across a broad spectrum of organisms has been made feasible by the cost reduction in DNA sequencing.

DNA synthesis



- The cost reduction in DNA synthesis has enabled us to screen for the most optimal protein function.

Applications of Synthetic Biology in remediation

Improvement in biological functions

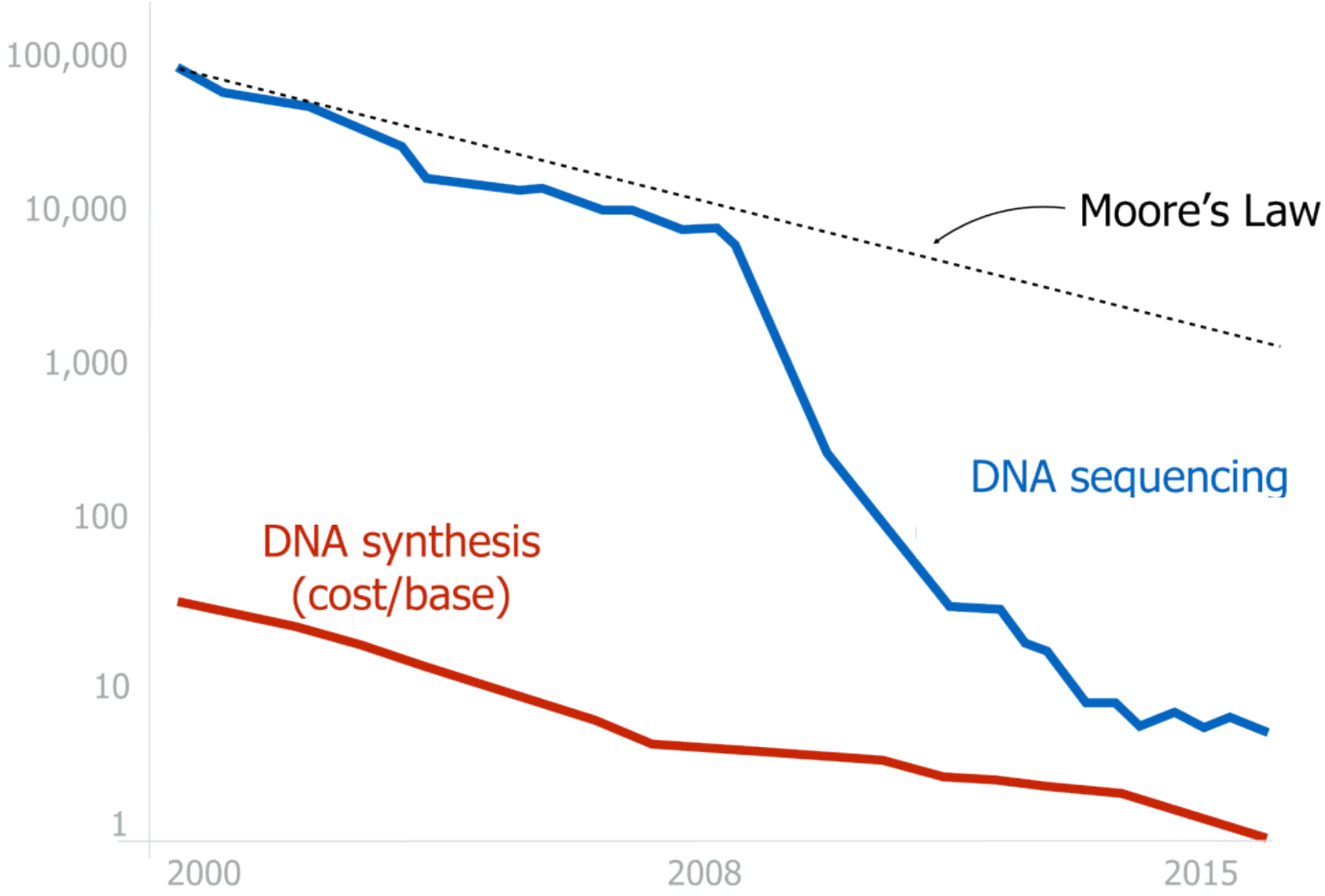
- **Host engineering:** for optimal function under environmental conditions
- **Enzyme engineering**: Protein and enzyme activity optimization (Aether)

Other Non-activity-based applications

- **Kill Switch:** Organisms can also be engineered to have a kill switch.
  - Microbes used in OSPW detoxification - only grow in the presence of Naphthenic Acids
- **Tracking:** Organisms can also be engineered to have a tracker.
  - 1,4 D degradation organism can be tracked in-situ
- **Deployment technology:** Organisms can also be engineered as a vehicle to deploy synbio solutions.
  - Protein surface display technology to deploy REE binding proteins

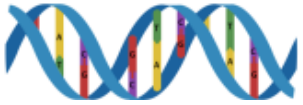
Thank You

Cost of DNA synthesis is although going down, it is still expensive

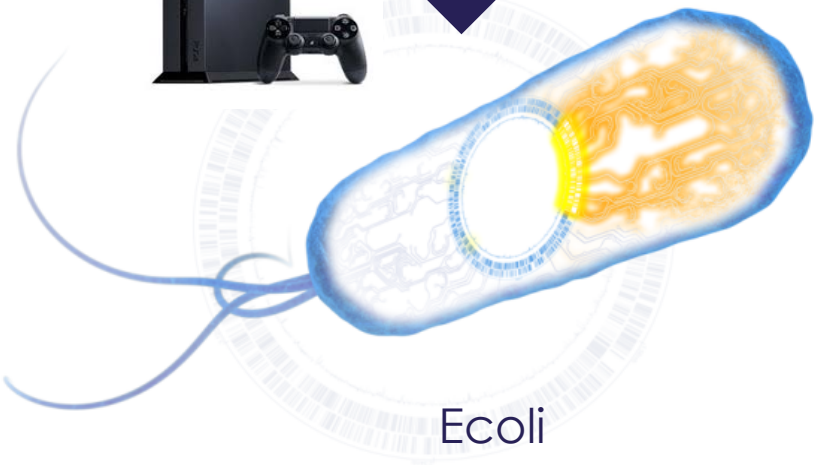


Gene recoding is needed to achieve most optimum activity in the desired host

Recoding



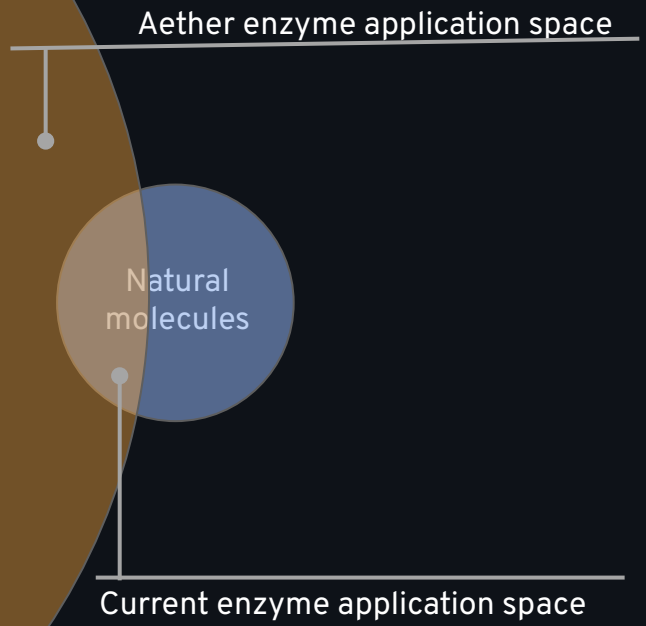
Genes identified with desired function



Ecoli

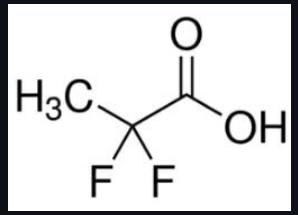
Aether is indexing chemistry that does not exist in nature

High value molecules

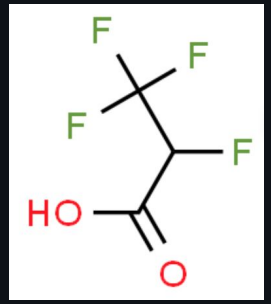


- Aether systematically explores the high value chemical reaction space by indexing a large set of substrates and enzymes combinations
- ML algorithm will use this dataset to design de-novo enzyme for new reaction

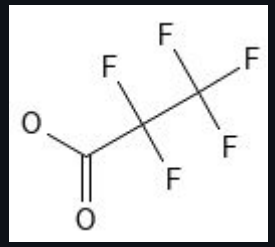
Our platform tests proteins against many different substrates in parallel



2,2-difluoropropionic acid

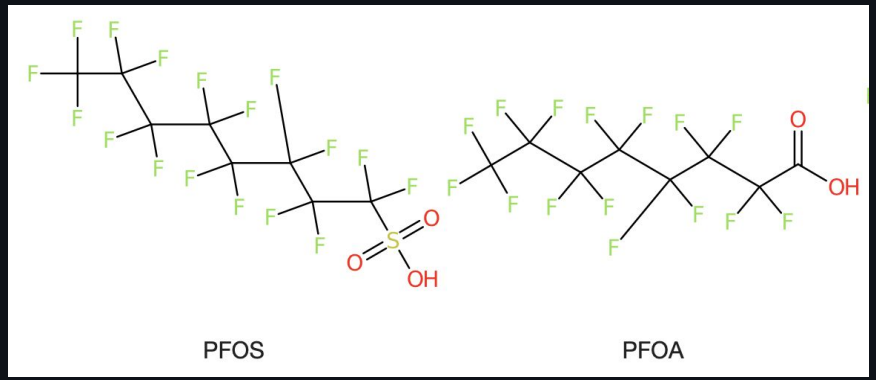


2,3,3,3-tetrafluoropropionic acid
Positive control with fully fluorinated beta carbon



pentafluoropropionic acid
Like positive control, but fully fluorinated

Dozen of comparable substrate mesh “walk” allow to explore and exploit enzymatic diversity without relying on positive control with PFOA or PFOS



We have an extensive pipeline of halogenated targets we're working on

	Tech scoping	POC	Min. viable product	Commercial
Enzymes targeting defluorination, dechlorination, decarboxylation, desulfonation, and removal of other groups	PFOS			
	PFOA			
	PFHxS			
	PFNA			
	PFBS			
	GenX			
	PCBs, other dioxins			
Peptides for selectively binding and mining critical minerals	Lithium extraction			
	Base metal extraction			
	REE separation			

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Opportunities and Challenges for Engineered Biology in Bioremediation

U.S. Regulatory Framework for Genetically Engineered Organisms

U.S. Coordinated Framework for Biotechnology

The Obama, Trump, and Biden Administrations have taken actions to modernize the U.S. regulatory approach to genetically engineered organisms.

See generally:

1. Keith Matthews, "Continuing Evolution of the Coordinated Framework: Implications for Agriculture Biotechnology," in *Navigating Legal Challenges in the Agrochemical Industry, American Chemical Society* https://pubs.acs.org/doi/10.1021/bk-2020-1362; and

U.S. Coordinated Framework for Biotechnology

2. Keith Matthews and Nur Ibrahim, https://www.wiley.law/alert-President-Biden-Signs-Executive-Order-14081-to-Promote-Biotechnology-and-Biomanufacturing (October 2022)

Coordinated Framework Regulatory Context

USDA	EPA	FDA

▪ Transport of plants
  ▪ Field testing
  ▪ Permits
▪ Notifications
▪ Determination of regulated status
	▪ Plant Incorporated Protectants (PIPs)
	▪ Agricultural GE Microbes
	▪ "Industrial" GE Microbes
		▪ Food and Feed safety consultation (voluntary)

Scope	**Scope**	**Scope**
All Potential Plant Pests	GE organisms	Food, Feed, Pharmaceuticals
Statutory Authority	**Statutory Authority**	**Statutory Authority**
Plant Protection Act (PPA) National Environmental Policy Act (NEPA)	Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Federal Food, Drug, and Cosmetic Act (FFDCA) Endangered Species Act (ESA) Toxic Substances Control Act (TSCA)	Federal Food, Drug, and Cosmetic Act (FFDCA) Endangered Species Act (ESA)

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US Coordinated Framework For Biotechnology

USDA

Mandate:

Evaluation of Plant Pest Potential

Data Evaluated:
- Gene/Protein Safety
- Compositional equivalence
- Ag/Pheno characterization
- Environmental Safety/NTO

Review Focus : Two main questions

Does the Product pose a significant Plant Pest Risk? (PPA)

NO → Does the product have the potential for a significant environmental impact? (NEPA)

YES → Determination of Regulated Status

NO → **Environmental Assessment (EA)**

YES → **Environmental Impact Statement (EIS)**

Finding of No Significant Impact (FONSI)

Public Comment (≥ 30 Days)

→ Determination of Non-Regulated Status

EPA

Mandate:

Evaluation of Pesticide Human Health and Environmental Risks

Data Evaluated:
- Gene/Protein Safety
- Allergenicity/Toxicity Non Target Organism Effects (NTO)
- Environmental Effects

Review Focus : Three Federal Statutes

Does dietary exposure to the Food/Feed derived from the product pose a health risk? (FFDCA)

→ **Conclusion:** There is a reasonable certainty of no harm from

Does the pesticide safety pose an unreasonable risk to the environment or human/animal safety? (FIFRA)

→ **Conclusion:** The pesticide safety does not pose a risk to the environment or human or animal safety

FDA

Mandate:

Evaluation of Food and Feed Safety

Data Evaluated:
- Gene/Protein Safety
- Allergenicity/Toxicity
- Compositional equivalence
- Consumption

Review Focus (FFDCA):
- Allergenicity/Toxicity
- Nutritional Composition

Conclusion:
The new food/feed is as safe as its non-modified counterparts

YES → Consultation Completed

NO → Additional Consultation with FDA → Consultation Completed

TSCA -- The Toxic Substances Control Act ("TSCA")

- TSCA regulates the manufacture (including importation) and use of chemical substances in U.S. commerce
- A chemical substance in commerce in the United States must be on the "TSCA Inventory"
- Microorganisms are regulated under TSCA as chemical substances
- Naturally occurring microorganisms are considered to be on the TSCA Inventory

TSCA -- The Toxic Substances Control Act ("TSCA")

"New" microorganisms that are not included on the TSCA Inventory include:

"Intergeneric" microorganisms (including bacteria, fungi, algae, viruses, protozoa, etc.) formed by combining genetic material from organisms in different genera

- **intergeneric microorganism:** a microorganism that is formed by the deliberate combination of genetic material originally isolated from an organism(s) in a different taxonomic genera.
  - Does *not* include: a microorganism that contains introduced genetic material consisting of only well-characterized, non-coding regulatory regions from another genus.

TSCA -- The Toxic Substances Control Act ("TSCA")

Intergeneric microorganisms: "a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera."

- Note: wrt chemically synthesized genes, if the genetic sequence of a synthetic gene is identical to a sequence known to occur in an organism in the same genus, the resulting microorganism is considered *intra*generic. Conversely, if the sequence of a synthetic gene is different than, or is not known to be identical to an existing sequence in the genus of the recipient microorganism, the resulting microorganism is considered to be intergeneric.

USDA Regulation of GE Microbes

USDA regulates microbes that are, or may be, plant pests under regulations at 7 C.F.R. Parts 330 and 340.

USDA regulates microbes generally under Part 330 and regulates GE microbes under Part 340.

But, sometimes, the distinction is not so clear.

USDA Regulation of GE Microbes

7 C.F.R. Part 330:

"Federal Plant Pest Regulations; General; Plant Pests, Biological Control Organisms, and Associated Articles; Garbage"

Plant Pest and Quarantine

7 C.F.R. Part 340:

"Movement of Organisms Modified or Produced Through Genetic Engineering"

Biotechnology Regulatory Service

USDA 18 May 2020 Part 340 Final Rule

On 18 May 2020, USDA published its final rule amending its 7 C.F.R. Part 340 regulations governing the interstate movement of certain genetically engineered organisms.

This completed a rulemaking effort first initiated in 2008.

USDA Part 340 Final Rule

Under the revised Part 340, product developers are required to obtain a permit for GE organisms if (1) the plant and trait mechanism of action (plant-trait-MOA) combination has not been previously evaluated by APHIS; (2) it is a plant pest; (3) it is a non-plant organism that has received DNA from a plant pest; (4) it is a microorganism that can control plant pests or is a parasite that can control invertebrate plant pests, and could be a plant pest risk; or (5) is a plant that produces a pharmaceutical or industrial use product.

USDA Part 340 Final Rule

- APHIS has developed a draft guidance document that details the information requirements and process for submitting permit applications for GE microorganisms.
- The draft guidance: *Guide for Submitting Permit Applications for Microorganisms Developed using Genetic Engineering Under 7 CFR part 340* is available at https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/regulatory-processes/permits/permits .
- The comment period for the draft Part 340 GE microorganisms guidance will close May 22.

GE Microorganisms in the EU – EFSA Survey

The EU Commission requested that EFSA provide a scientific opinion regarding microorganisms produced by new techniques of biotechnology. EFSA initiated a stakeholders survey on March 7 requesting interested parties to share information on "microorganisms produced by new developments in biotechnology that are intended for food and feed." EFSA will use the information collected through the survey to develop its scientific opinion on the potential novel hazards and risks of such microorganisms, and to assess the adequacy of the current EU risk assessment guidance. The survey closed on April 30.

http://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=European%20Food%20Safety%20Authority%20Launches%20Stakeholder%20Survey%20on%20Microorganisms%20Produced%20by%20Biotechnology_Brussels%20USEU_Belgium_BE2023-0001.pdf

U.S. Federal Regulation of GE Microorganisms-2023

For questions contact:

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GINKGO BIOWORKS

Regulatory Considerations for Field Research with Engineered Microorganisms

Battelle Symposium

Tammy Zimmer

May 10, 2023

Biotechnology Enables Development of Precision Microorganisms

Sustainable

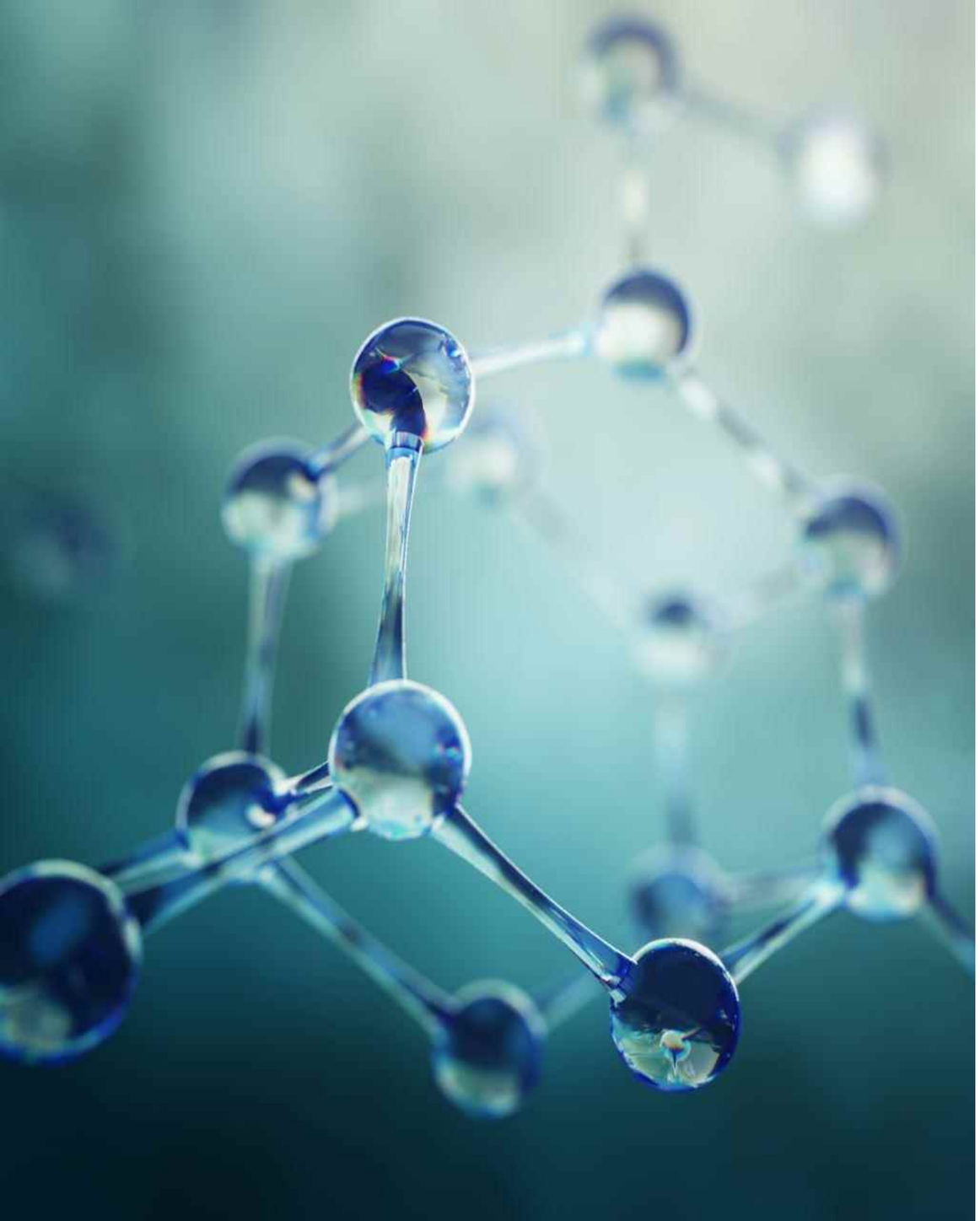
Improved Performance

Tailored Performance

Knock out undesirable properties

Engineered Microbes for Sustainable Agriculture

- Sustainable solutions to help address impacts of climate change
  - Reduce synthetic fertilizers and pesticides
  - Increase nutrient use efficiency
  - Increase crop resilience to abiotic stress
  - Carbon sequestration
  - Enhance soil health



Regulatory Considerations for Field Research

- Regulatory Requirements
- Application & Timelines
- Permit Conditions

Field Trial Regulation

Small-scale release of engineered microorganisms (<10 acres)

- EPA Biopesticide Pollution Prevention Division (BPPD) regulates microbial pesticides
- EPA Office of Pollution Prevention and Toxics (OPPT) regulates INTERgeneric microbes
- USDA Animal Plant Health Inspection Services (APHIS) Biotechnology Regulatory Services (BRS) regulates GE microbes considered to be a plant pests

Microbe/Product Type	Biocontrol	Biofertilizer/ Biostimulant
INTRAgeneric or Cisgenic	USDA APHIS BRS	USDA APHIS BRS*
INTERgeneric or Transgenic	USDA APHIS BRS + EPA BPPD Biotech Notification	USDA APHIS BRS* + EPA TSCA Environmental Release Application (TERA)

*If a plant pest risk (7 CFR § 340.3 The potential for direct or indirect injury to, damage to, or disease in any plant or plant product resulting from introducing or disseminating a plant pest, or the potential for exacerbating the impact of a plant pest)

Revised USDA APHIS BRS regulations

- Published in May 2020
- Revision intended to be risk focused and enable development of new technologies
- No guidance or implementation plan for microbes
- Most impactful change is BRS' expanded jurisdiction over GE microorganisms
  - *(d) Is a microorganism used to control plant pests, or an invertebrate predator or parasite (parasitoid) used to control invertebrate plant pests, and could pose a plant pest risk*

Application Process and Timelines

APHIS BRS Release Permit 7 CFR § 340
Apply via eFile
Agency Review - 120 days

EPA TSCA TERA 40 CFR § 725.250
Apply via CDX
Agency Review - 60 + 30-day screen

EPA Biotech Notification 40 CFR § 172.43
Apply via CDX
Agency Review - 90 days

Application Requirements

- genetic modifications
- ecological characteristics
- human & environmental Safety
- proposed research activity
  - Amount released
  - Application methods
  - Test sites / dates / duration
- containment practices
- monitoring plans
- analytical methods

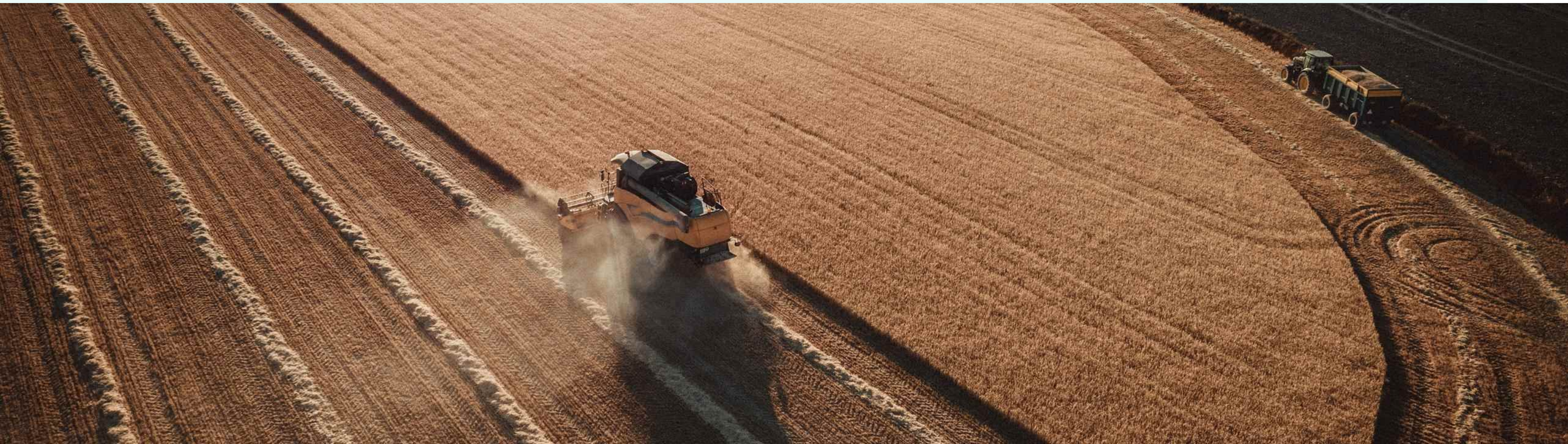
Permit Conditions

Containment

Devitalization

Monitoring

Reporting



Tips for Navigating Framework

- Know relevant regulatory requirements
- Engage with regulators
- Data to support applications may reduce requirements
- Engineering strategies
- Submit early

THANK YOU