GINKGO
BIOWORKS

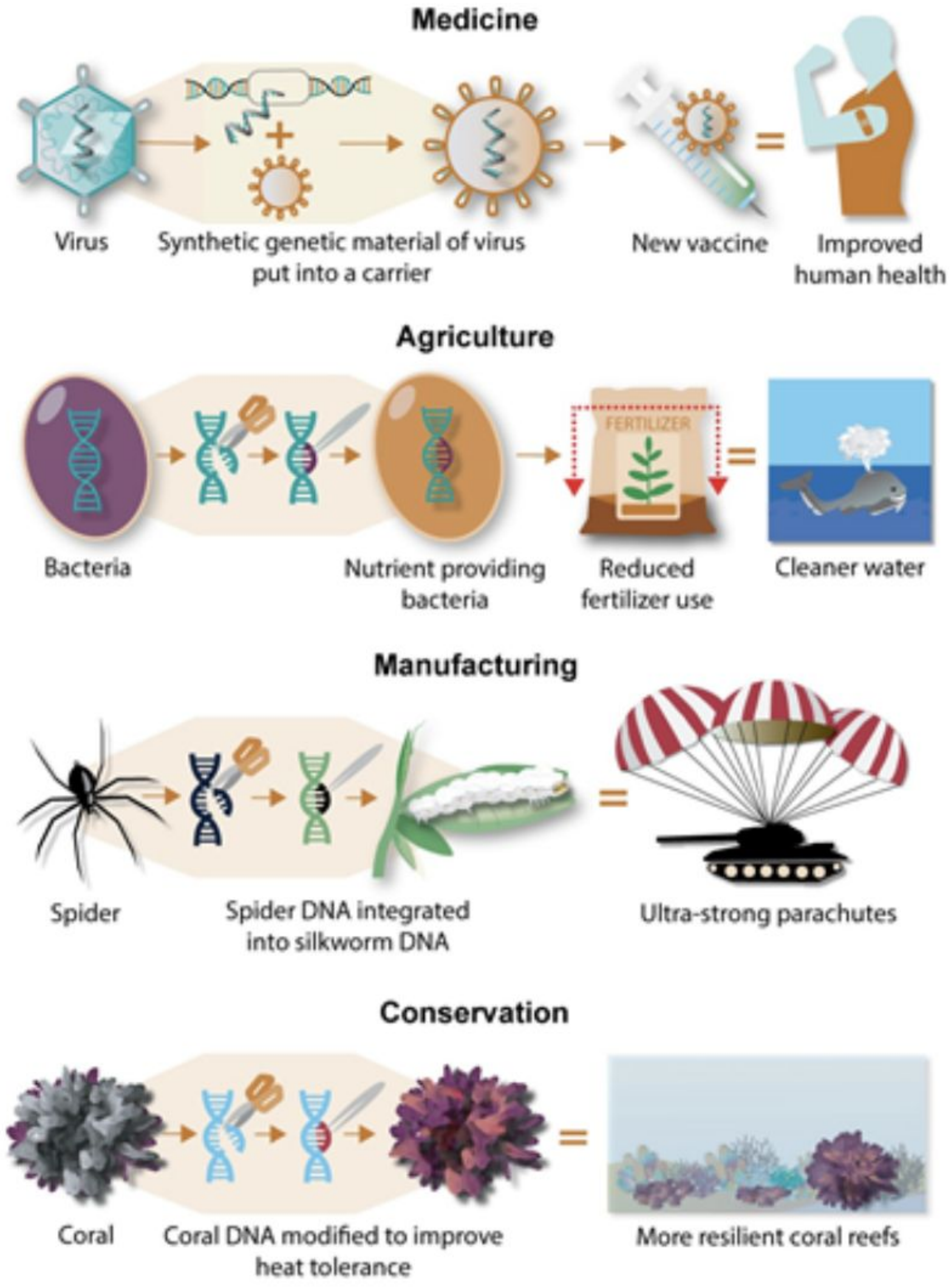
Harnessing Synthetic Biology for Agriculture

January, 2024

Synthetic biology harnesses large datasets to transform therapeutics, manufacturing, conservation and agriculture

An area of scientific research that involves the purposeful **editing and redesigning** of genetic components of organisms such as bacteria, yeast, and fungi to make new material–**proteins, small molecules, and microbes**–with altered functionality.

Examples of the Power and Impact of Synthetic Biology



Source: GAO analysis (scientific literature). | GAO-23-106648

The pressure is on for growers to...

Meet demand without increasing land in use

Adhere to new regulations

Grow crops more sustainably

Control increasingly persistent pests

Adapt to climate change

Manage price volatility of inputs

Growers need innovation that delivers high-performing products to meet the moment

...and therefore the pressure is on for innovators

Biology can meet these challenges, but the next generation of plant traits and biological products must be efficacious, consistent, and affordable to implement in the field

Innovators need:

Better starting points from wild type biology
Search broad evolutionary space for beneficial properties

Better performance
Design and optimize biological solutions

Better consistency
Deliver innovative products that growers can trust

Synthetic biology offers:

Machine-learning guided design & discovery

Protein, gene expression, and Fermentation Optimization at Scale

...and more

Ginkgo Bioworks’ horizontal platform capabilities enable our partners across the life sciences

Since 2008, building integrated automation, software, and biological tools

In 2021, began trading publicly on the NYSE ($DNA) after raising $1.6 billion

Entered strategic partnership with Google in 2023 to develop industry-leading Generative AI models for biology

Case Studies - Use of Synthetic Biology for Ag Biologicals R&D

An end-to-end R&D platform to successfully bring novel ag biologicals...

Fast-tracking innovation pipeline

DISCOVERY

Identify unique microbes and genes with our proprietary **200,000+ member strain library** and metagenomic database of **5B+ genes**

STRAIN OPTIMIZATION & ENGINEERING

Improve **biologicals' performance** with best-in-class strain optimization using non-GM and GM techniques

FERMENTATION & FORMULATION DEVELOPMENT

Industry-leading **formulation expertise** across multiple product concepts. Test at scale with **3,000L pilot plant** facility

LAB-TO-FIELD TESTING

Test *in planta* with **rapid phenotyping** in our **175k ft^2 plant science facility** with growth chambers & greenhouses

...led by a team commercially and industrially focussed

Addressing relevant market and solutions

175,000 FT^2 *IN PLANTA* TESTING FOOTPRINT

LED BY THE TEAM THAT BROUGHT MULTIPLE PRODUCTS TO MARKET

Serenade®
Broad-spectrum fungicide

Poncho® Votivo® 2.0
Nutritional Nematicide

Requiem® Prime
Insecticide

Sonata®
Fungicide

PILOT PLANT

Ginkgo's R&D platform leverages synthetic biology to bring **solutions to ...**

Accelerate time to market for new biological products and second generation products with **significantly improved performance**

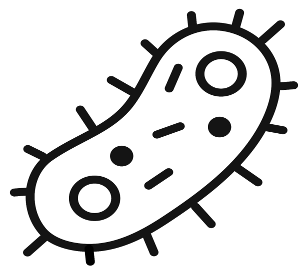
Expand market access by **reducing COGS** of biological products in or near-to commercial distribution

Earn growers' trust by **enhancing stability and shelf-life** of a biological product

Synthetic Biology allows to optimize and formulate microbes in a variety of ways to meet regulatory and commercial goals

Microbes – Live or Dead (applied to the field as “whole or intact-cells”)

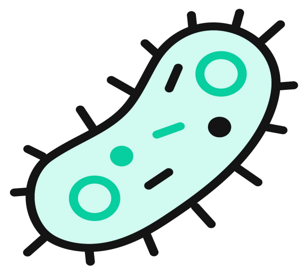
Generation 1
Wild Type Microbe



Entry point for most product classes

Products
Live microbes or spores in formulation

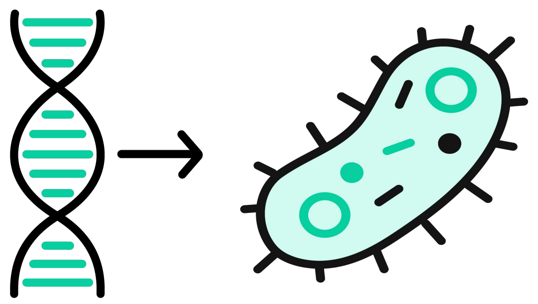
Generation 2
Randomly Mutagenized Microbe



Increased potency with reduced regulatory burden

Products
Live microbes or spores in formulation

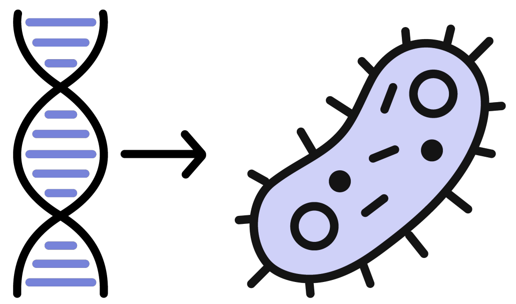
Generation 3
Engineered Microbe Cisgenic Approaches



Maximized species potency for select markets

Products
Live microbes or spores in formulation

Generation 4
Engineered Microbe Transgenic Approaches

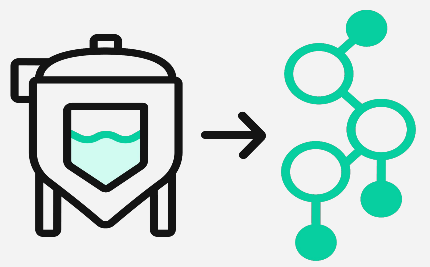


Maximized potency for select markets

Products
Live microbes or spores in formulation

Biochemicals

Wild, Cisgenic or Engineered Microbe



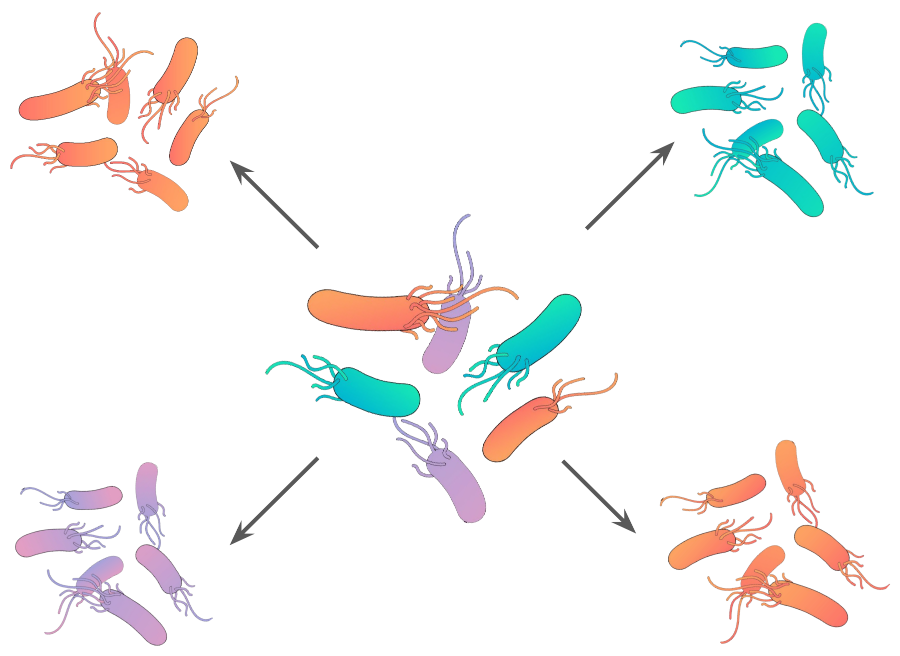
Discrete & bespoke production of natural compounds

Products
Biochemicals, enzymes, RNA produced through fermentation

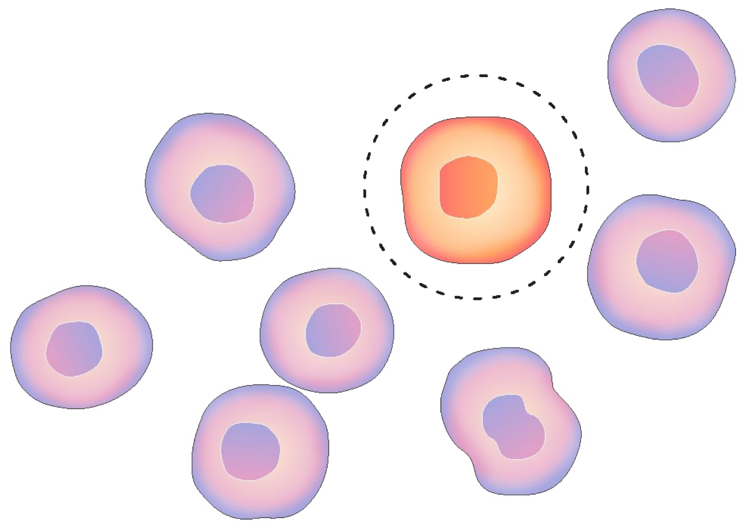
RAPID STRAIN OPTIMIZATION

Combine mutagenesis and ultra high throughput screening for 1.5-5x performance improvement

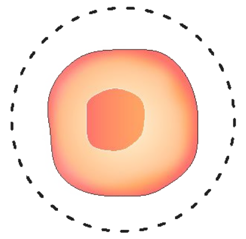
Random mutagenesis techniques rapidly generate millions of strain variants



But conventional methods to screen millions of strains are costly and time-consuming



Screen up to **1 million variants per day** with EncapS: Ginkgo's proprietary encapsulation and screening technology



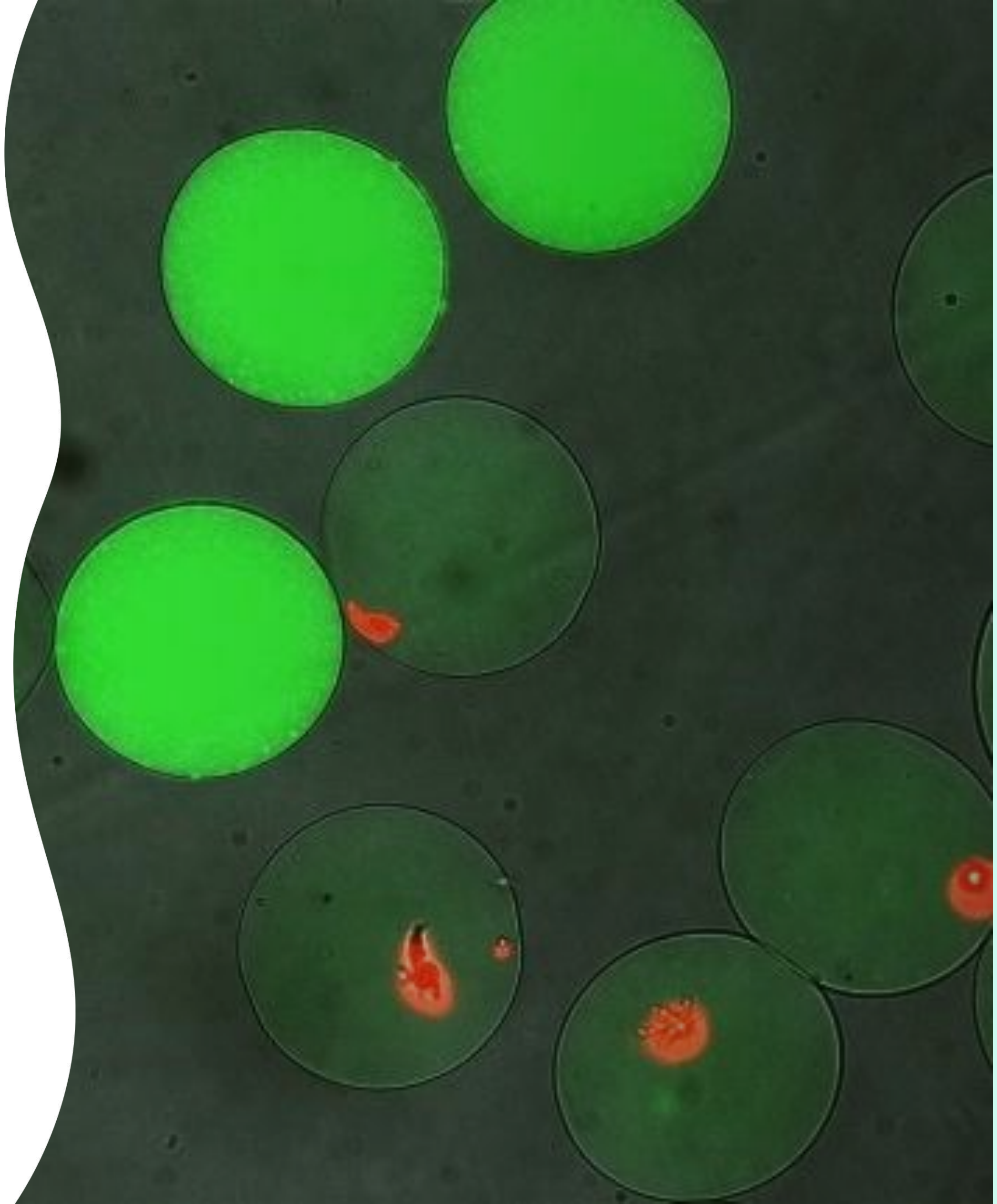
Find the needle in the haystack typically in less than one year

RAPID STRAIN OPTIMIZATION

EncapS (Encapsulation & Screening) performs phenotypic selection at unprecedented speed and scale

Our proprietary technology:

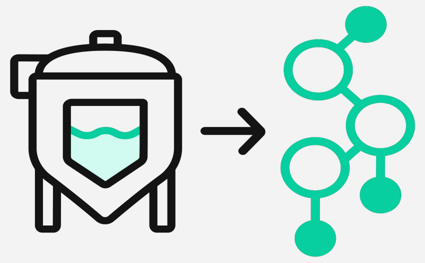
- Captures up to **1,000,000 cells per run** in nanoliter-scale reactors.
- Works with **many different cell types**.
    - Bacteria, yeast, filamentous fungi, mammalian cell lines.
- Cultivates microcolonies for reduced noise relative to single-cell approaches.
- Sorts and isolates high-performing microcolonies using **fluorescence assays**.
    - Diverse phenotypes.
    - **Secreted products.**
    - Secreted enzyme activity.



The same tools can be applied to optimize biochemical products

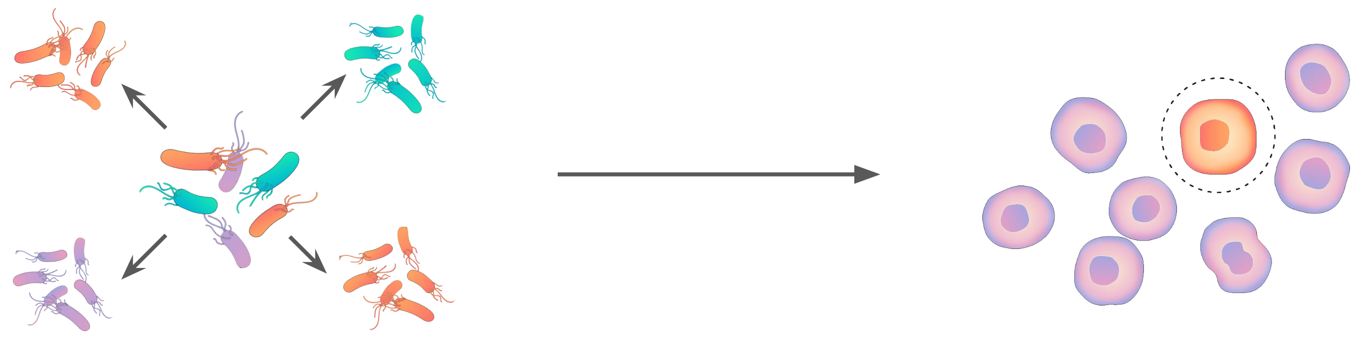
Biochemicals

Wild Type, Mutagenized or Engineered Production Strain



Discrete & bespoke production of natural compounds

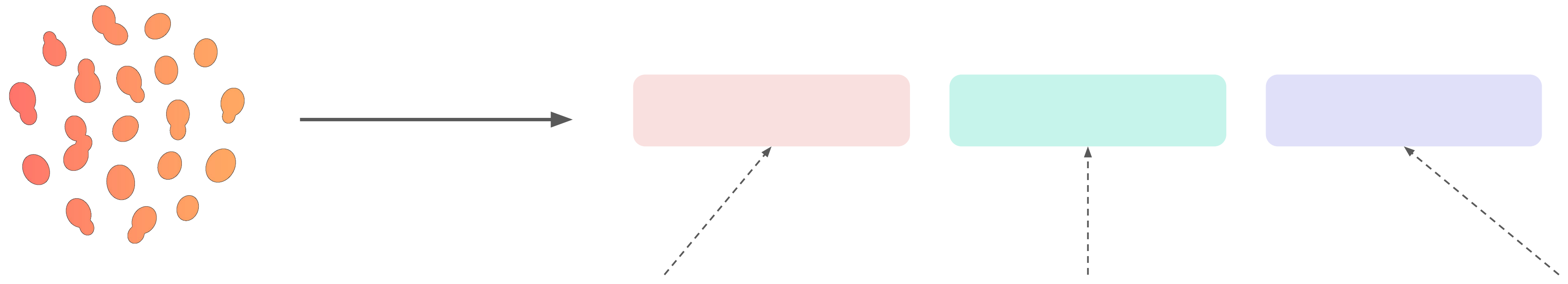
RAPID STRAIN OPTIMIZATION



Base strain

Mutated strains

RATIONAL STRAIN ENGINEERING



Fermentation-friendly strains

Overexpression constructs

Target Pathway

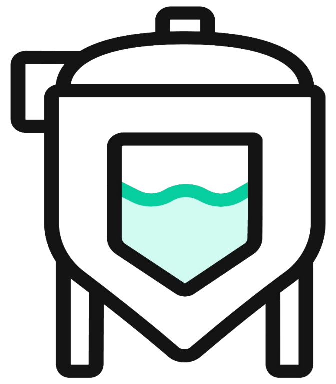
Targeted deletions

FERMENTATION PROCESS OPTIMIZATION

Design of experiments (DoE) campaigns

Process design

Final strain and process: commercially viable titer



Discovering novel peptides with significant antimicrobial activity

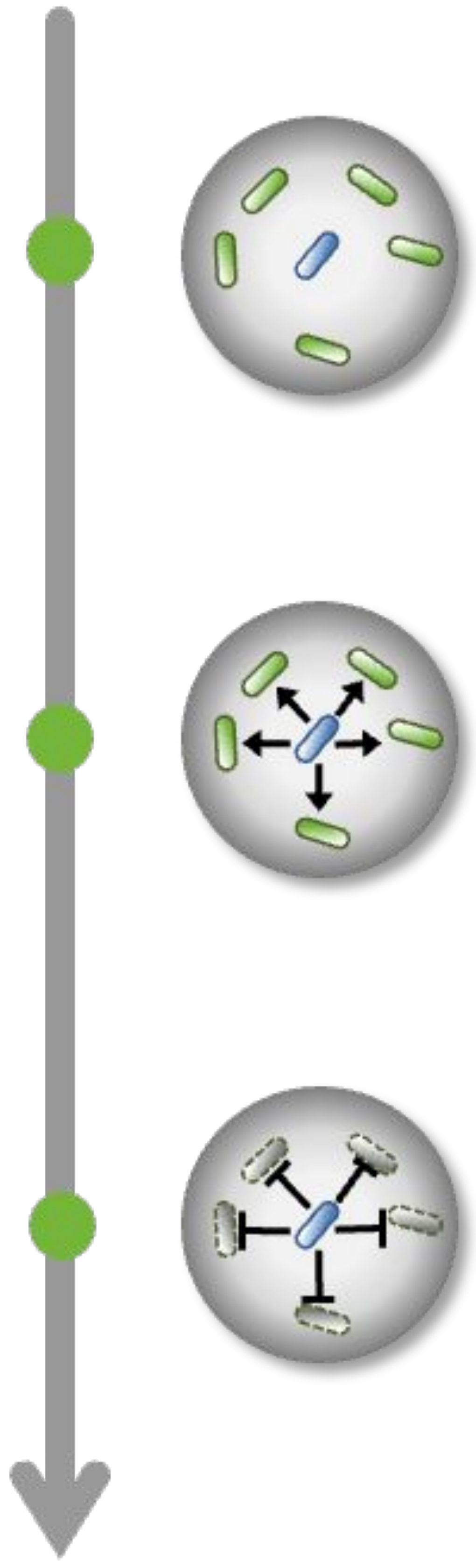
The Challenge

Discovering microbes that produce novel peptides requires the analysis of large combinatorial libraries to select improved strains.

Our Work

Our team co-encapsulated each variant in the library with a target pathogen in nanoliter reactors. The team screened each variant's ability to inhibit growth of the pathogenic strain. The screening campaign resulted in 126 hits

Co-encapsulation assay design



NLRs contain **candidate cell** (blue) and **pathogenic sensor cells** (green)

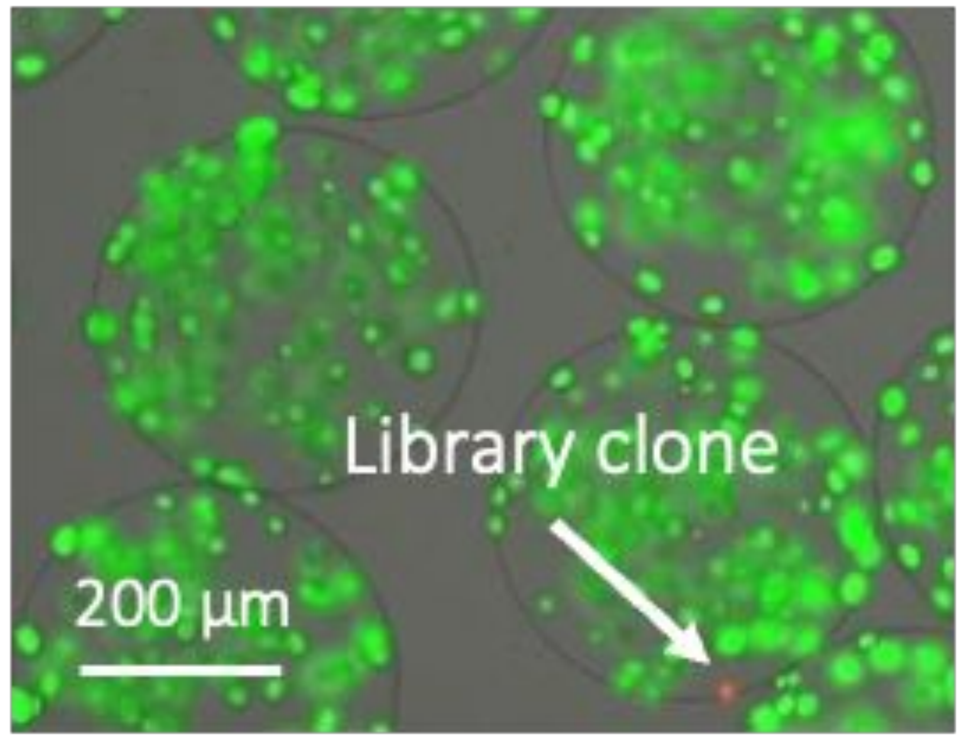
Cells proliferate and candidate cells secrete lantibiotic peptides

Fluorescence correlates with inhibition of pathogenic sensor cell growth by secreted lantibiotic peptides

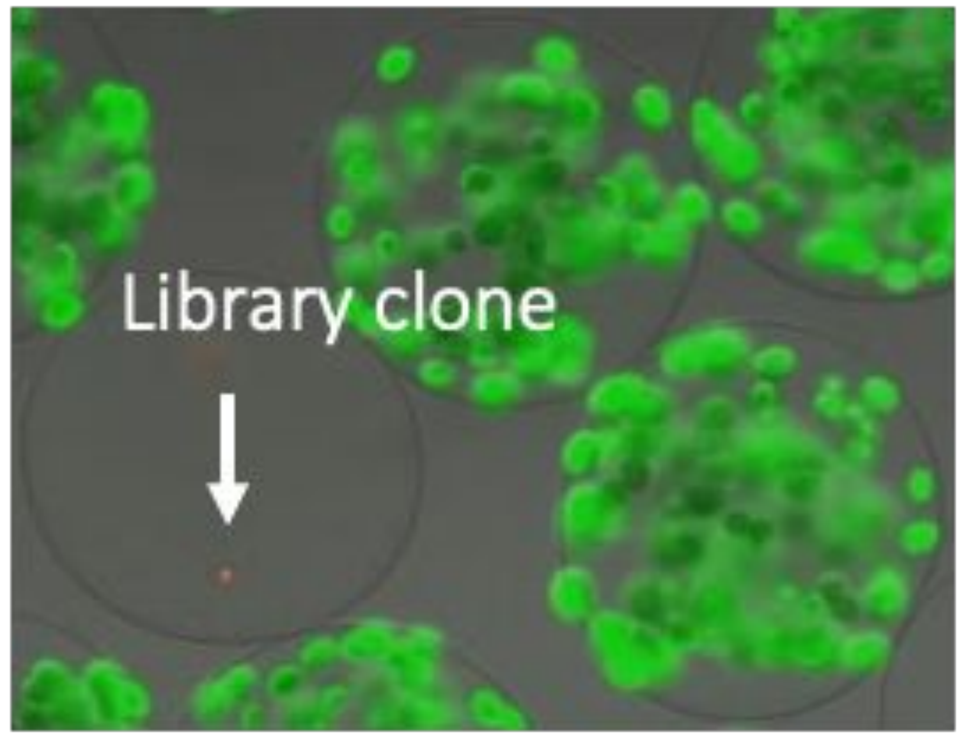
Discovering novel peptides with significant antimicrobial activity

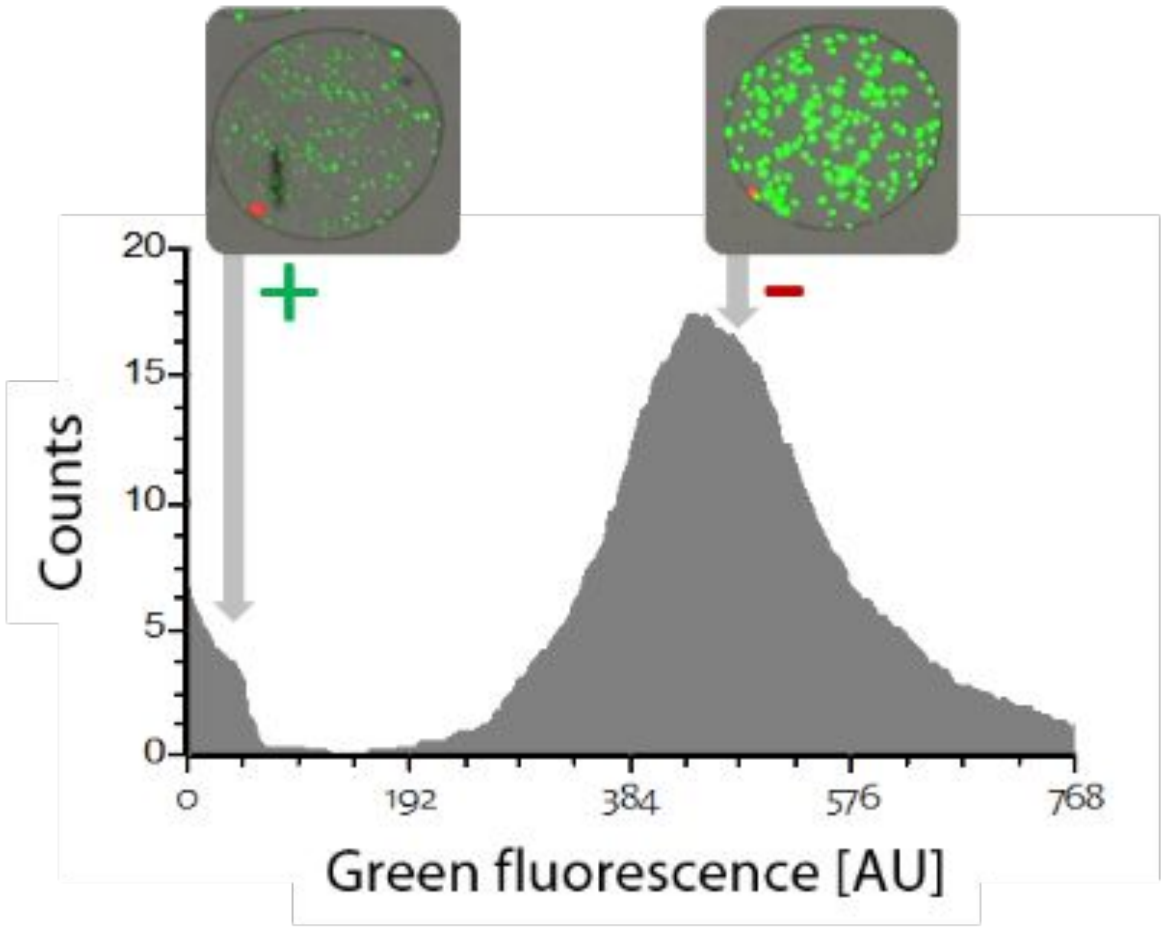
Selection and characterization of candidates

No activity



Active lantibiotic





	S.aureus CAL	*S.aureus MW2*	*E. faecium LMG16003*	*B. cereus ATCC14579*	*E. faecalis BE14089*	*S. pneumoniae DS9*	*S. pneumoniae TIGR-4*
SC5.0069.1	2.8	2.1	7.0	44.3	7.0	0.3	0.3
SC5.1743.1	7.3	6.1	0.5	2.4	0.8	0.4	3.4
SC5.1057.1	3.6	3.6	0.9	2.4	0.9	0.8	0.6
Nisin	3.1	1.9	0.3	2.7	0.6	3.7	4.0

MIC [µg mL^{-1}]

126

lantipeptide designs with significant antimicrobial activity

(Schmitt *et al.*, Nat Chem Biol. 2019 May;15(5):437-443)

Case Studies - Use of Synthetic Biology for Plant Traits R&D

Ginkgo's platform is well-positioned to support discovery, expression & optimization of novel input traits

Input Traits

- Insect Protein Control
- Herbicide Tolerance
- Disease Resistance

Agronomic Traits

- Drought
- Flood
- Salinity
- Yield

Early R&D to Late R&D → Plant Transformation → Regulatory & Plant Breeding → Field Trials & Commercialization

Trait Discovery → Gene and Protein Expression Engineering → Construct Design Optimization → Efficacy Testing

TRAIT DISCOVERY

We mine our industry-leading metagenomic library with patented tools to find next novel trait

Our existing metagenomic libraries contain significant diversity and sources of value that have not been discovered by others

Market leading size.
>3.75 terabases, 2 billion genes*, and 8.38 million natural product gene clusters**

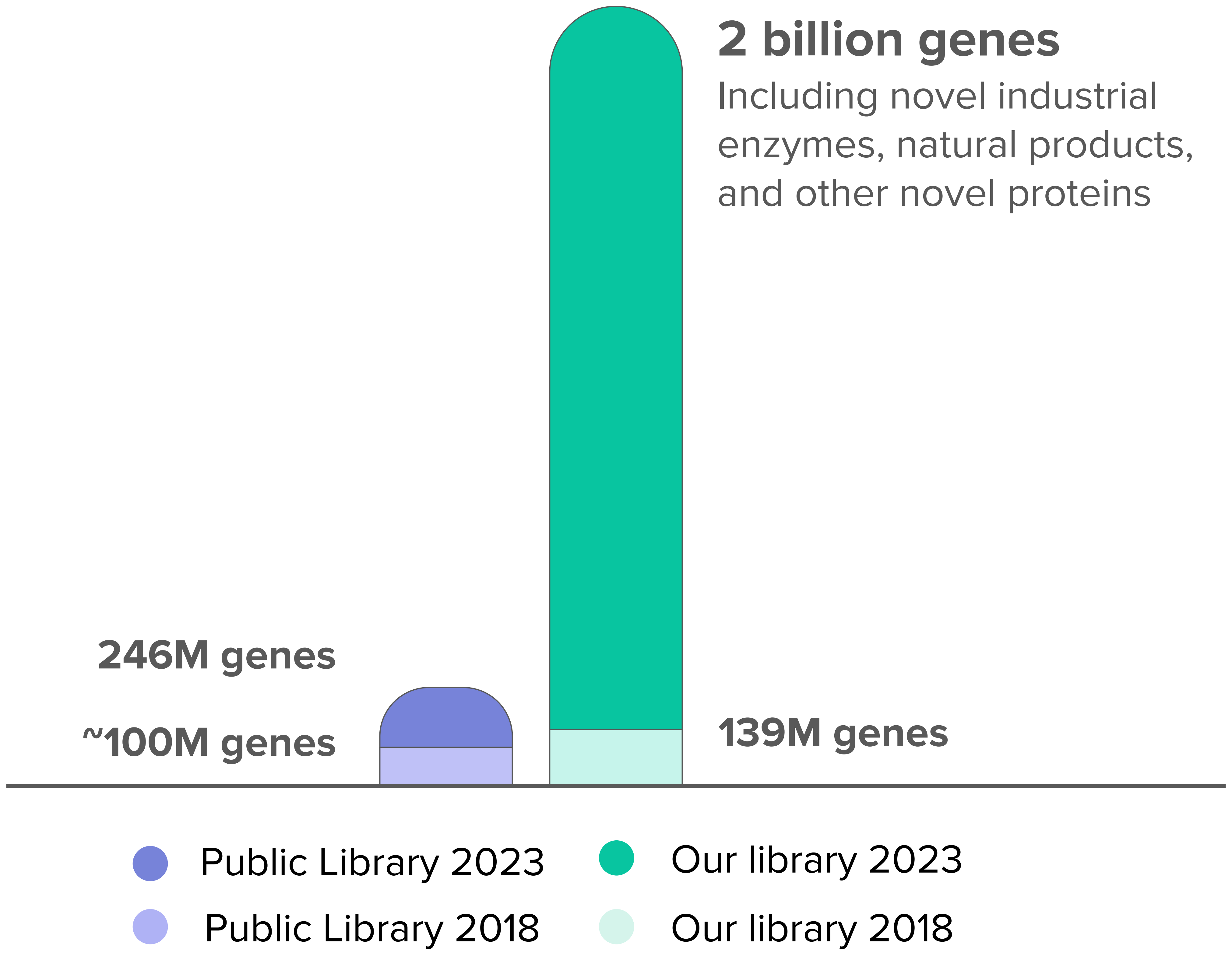
Long continuous sequences.
>2 terabases of total sequence contained on contigs >10kb

High novelty.
<5% overlap with public sequence

Sourced from uncultured microbes.
>99% unknown to science

Physical as well as digital.
Samples are physically arrayed, for rapid access, facilitating expression and testing of samples

Expanding and improving.
Proprietary library creation methods enable us to expand and enrich libraries



*clustered at 90% aa id per library | **non-deduplicated

TRAIT DISCOVERY- CASE STUDY

Finding novel, unencumbered insect control proteins (ICPs) for new MoA

CHALLENGE

A Tier-1 agricultural company was seeing increasing pest resistance to its traited ICPs. Meanwhile, it seemed like its existing protein library wouldn't yield new proteins with full FTO and new MoA. Could Ginkgo's use machine learning and enrichment in its megategenomic database to find a novel protein and reset the resistance clock?

OUTCOME

Ginkgo combed through its metagenomic database using a query demand based on known ICPs and used its patented enrichment and amplification techniques to find 220 candidates out of thousands. Further criteria narrowed it down to 92 putative proteins that Ginkgo produced and purified for diet bioassays. While 1 constituted "a homerun", Ginkgo found 3 proteins that worked in the killing assay, showing potential new MoA.

1,000s
of protein possibilities

↓

220
candidates

↓

92
selected for diet bioassy

↓

3
showing killing effectiveness — **2 more** than the client hoped for!

Ginkgo uses its cell engineering expertise to optimize plant trait gene construction & expression

Multi-gene constructs

- Requires scale of ~4,500 constructs
- Designs are largely bespoke to the groups generating them
- Best practices on design are not shared between the various program teams

Speed

- Transformation is determined by narrow windows upstream of greenhouse and field experiments
- Turn around time (TAT) is important to recover quickly when transformation fails

Quality

- Improving the quality of multi-gene designs to remove repetitive sequences
- Backup designs allow “bouncing back” from failed transformation

Our Design and Build teams have deep expertise in complex gene constructs

Onboard and standardize constructs across different programs to allow flexibility in DNA design

Improve quality of multi-gene designs (e.g. applying heuristics to avoid repetitive sequences)

Deliver **multiple backup designs** in parallel with preferred designs

Offer **TAT** for multi-gene constructs on the order of ~3 weeks

Ginkgo utilizes best-in-class codon optimization tools for reliable gene expression

Our codon optimization algorithms outperform external vendors and literature state-of-the-art

Proteins codon optimized using:

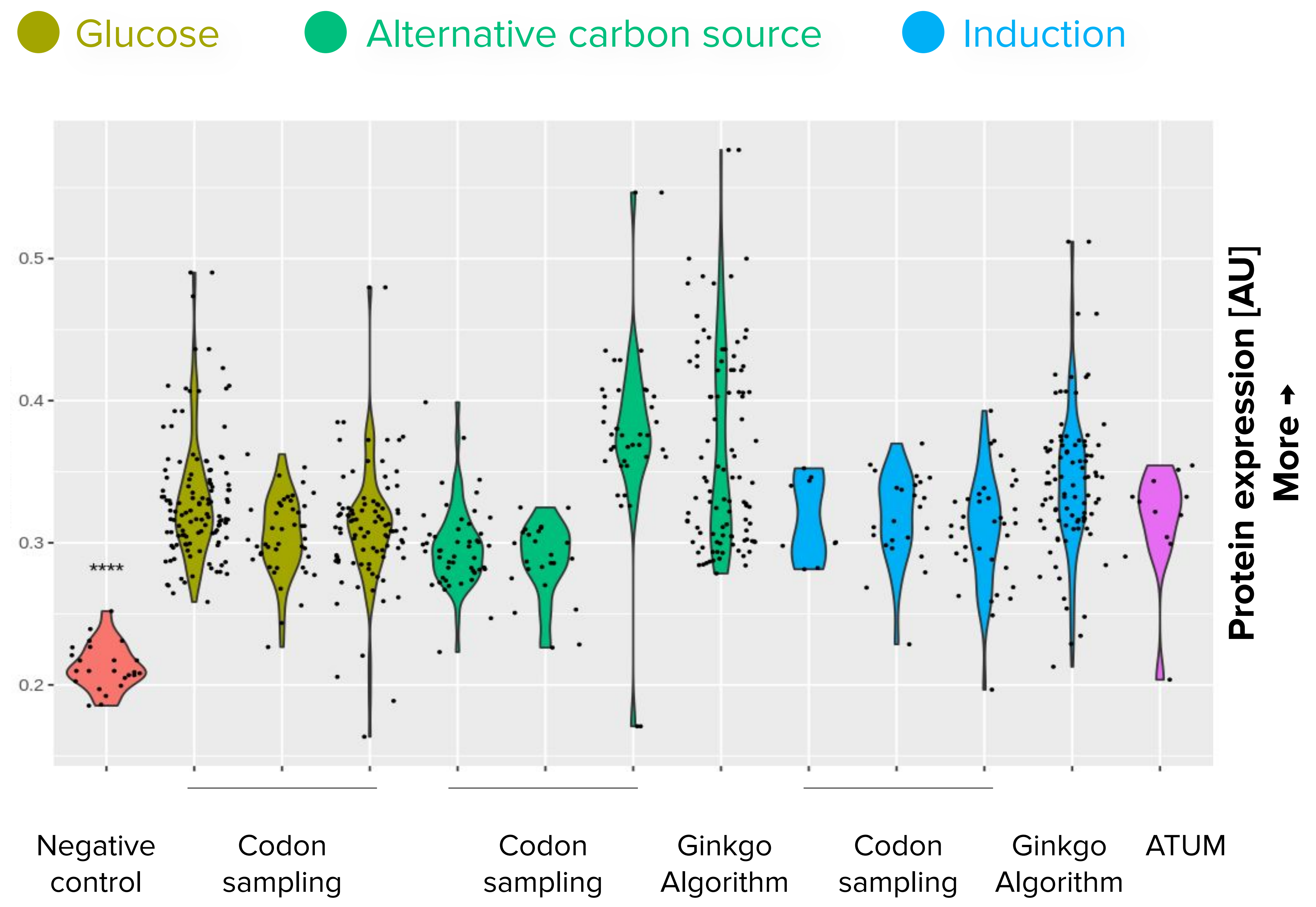
- Sampling from codon table (common practice)
- Ginkgo ML algorithm
- ATUM (formerly DNA 2.0)

Ginkgo ML algorithm has:

- Higher median expression than all others
- Higher top expression

Protein expression in *Pichia pastoris*

Codon tables & ML algorithm tailored to expression under distinct fermentation conditions



CASE STUDY: PROMOTER OPTIMIZATION

Best in class promoter optimization for reliable protein expression in eukaryotes

CHALLENGE

Similar to plant traits, cell & gene therapies **must deliver payloads only in target tissue types, at the right amounts, and at the right times to be safe and efficacious**. Like plants, the rules governing mammalian gene expression are complex.

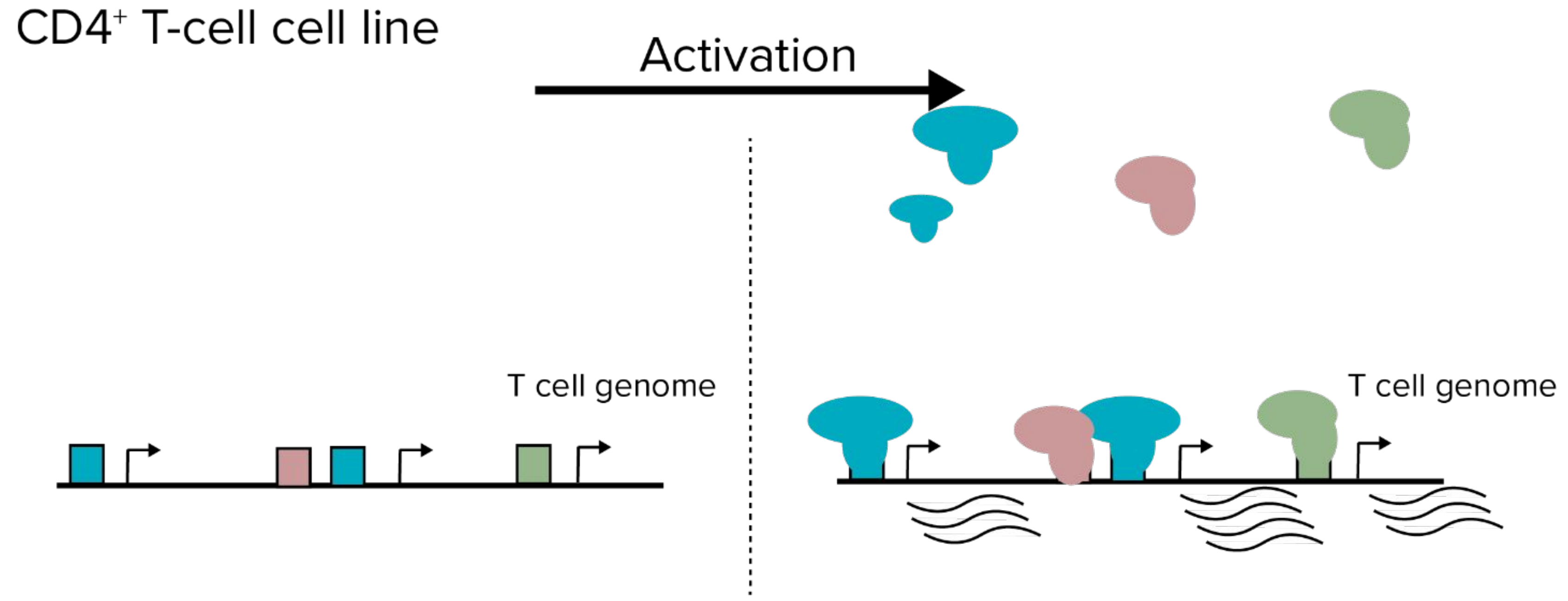
In this project, we designed promoter sequences that only induced gene transcription after receiving a chemical signal through a specific cell surface receptor.

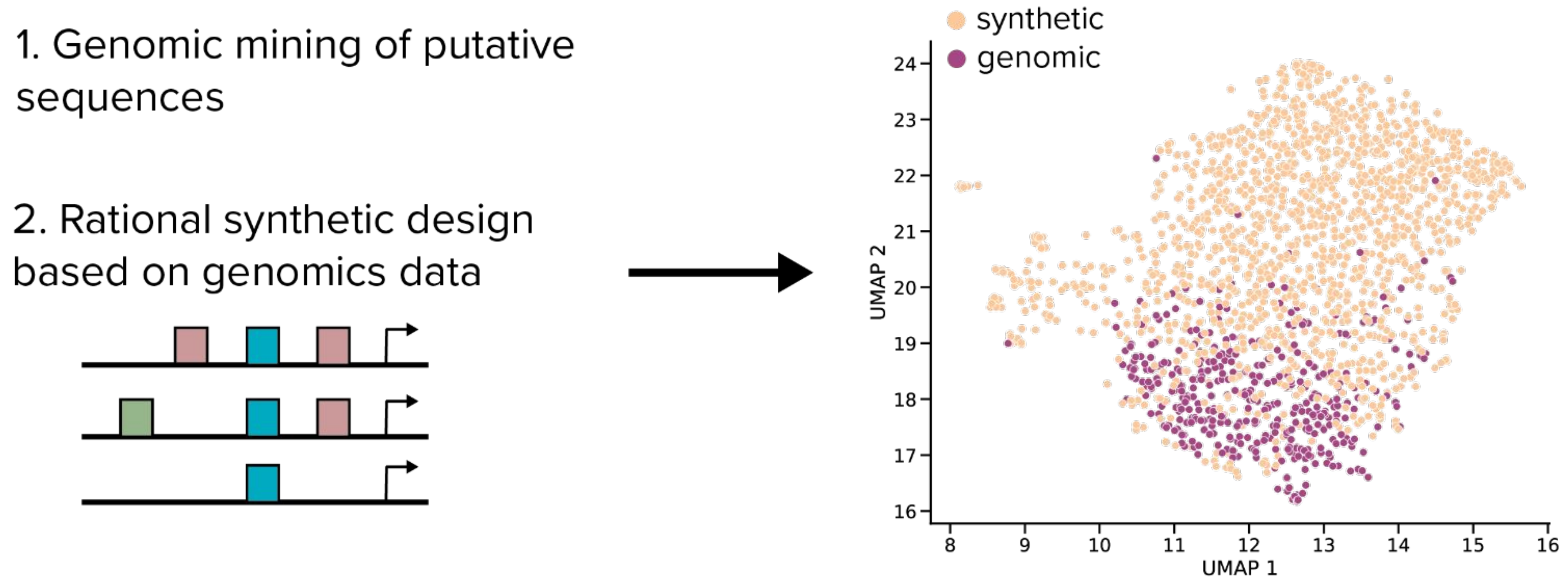
APPROACH

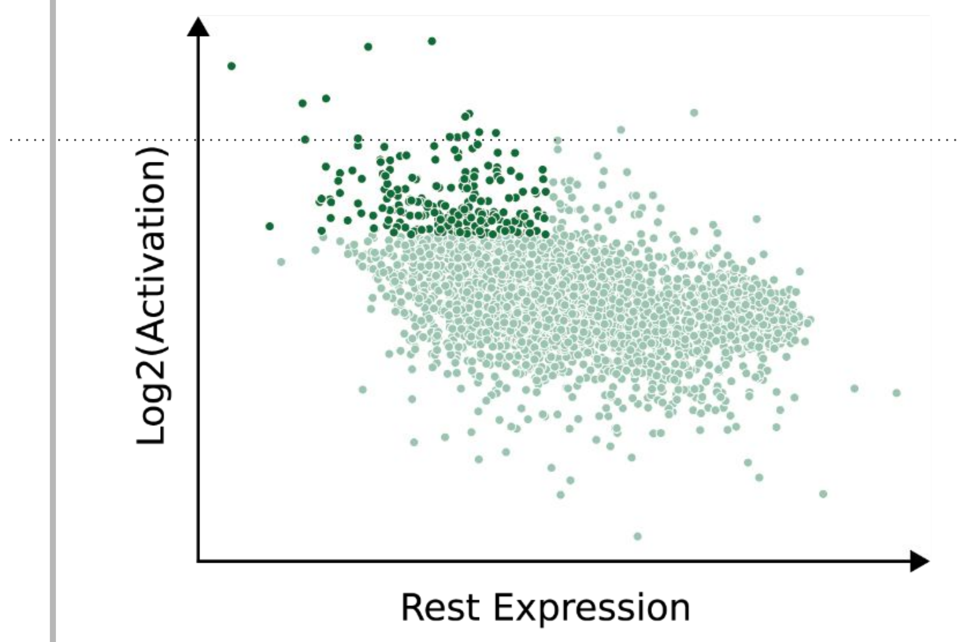
Leveraging highly multiplexed assays, Ginkgo designed a library of **10,000 enhancer-promoter sequences**, including natural and synthetic variants, and assessed their activity under inducing and non-inducing conditions.

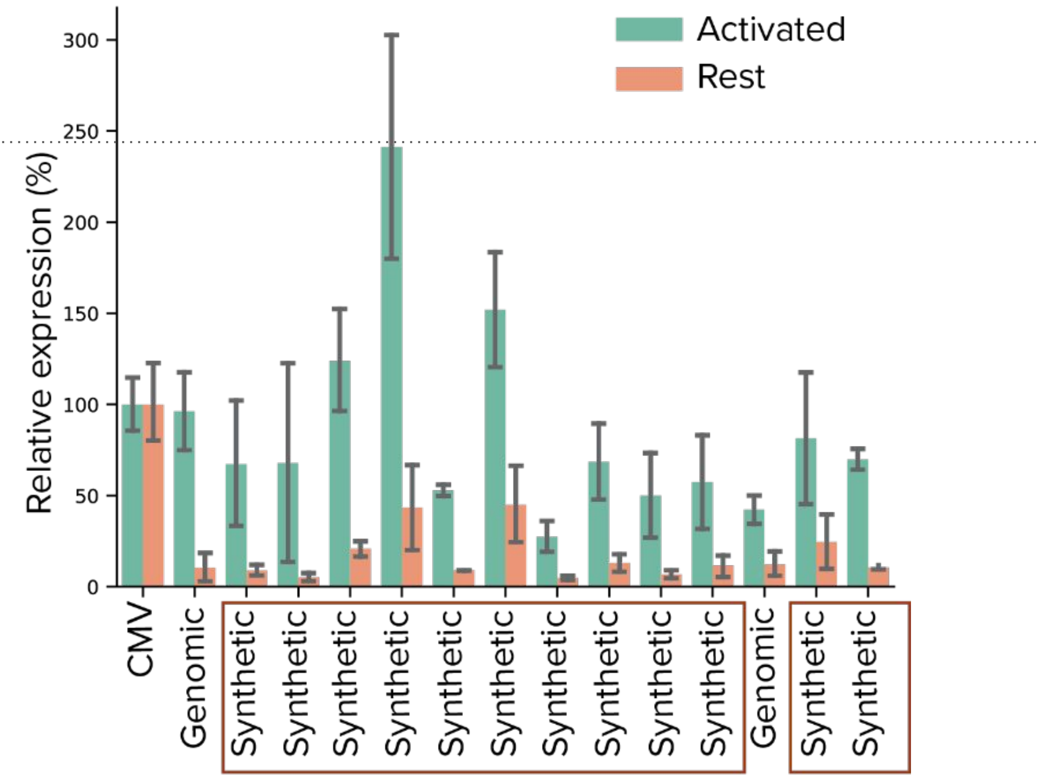
OUTCOMES

In **<4 months** from conception through results, the team identified **271 sequences** that are significantly activated. **14 of these showed strong expression and greater than fourfold induction** by the chemical signal.









Considerations and Future Prospects

To successfully harness Synthetic Biology for Agriculture

Ethical Concerns

Environmental Impact:
- Alteration of ecosystems due to the release of genetically modified organisms.
- Potential unintended consequences on non-target organisms.

Ownership and Access:
- Issues related to patenting and ownership of genetically modified seeds.
- Concerns about access to technology and its affordability for small-scale farmers.

Social and Economic Equity:
- Possible exacerbation of existing social and economic inequalities.
- Unequal distribution of benefits and risks among different communities.

Unintended Consequences:
- The possibility of unintended ecological or health consequences from genetic modifications.
- Long-term effects on biodiversity and ecosystem stability.

Risks

Biosafety:
- Accidental release of genetically modified organisms into the environment.
- Spread of modified genes to wild populations.

Health Concerns:
- Unknown effects of consuming genetically modified crops on human health.
- Potential development of allergens or toxins.

Resistance and Evolution:
- Development of resistance in target pests or pathogens.
- Evolutionary responses leading to new challenges in pest management.

Dependency on Technology:
- Increased reliance on synthetic biology for agriculture.
- Vulnerability to failures or unintended consequences of the technology.

Regulatory Framework

Risk Assessment:
- Establishing robust risk assessment protocols for genetically modified organisms.
- Continuous monitoring and evaluation of environmental and health impacts.

Transparency and Labeling:
- Ensuring transparency in the development and deployment of synthetic biology applications.
- Implementing clear labeling for genetically modified products.

International Cooperation:
- Developing international agreements and standards for synthetic biology in agriculture.
- Collaboration on research and regulatory frameworks to address global challenges.

Public Engagement:
- Involving the public in decision-making processes related to synthetic biology.
- Educating and informing stakeholders about the technology and its implications.

CCTTCAGCTAGTGGAAGATATT
GTTTAGTCGAGAGAGTTGCACA
CTTCACTGAAGTTCTGCAAATT
ATACATGAGCTGTACCCTGATT
TGCAACTTCCAGAGAAATGTTC
GGATGATAAACCTTTTGTGCCA
ACATATCAGGTGTCCAAAGAAA
AGGCAAAGAGCTTGGGAATTGA
GTTTATTCCATTAGACATTAGC
CTCAAGGAAACAATTGAAAGCT
TGAAGGAAAAGAGTATCGTCAG
CTTCTGAATGAGCAACAAGGTG
GTCTGCGTCACGGGTGCCTCCG
GCTACATTGCTTCATGGCTCGT
CAAGCTCCTCCTCCAACGCGGC
TACACTGTCAAGGCCTCTGTTC
GCAACCCAAATGATCCAACAAA
GACGGAGCACTTGCTCGCACTT
GATGGAGCTAAGGAGAGACTTC
AACTTTTCAAAGCAGATCTATT
AGAAGAAGGTTCTTTTGACTCT
GCTGTTGAGGGCTGTGAGGGTG
TTTTCCACACTGCATCCACTGC

Future Prospects - biology is fundamentally **programmable**

Teaching AI to Speak DNA

GINKGO BIOWORKS × Google Cloud

Ginkgo Bioworks and Google Cloud Partner to Build Next Generation AI Platform for Biological Engineering and Biosecurity

PR Newswire Aug 29, 2023

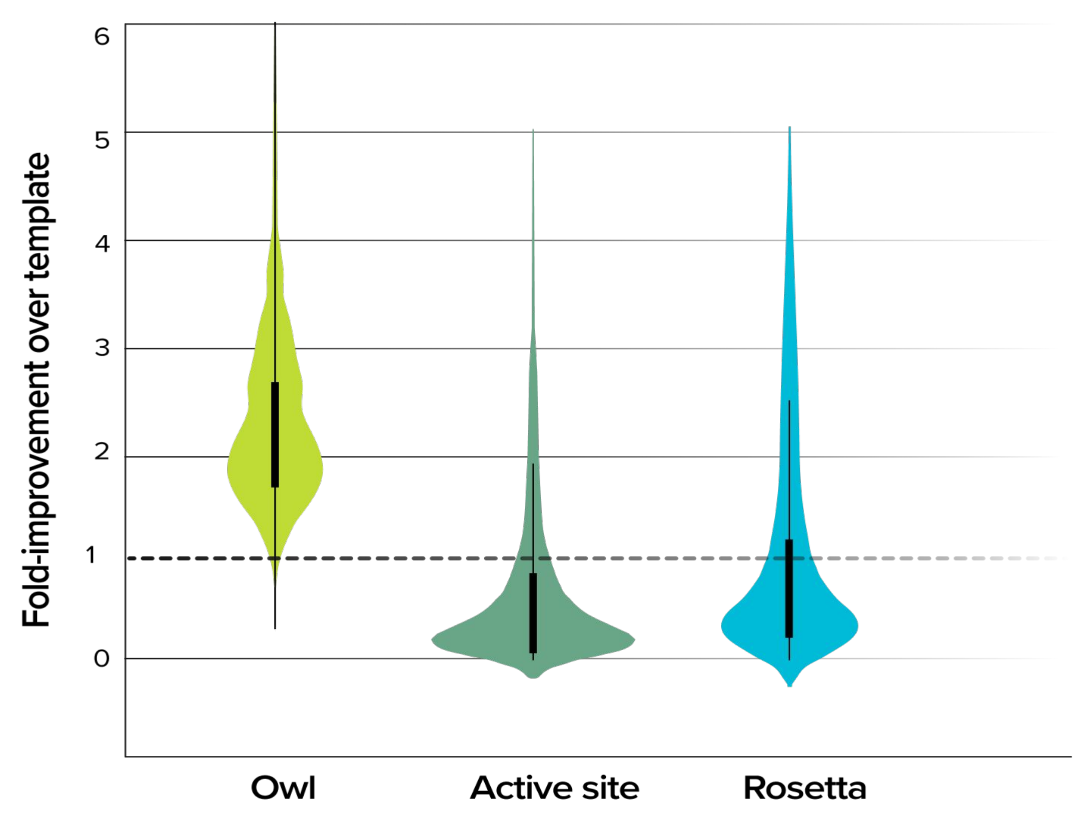
Ginkgo Enzymes Services are powered today by AI/ML design

AI platforms are only as good as the data they are trained on. Ginkgo has 2B+ protein sequences and 5M+ enzyme designs built and tested in our proprietary DNA database.

Ginkgo uses AI/ML today to drive enzyme design and discovery. Now, our partnership with Google Cloud will build foundation models for generative AI to supercharge how agricultural solutions are discovered and optimized.

LEAD PROTEIN OPTIMIZATION - CASE STUDY

Improving enzyme k_{cat}/K_M 10x via iterative ML-guided protein design



Large experimental datasets coupled with Owl ML-guided design yield more variants with improved performance as compared to traditional methods.

	Gen 1	Gen 2	Gen 3	Gen 4
Library Size (unique enzymes)	2,000	2,000	4,000	100
Design Approach	• Sequence-based (self-supervised) model predictions • Active site mutagenesis • Rosetta docking & design	• Hit recombination • Sequence-based (self-supervised) model predictions • Rosetta docking & design	• Owl ML-guided design • Active site mutagenesis • Molecular dynamics • Rosetta docking & design	• Owl ML-guided design
Experimental Screening Methods	• Arrayed activity testing	• Arrayed activity assay • uHTP pooled abundance screening on comprehensive site-saturation libraries	• Arrayed activity assay • uHTP pooled abundance and activity screening on large ($>10^6$) combinatorial libraries	• Arrayed activity assay
Fold Improvement over Wild Type	**1.5X**	**3.9X**	**4.5X**	**10X**

Synthetic biology in Agriculture holds great promise for addressing challenges and contributing to sustainable and resilient food production systems.

It is essential to approach these advancements with a commitment to ethical considerations, regulatory compliance, and inclusive engagement with stakeholders.

Thank you!!!!