Synthetic Biology of Natural Products

New Tools for Unlocking Natures Medicine Cabinet

terra bioforge

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$1T Natural Products Market and Problem

Pharma Biotech

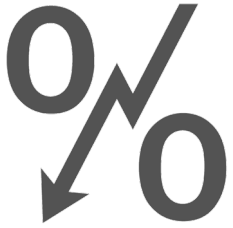
Agriculture

Animal Health

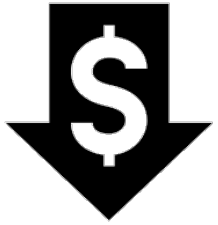
Consumer

“Why is NP production so expensive?”

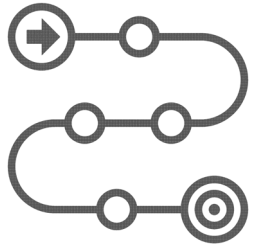
“Why does commercialization take so long?”



Low Yields



Low Margins



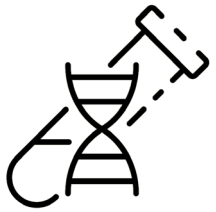
Long Timelines

Advantages of Engineered Natural Products

More affordable supply $$$$

Faster commercialization

Superior purity and quality

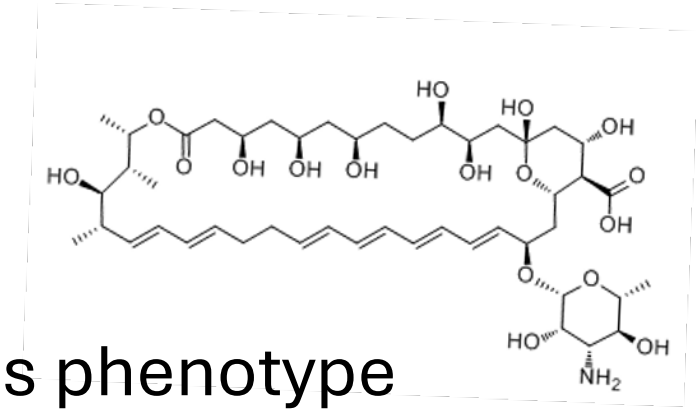


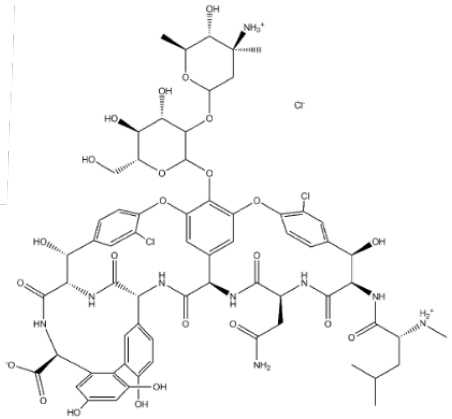
Potential for modification exploiting NP diversity

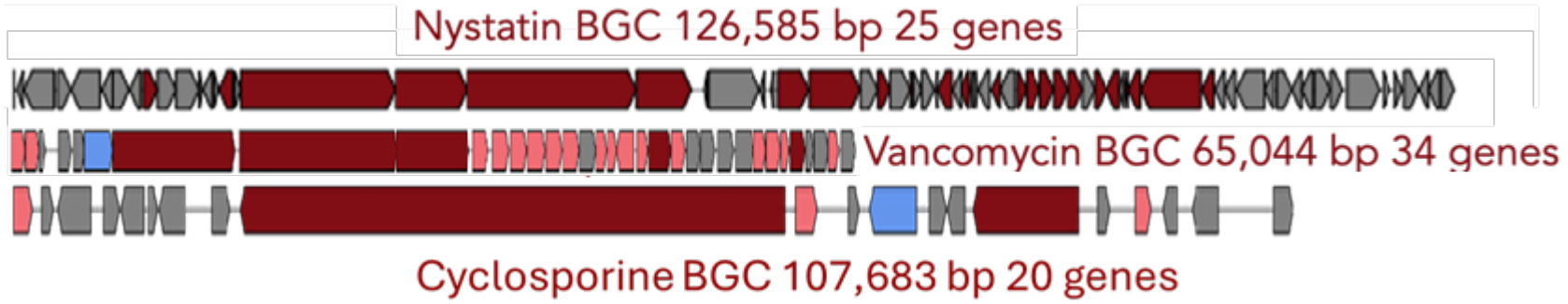
NP Discovery/Production Is Challenging

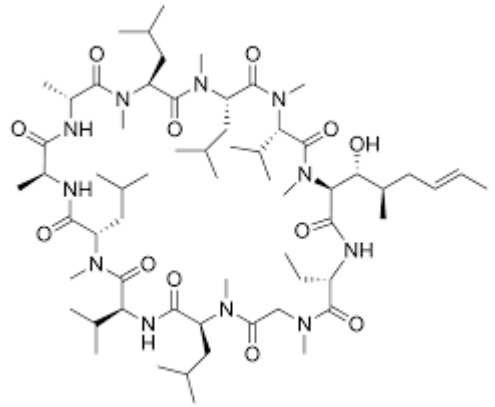
Biosynthetic Gene Clusters are:

- **Large** – up to 400,000 base pairs
- **Complicated** – dozens of genes
- **Difficult to discover** – genotype versus phenotype
- **Difficult to express** – many pathways are cryptic
- **Variable culture** – native host may not always be useful









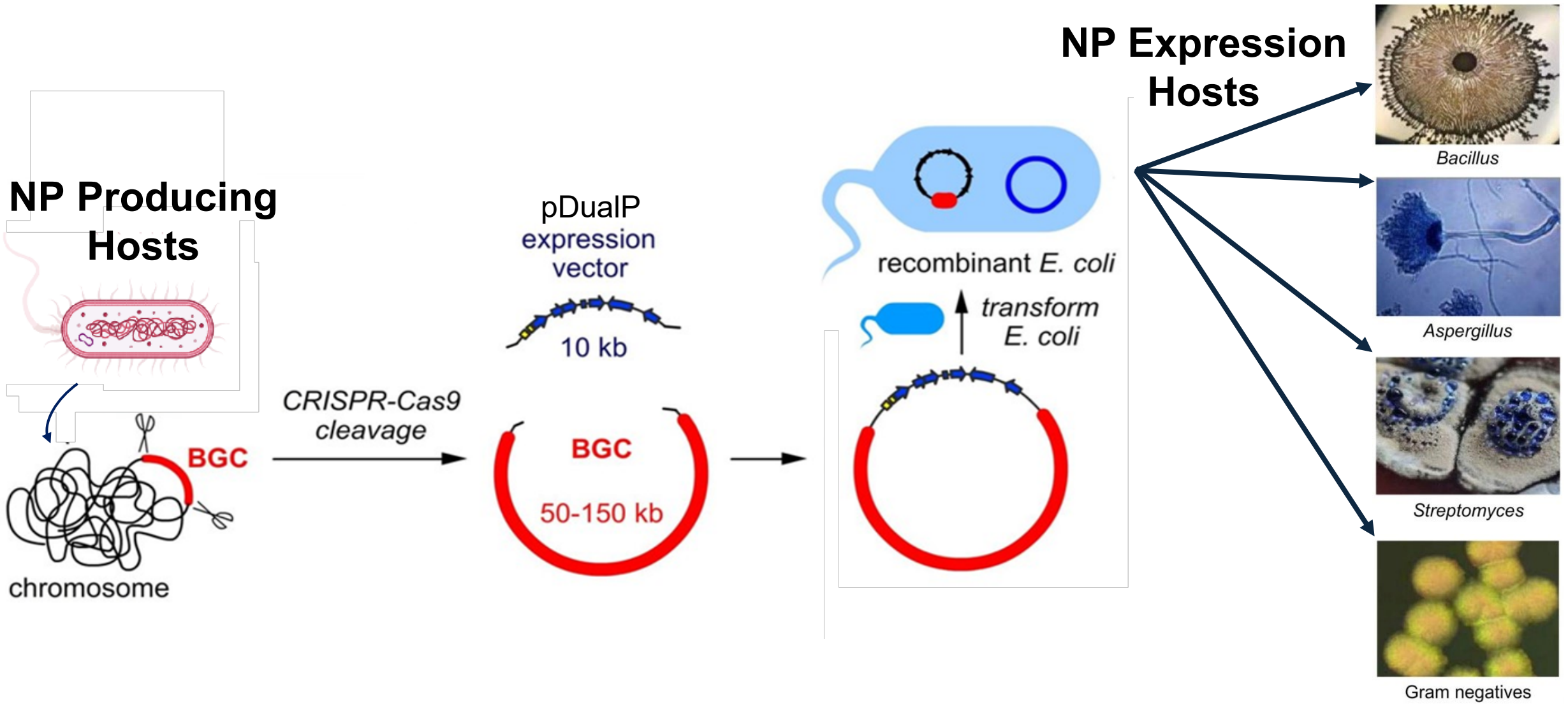
Cloned and expressed by Terra Bioforge

DNAtrap™
Microbial BGC Cloning

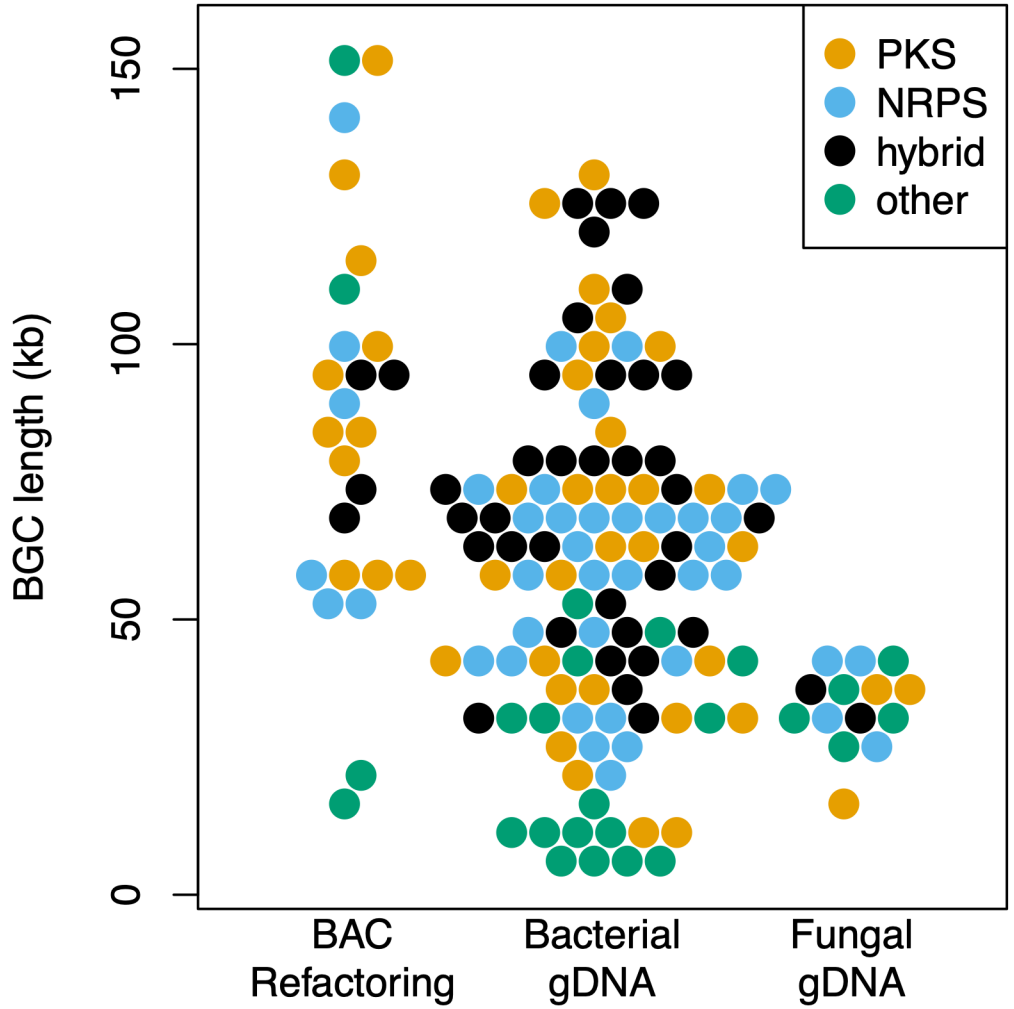
terra bioforge®

terra bioforge

PIONEERED “BIG DNA” TOOLS CAPTURING $B NP PATHWAYS



Terra Bioforge Captures Intact BGCs



- >150 BGCs cloned from >100 strains
- ~70% from Actinomycetota (high GC)
- ~10% from Bacillota
- Clusters from filamentous fungi (30-40 Mb genomes)
- Cluster sizes range from ~10–130 kb
- Refactoring BGCs
  - subcloning
  - insertions/deletions
  - promoter swaps

pDualP
Bacterial BGC Activation

terra bioforge®

terra bioforge Inducible Azaserine Expression in Streptomyces

GDCh Communications Angewandte International Edition Chemie www.angewandte.org

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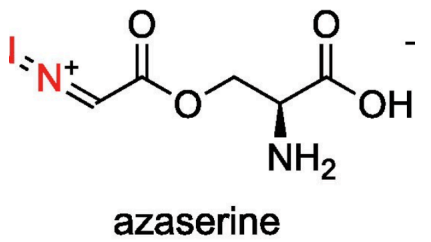
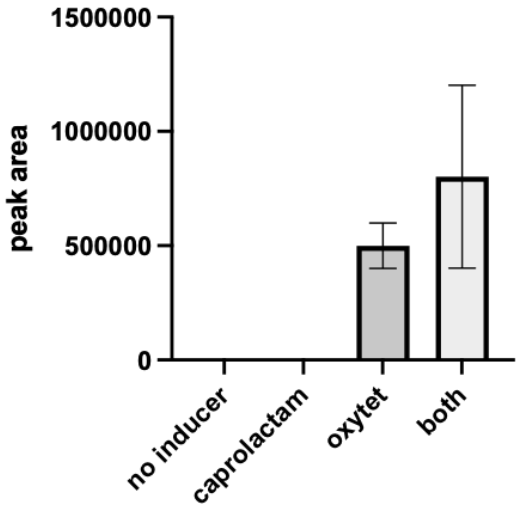
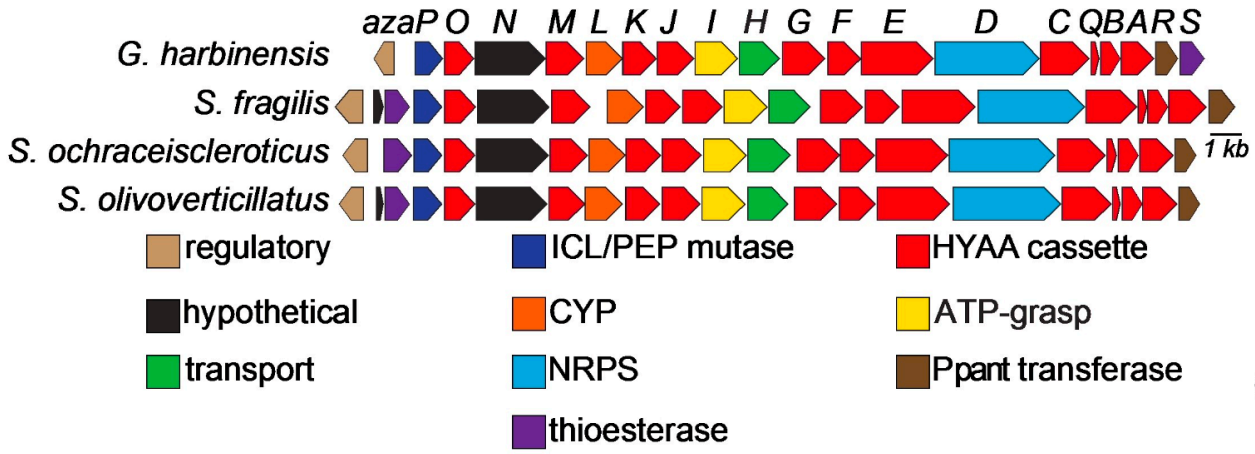
Biosynthesis

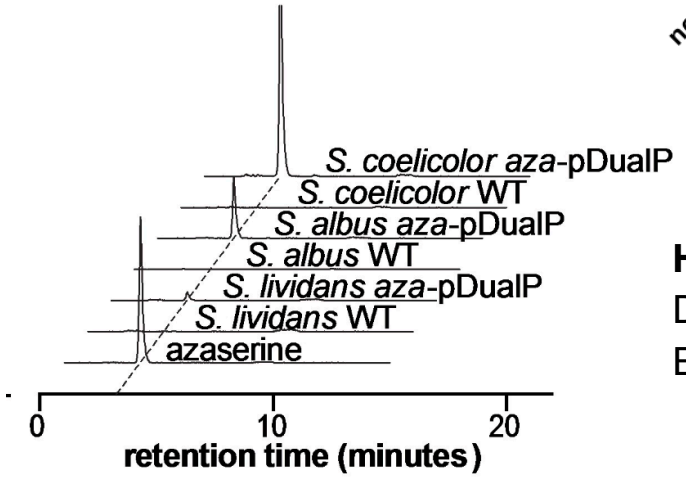
Discovery of the Azaserine Biosynthetic Pathway Uncovers a Biological Route for α-Diazoester Production

*Devon Van Cura, Tai L. Ng, Jing Huang, Harry Hager, John F. Hartwig, Jay D. Keasling, and Emily P. Balskus**





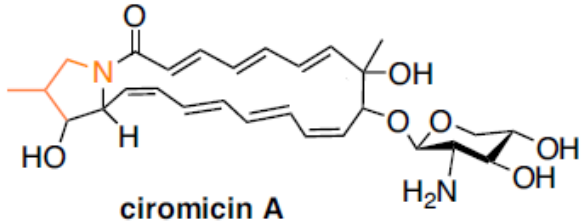




Harvard University
Devon Van Cura
Emily Balskus

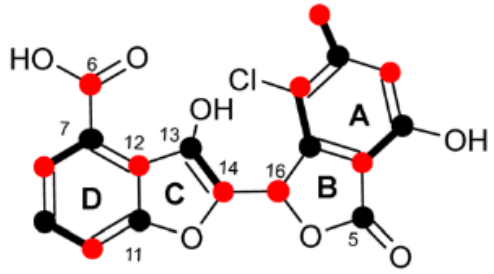
Inducible BGC Expression in Actinobacteria

Discovery of unreported β-amino acid macrotermycin derivatives



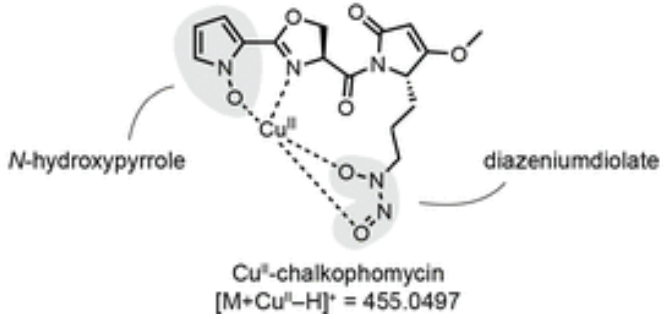
https://www.nature.com/articles/s42004-023-01034-w

Heterologous expression of seongomycin allowed assessment of its antibacterial, antiproliferative, and antiviral activities and revision of its structure



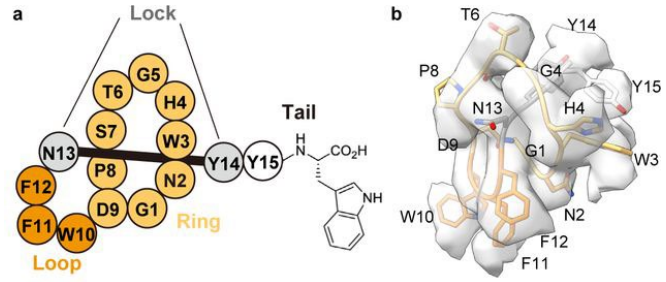
https://pubs.rsc.org/en/content/articlehtml/2023/ra/d3ra05931f

Chalkophomycin BGC Expression Identified New N-nitrosating Enzymes



https://www.biorxiv.org/content/10.1101/2024.01.24.577118v1.abstract

Structure of a lasso peptide bound ETB receptor provides insights into the mechanism of GPCR inverse agonism



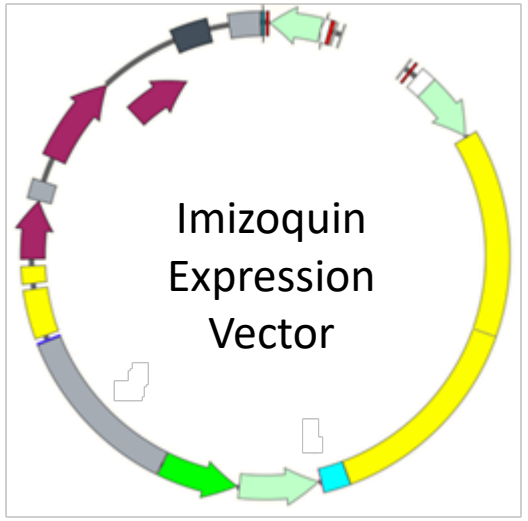
https://www.biorxiv.org/content/10.1101/2023.12.30.573741v1.abstract

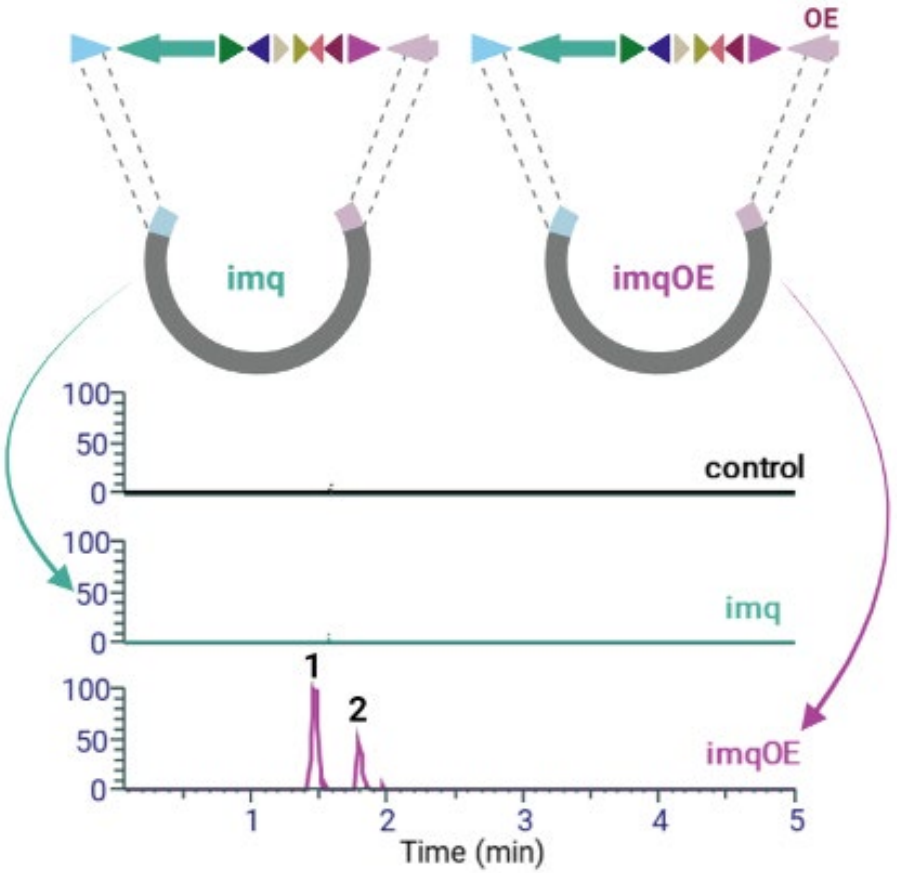
Targeted Cloning and Heterologous Expression of Fungal Clusters

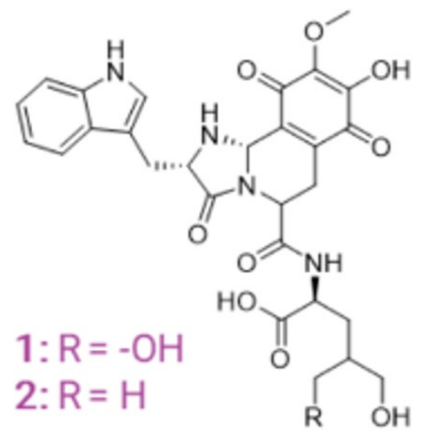
BGC manipulations using AMA1

Native imizoquin (img) cluster fails to heterologously express in *A. nidulans*

Promoter replacement (OE) activates a silent BGC for heterologous imizoquin production

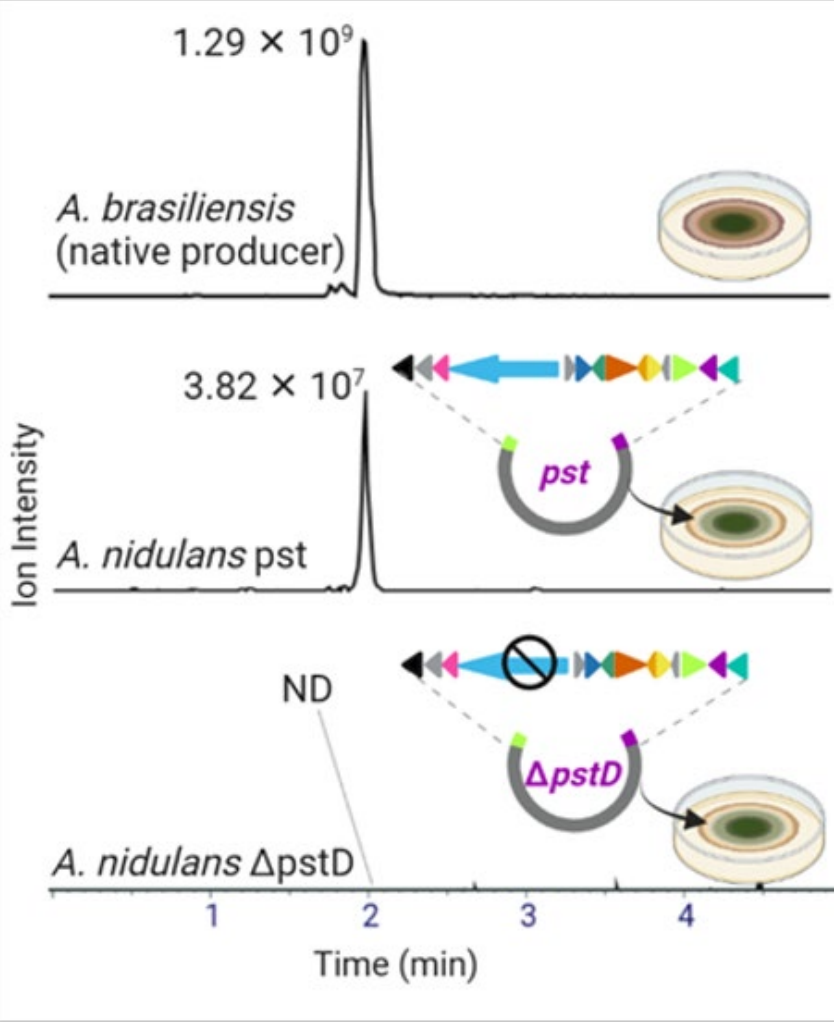






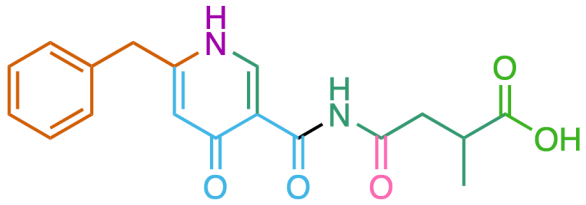
https://www.nature.com/articles/s41589-023-01276-8

Targeted Cloning and Metabologenomics Demystifies Pestalamide Biosynthesis



nature chemical biology

Correlative metabologenomics of 110 fungi reveals metabolite–gene cluster pairs



Pestalamide B

Validated Metabologenomics Platform

Resolved correct biosynthetic pathway for pestalamide

https://www.nature.com/articles/s41589-023-01276-8

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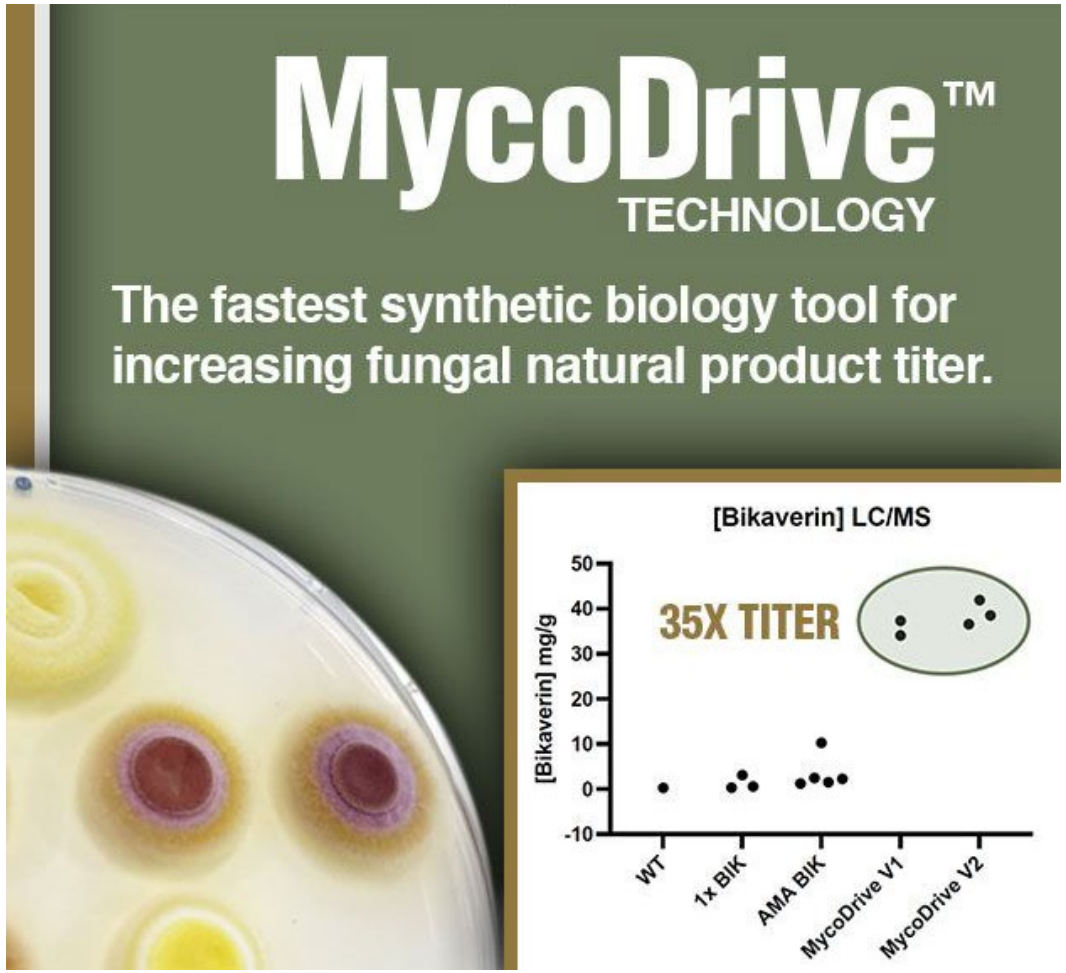
MycoDrive Technology
Fastest Synbio Tool for Increasing Fungal NP Titers



Applications & Benefits Include:

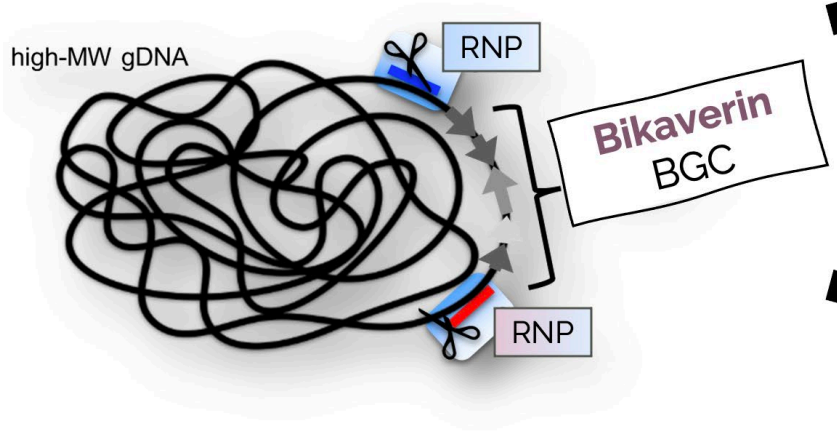
- Higher titer screening of biosynthetic pathways
- Flexible engineering in native or heterologous hosts
- COGS savings on existing products
- Faster prototyping and scaleup

MycoDrive Consistently Generates Multiple Genome Copies

Payloads		Payload Copy Number			Heterologous Production?
		Lowest	Average	Highest	
BGC	BIK (18.6 kbp)	5.4	18.7	48.2	Yes
	PST (37.9 kbp)	1.4	6.3	24.8	Yes
	ENF (42. 3 kbp)	1.4	7.8	28.9	No
	CsA *simA* (45.8 kbp)	2.2	12.8	27.7	Yes
	CsA 2nd 1/2 (47.9 kbp)	7.4	15.2	30.9	
Enzyme	XynA (2 kbp)	1.0	4.3	31.6	Yes

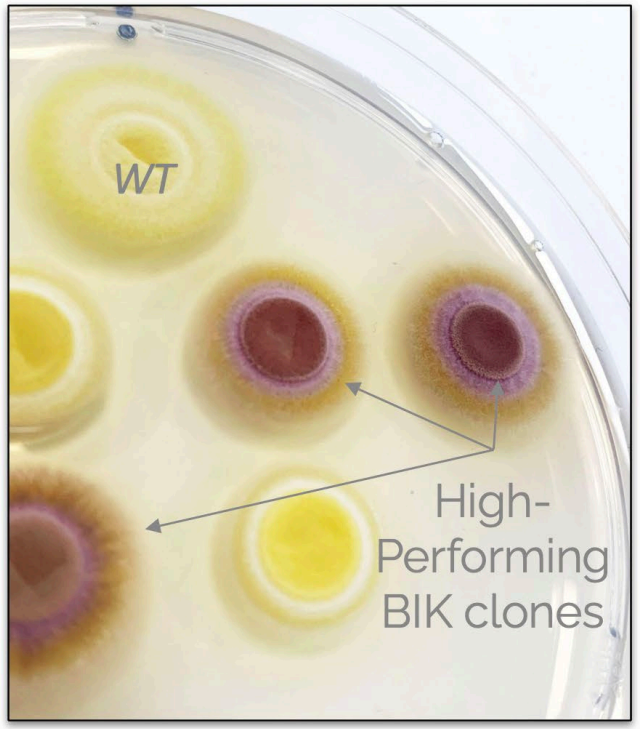
MycoDrive Case Study
Heterologous Production of Bikaverin

Captured intact Bikaverin BGC from *F. oxysporum* gDNA



Cloned 3 Ways

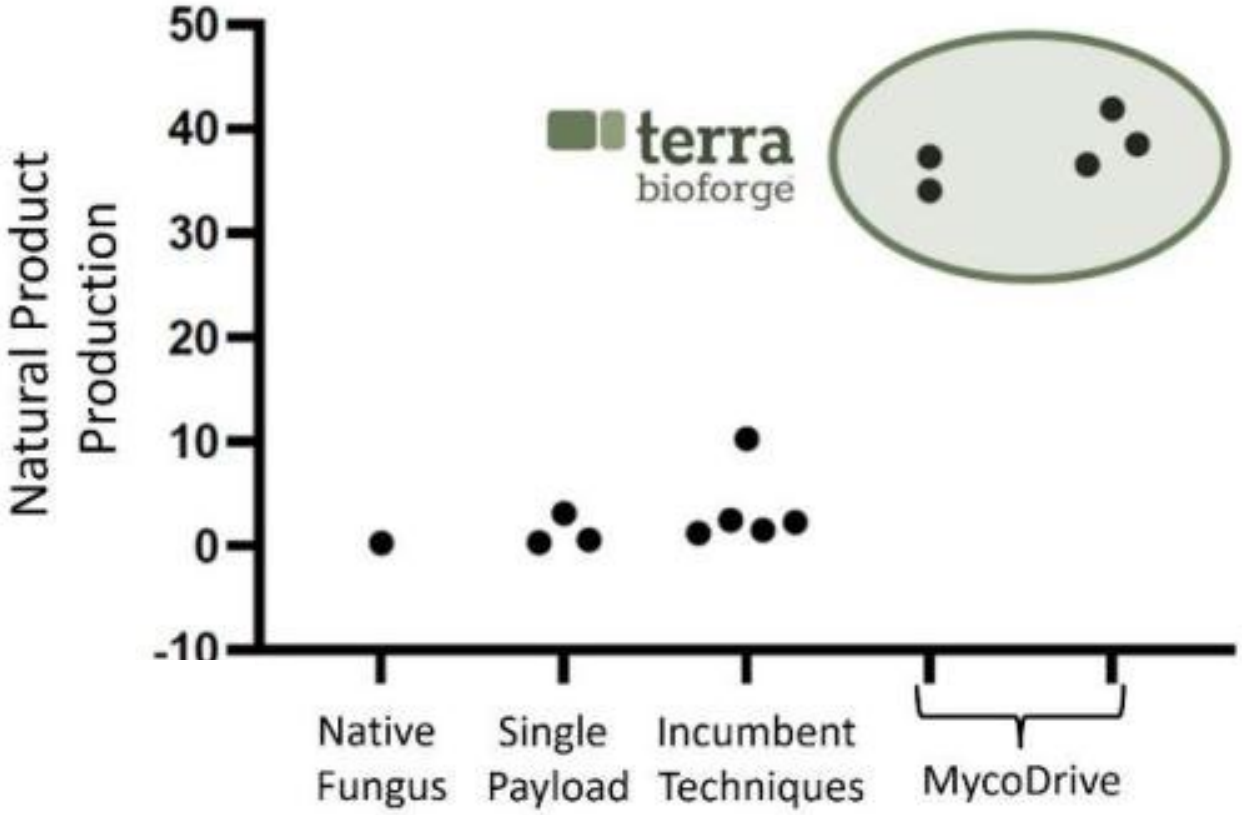
- Targeted Integration
- Episomal (Plasmid)
- MycoDrive™



MycoDrive™ quickly attains highest titer

- No BGC refactoring – this example
- Straightforward cloning – no HOM arms or dealing with AMA1 repeats
- More high-producing clones per transformation – less time screening

PIONEERED “BIG DNA” TOOLS FOR PRODUCING $B DRUGS

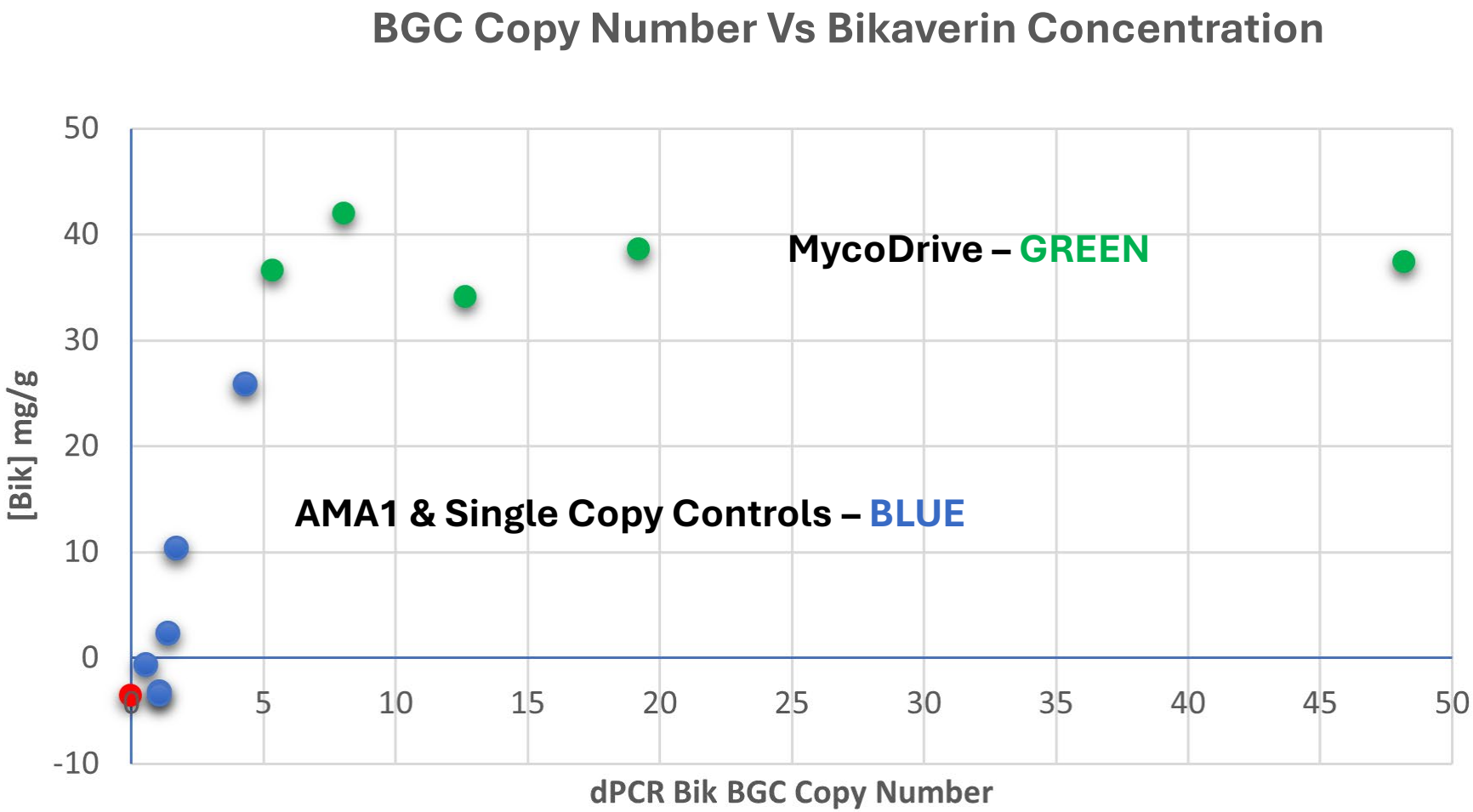


MycoDrive™ Technology

35X MORE PRODUCT
Higher profits

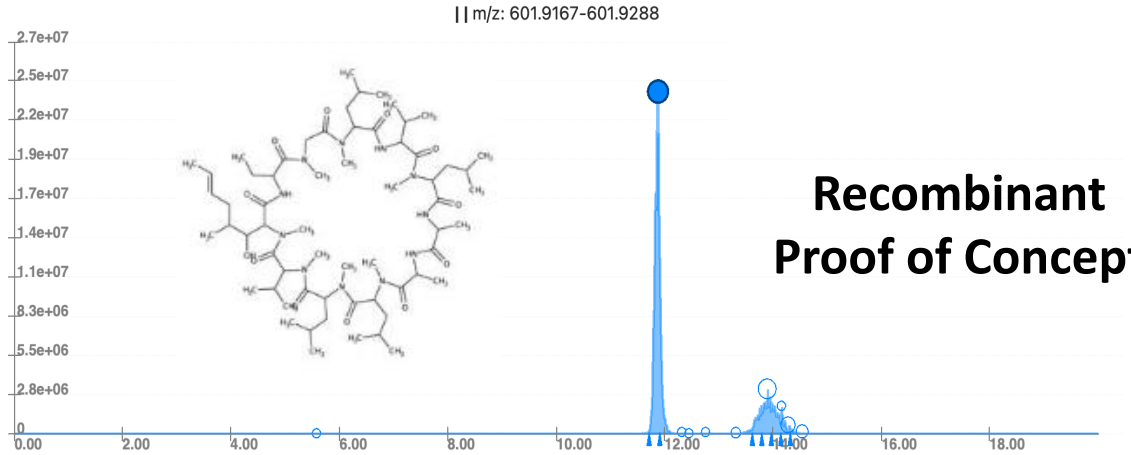
10-20X FASTER
Months NOT Years

BGC Copy Number Positively Correlates with NP Production Levels



Wild-Type – RED

MycoDrive Recombinant Cyclosporine Accelerates Drug Production



Recombinant Proof of Concept

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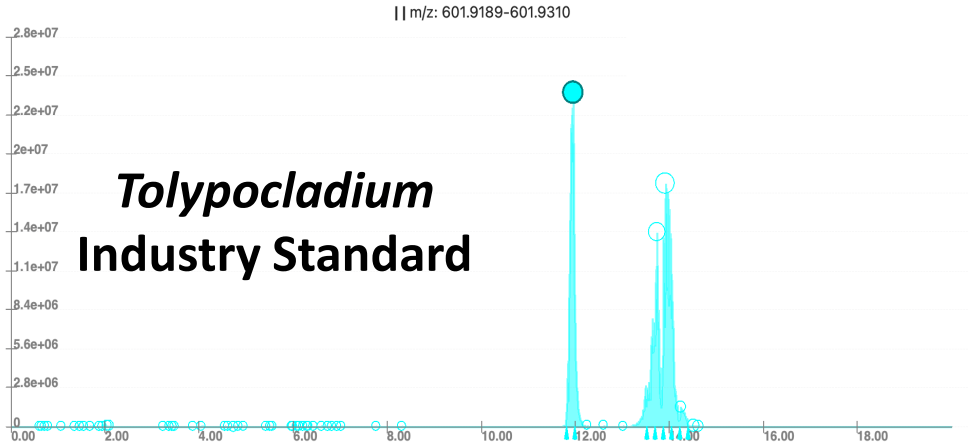
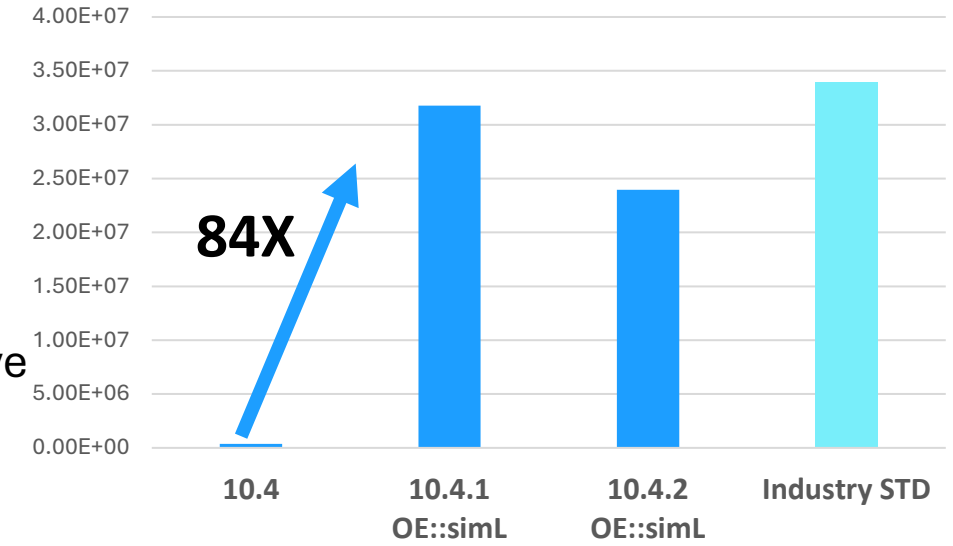


***Tolypocladium* Industry Standard**

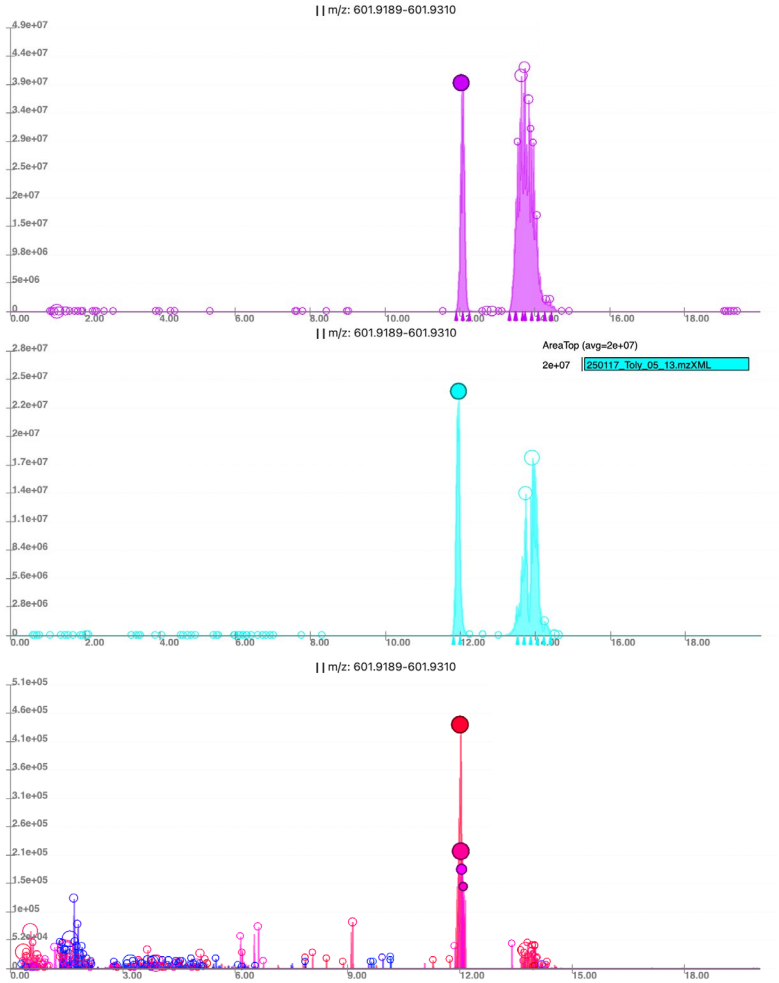
Strain 10.4 was wild type and barely made CsA

Strain 10.4.1 and .2 was a constitutive promoter swap in regulatory gene
That improved yield 84X, same as native strain!

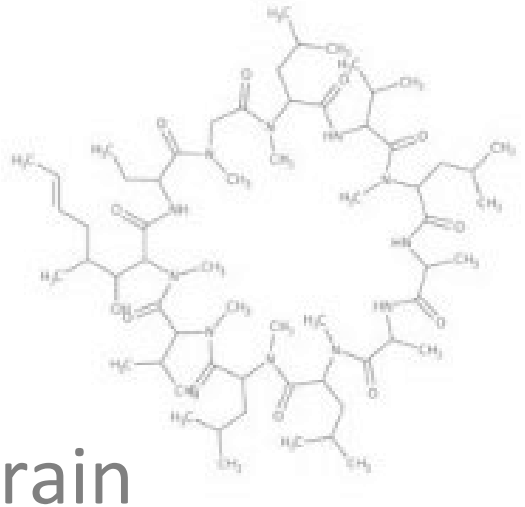


3d = Terra Bioforge
14d = Industry Std
10X Yield = Next Gen

Recombinant Cyclosporine!

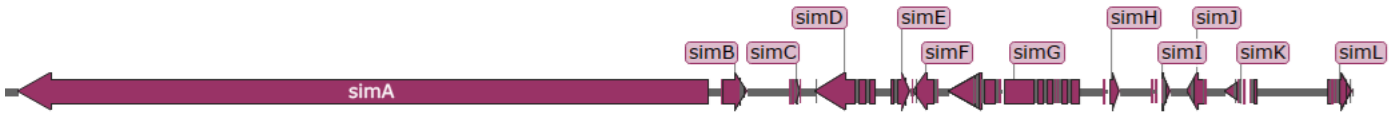


Cyclosporine Standard



Tolypocladium producer strain

Recombinant Cyclosporine



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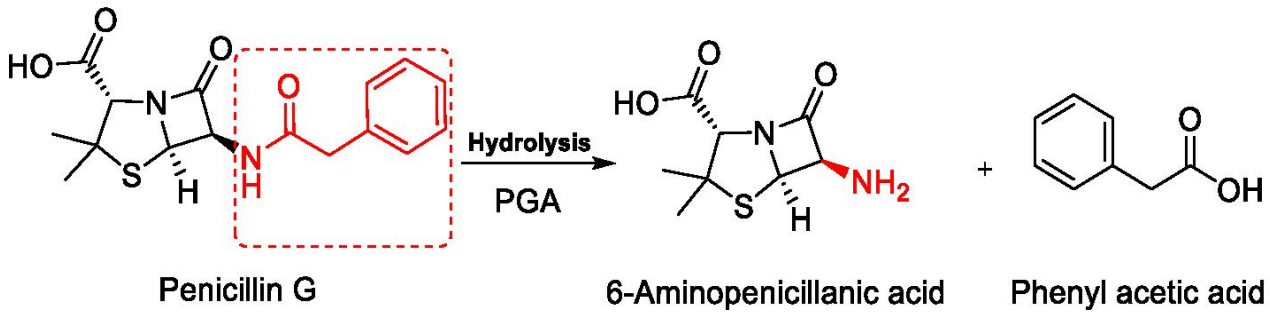
MycoDrive™ Fungal Engineering
A New SynBio Tool for High Product Titers

	Integration Mechanism	Overexpression Strategy	Genetic Stability	Titer Potential	Scalability/ Throughput	Technical Complexity	Cost/ Burden
MycoDrive	**Proprietary**	**Multiple Copies**	**High**	**High**	**High**	**Low**	**Low**
Incumbent Technologies							
Targeted Integration	**DNA Repair**	**Strong/Inducible Promoters**	**High**	**Low**	**Low**	**High**	**High**
Random Integration	**DNA Repair**	**Copy Number**	**Medium**	**High**	**Medium**	**Low**	**Medium**
Ama1	**n/a**	**Strong/Inducible Promoters**	**Low**	**Medium**	**High**	**Medium**	**Medium**

Conventional 6-APA Production from Pen G



Penicillin G | 6-Aminopenicillanic acid | Phenyl acetic acid

6-APA (6-aminopenicillanic acid) is made by enzymatic removal of the side chain from penicillin G using penicillin G acylase, followed by purification steps to isolate the product for use in antibiotic synthesis.

- Fermentation to produce penicillin G which is recovered, purified, and crystallized.
- Enzymatic hydrolysis of penicillin G using *penicillin G acylase*, yielding 6-APA and phenylacetic acid as products.
- Separation of 6-APA from enzyme and byproducts which is crystallized.

Penicillium rubens Strain Engineering to Make 6-APA and/or Amoxicillin and Ampicillin Directly

P. rubens is the "ancestral" penicillium strain and an ideal host for adding the right balance of pathways to achieve new capabilities:

- Fermentation to produce 6-APA directly.

-or-

- Fermentation to produce Amoxicillin or Ampicillin directly.

Benefits of Terra Bioforge Fungal Genome Engineering

Streamlined strain engineering

- BIGDNA™ capabilities are ideal inputs for size-agnostic payload integrations
- Proven pSMART BAC vectors are now updated for higher heterologous titer
- Bypasses limitations of traditional multicopy and single copy integration methods generating high-value production strains faster than ever before

Highest capacity for transcriptional overexpression

- Guided integration into active chromatin
- Select for a range of integrations for fine-tuned performance
- Can easily clone new promoters in front of key BGC components (i.e., TFs, core enzyme, precursor bottleneck, etc…) for additional layers of control

High, stable production titers

- Stable maintenance of heterologous cargo independent of selection
- Identify titer improvements and commercial viability early in the R&D process
- Alleviating existing limitations of attaining high, stable transcription accelerates downstream bottleneck identification and abrogation