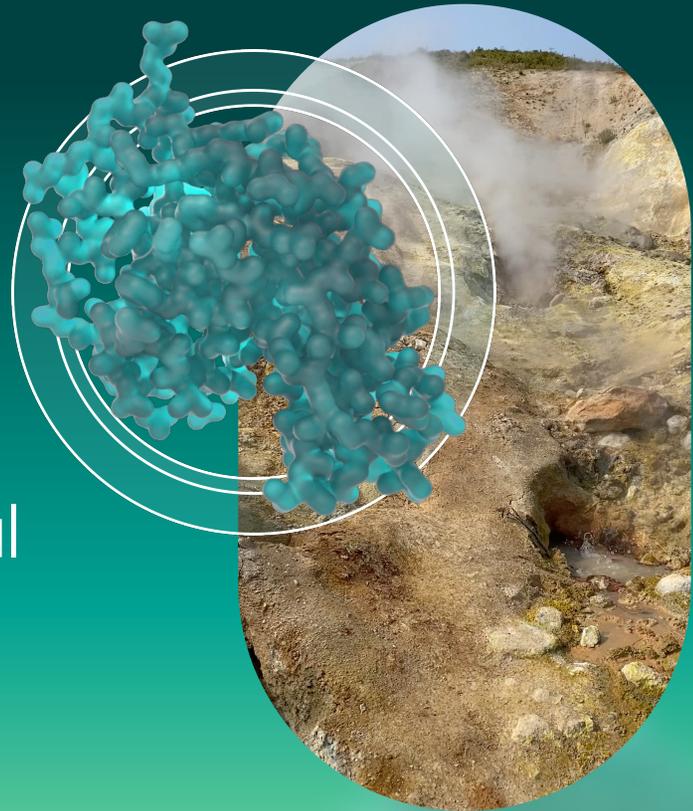
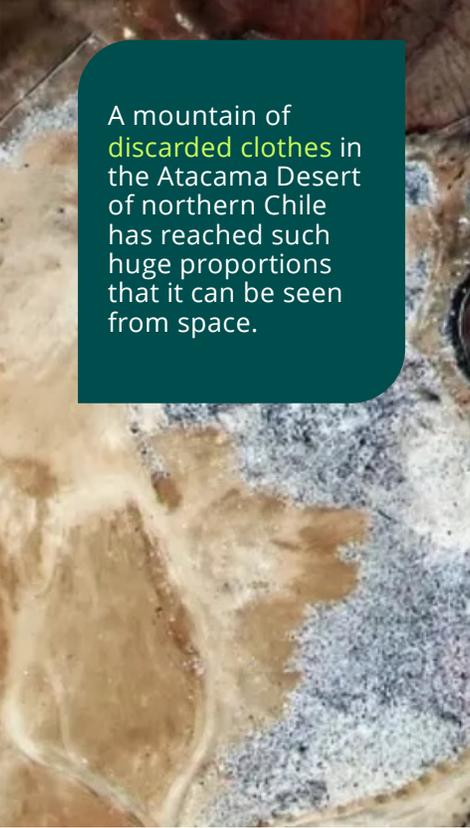




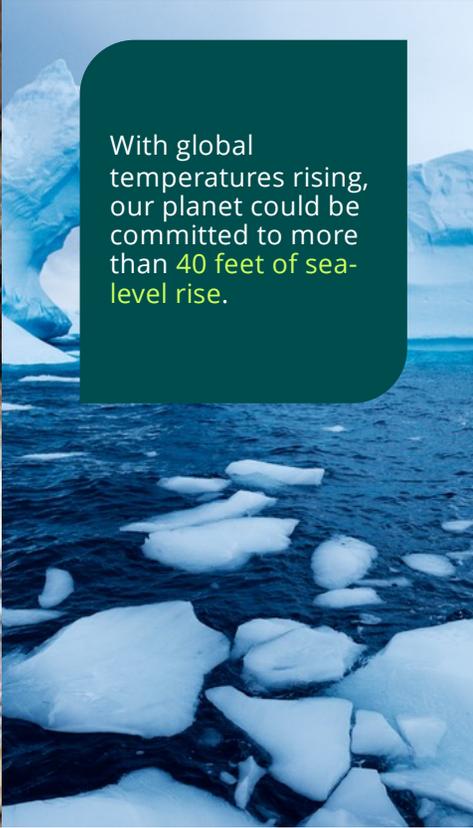
Enzyme Discovery and
Engineering
using bitBiome's Microbial
Genome Database



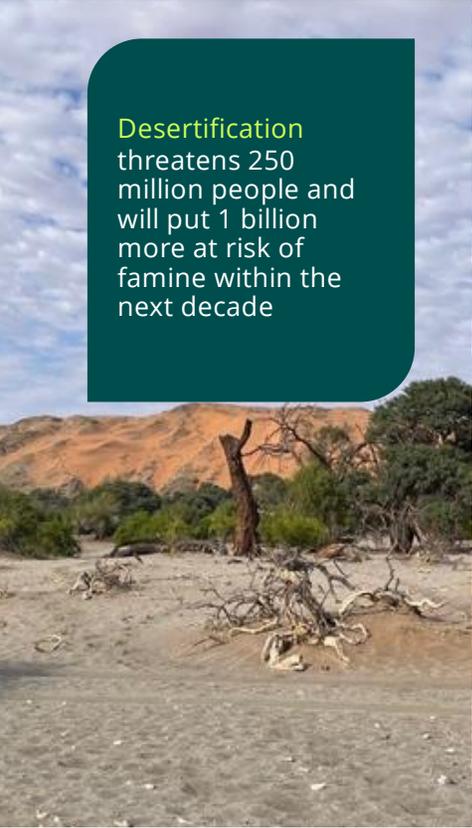
The bioeconomy is our planet's best chance for solving some of our most pressing global crises.



A mountain of discarded clothes in the Atacama Desert of northern Chile has reached such huge proportions that it can be seen from space.



With global temperatures rising, our planet could be committed to more than 40 feet of sea-level rise.



Desertification threatens 250 million people and will put 1 billion more at risk of famine within the next decade.



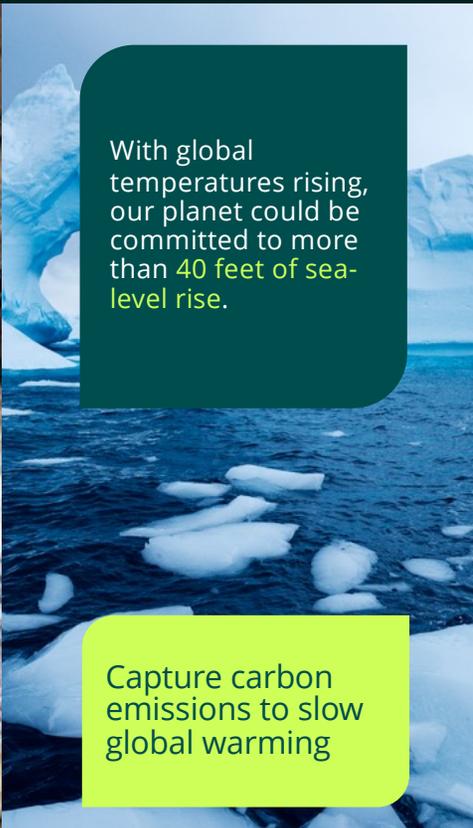
At current rates, plastic is expected to outweigh all the fish in the sea by 2050. Nearly 700 species eat and get caught in plastic litter.

Powered by **microbes**, we can imagine a brighter, more resilient future for all of us.



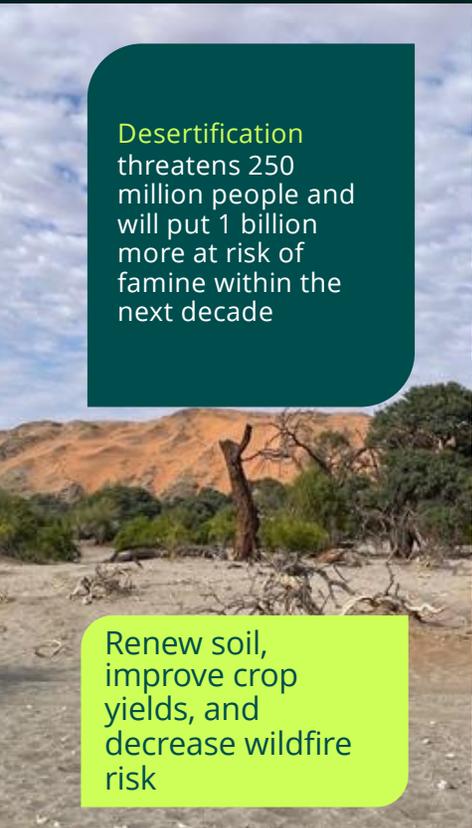
A mountain of **discarded clothes** in the Atacama Desert of northern Chile has reached such huge proportions that it can be seen from space.

Create better, more sustainable materials



With global temperatures rising, our planet could be committed to more than **40 feet of sea-level rise**.

Capture carbon emissions to slow global warming



Desertification threatens 250 million people and will put 1 billion more at risk of famine within the next decade

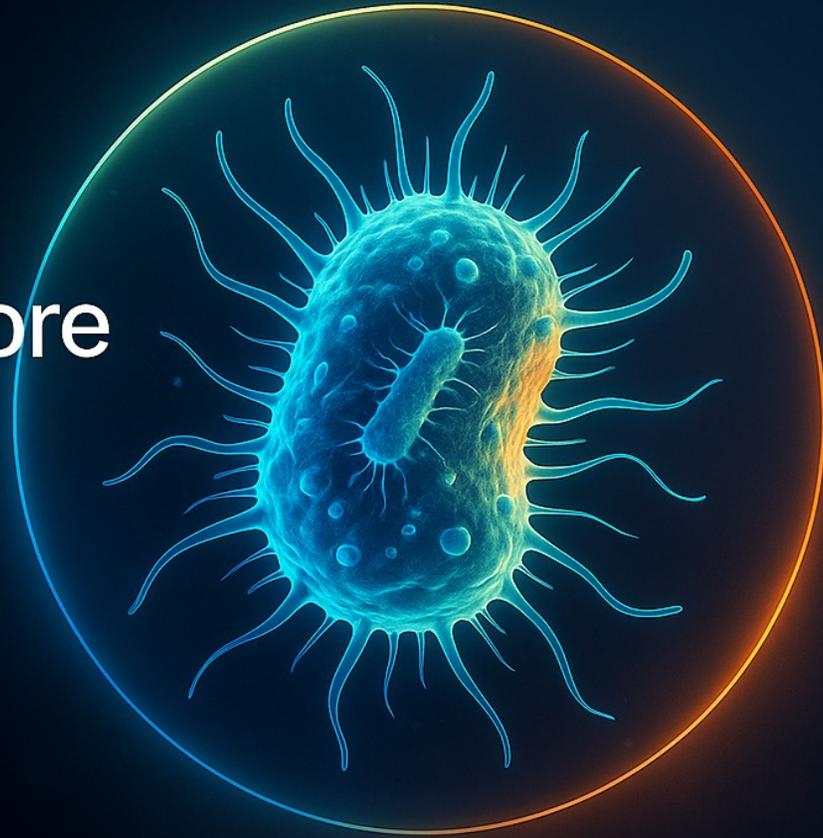
Renew soil, improve crop yields, and decrease wildfire risk



At current rates, **plastic is expected to outweigh all the fish in the sea by 2050**. Nearly 700 species eat and get caught in plastic litter.

Dissolve plastic waste and build renewable alternatives

We've explored more
of the moon than
we have of the
microbial world.



Many have focused on technologies to harness
0.001% of their power...

**What if we can access
and use 100%?**

bitBiome's platform technologies generate **the data to power innovation** in biomanufacturing



SEQUENCE

bit-MAP

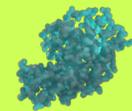
The **one and only single-cell sequencing** for microbes that's 100x more efficient than conventional methods



CATALOG

bit-GEM

The **world's most diverse database**—2.4 billion sequences and growing—built on single-cell sequencing of microbes from natural samples



EVOLVE

bit-QED

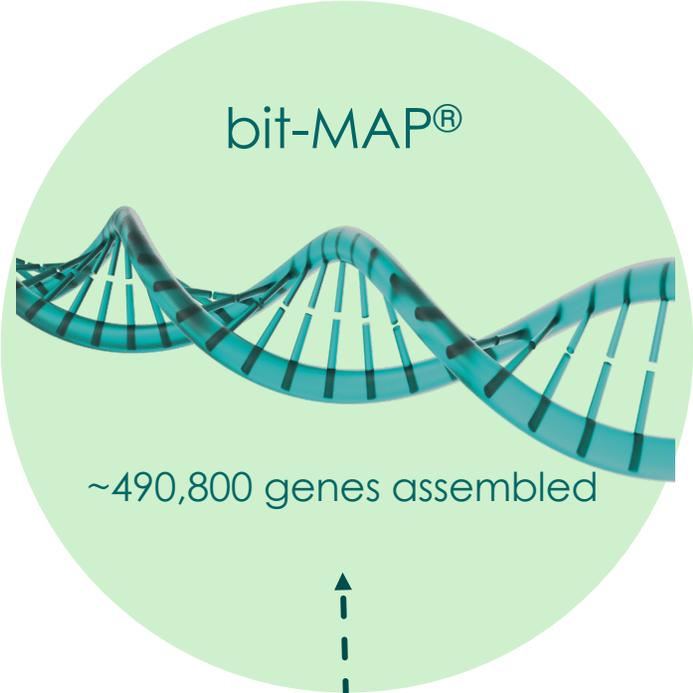
Customized bio- and cheminformatics and AI-driven enzyme engineering, strain development and ingredient manufacture

bit-MAP[®] is the most advanced platform for the discovery of microbial dark matter genes

From 10,000+ years to just a **single generation** to identify genomes and sequences from yet to be discovered microbes

Comparison of bit-MAP[®] vs shotgun metagenomics

- Soil microbiome sequencing results from a single source
- Data size: 5 Gbp (both)
- # of CDS obtained (>200 a.a. residues)



Our method is over **100x more efficient** at constructing and identifying genes



Ratio of reads utilized for assembly

73%

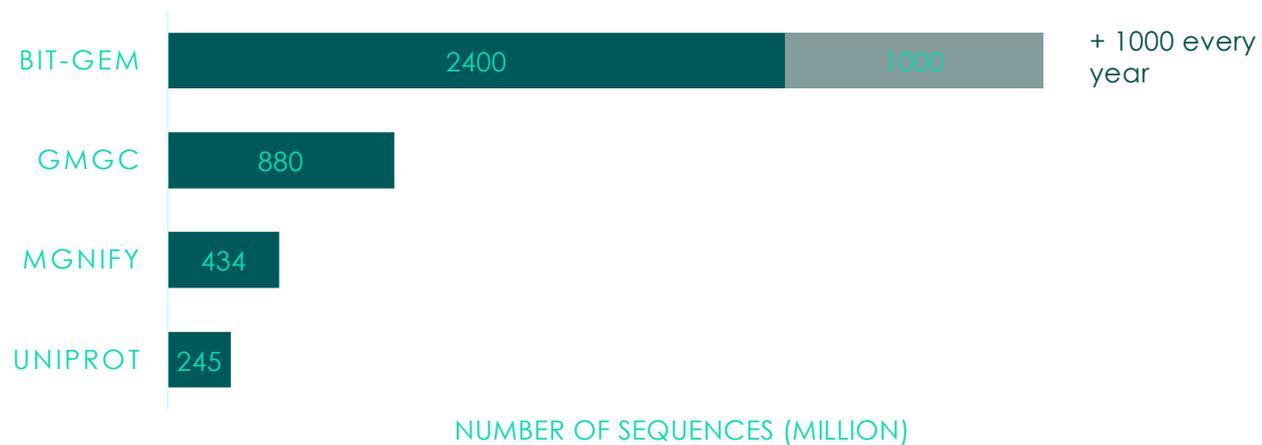
8%



2.4 billion genes—and growing

EXPANDING BY 1 BILLION EVERY YEAR

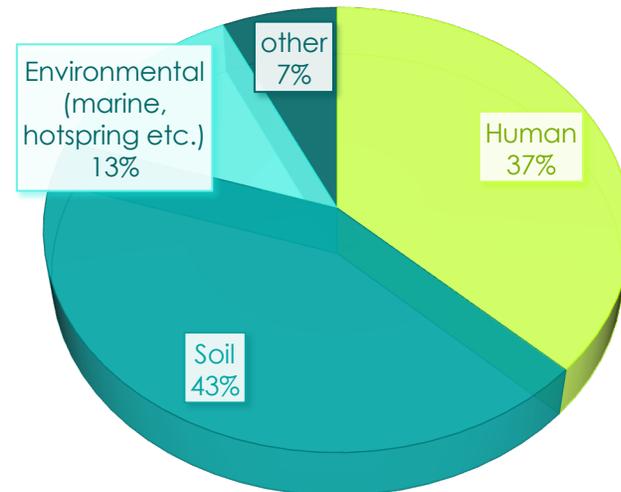
Collected by our proprietary methods, which enables us to obtain full length genes



Diversity powered by nature

- Large collections of extremophiles with potentially novel enzyme activities and functional requirements
- Unique, free-to-operate, patentable sequences in accordance with the Nagoya Protocol
- Industry-wide applications, including healthcare, pharma, sustainability, food, materials, and chemicals

56% from environmental samples including soil, marine water, hot spring, mining site etc.



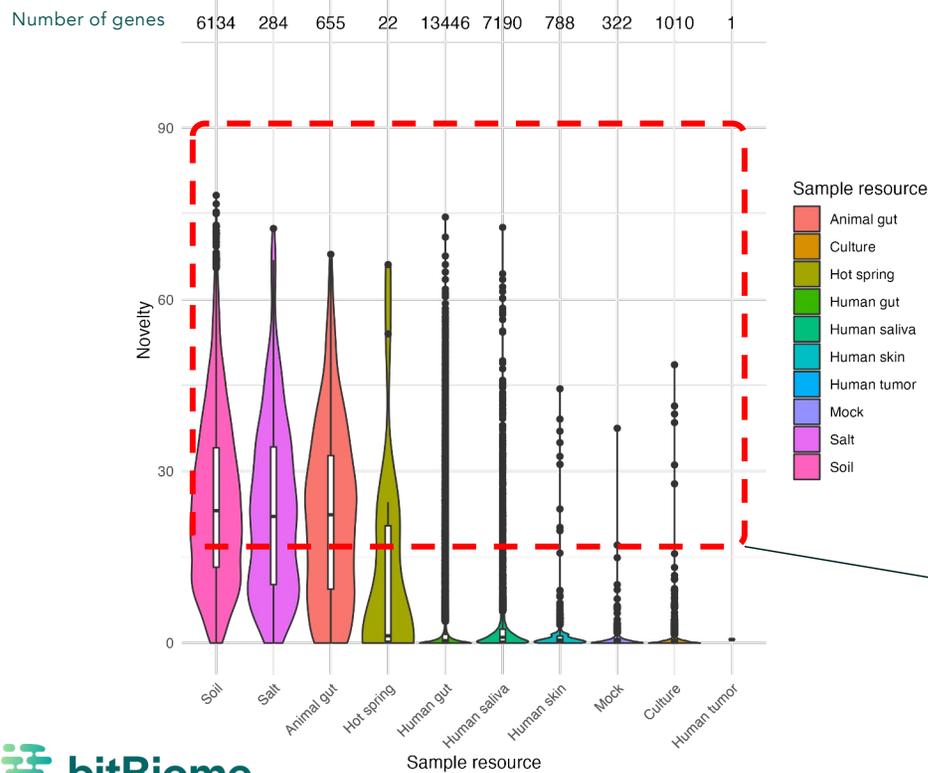
Many relevant enzyme candidates were discovered from our database, bit-GEM

Biocatalysis Enzymes	Number of Sequences
Aldo-keto Reductase	24,700
Aldehyde Dehydrogenase	150,400
Alkali Phosphatase	124,100
Amine Dehydrogenase	34,300
Carbonyl Reductase	34,900
Ene Reductase	4,600
Monoamine oxidase	4,300
Nitrile Hydratase	700
Nitrilase	20,400
Imine Reductase	14,400
Transaminase	55,700
Nitroreductase	33,100
P450	11,200
Squalene-Hopene Cyclase	700
Flavin-dependent Halogenase	1,500

Life Science Enzymes	Number of sequences
Cas3	1,100
Cas9	9,700
Cytosine Deaminase	499
DNA polymerase	10,654
Large Serine Recombinase	25,432
Poly(A) Polymerase	13,638
Primase	637
RNA Ligase	347
Single Subunit RNA Poly	1,452
Tn5 Transposase	1,320
Transglutaminase	11,914

Bio Industrial Enzymes	Number of sequences
Alpha-Amylase	120,200
Amidase	87,300
Esterase	39,500
Laccase	35,700
Lipase	20,000

Value comes from the uniqueness of sequences in our database



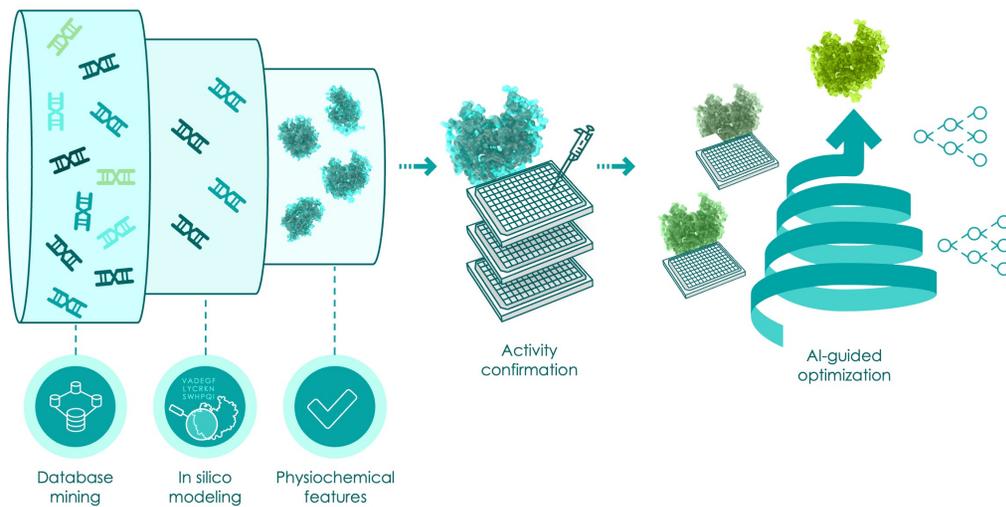
Malate dehydrogenase as example case:

- Essential enzyme for TCA cycle
- Used as a proxy to represent novelty of genes in our DB

- Represents sequences only bitBiome has access to
- Sequences with a novelty score over 20 represent sequences with IP potential

The uniqueness of our database means we have higher success rates in finding novel genes and activities of interest

Microbe data plus AI makes faster, more robust enzyme engineering possible

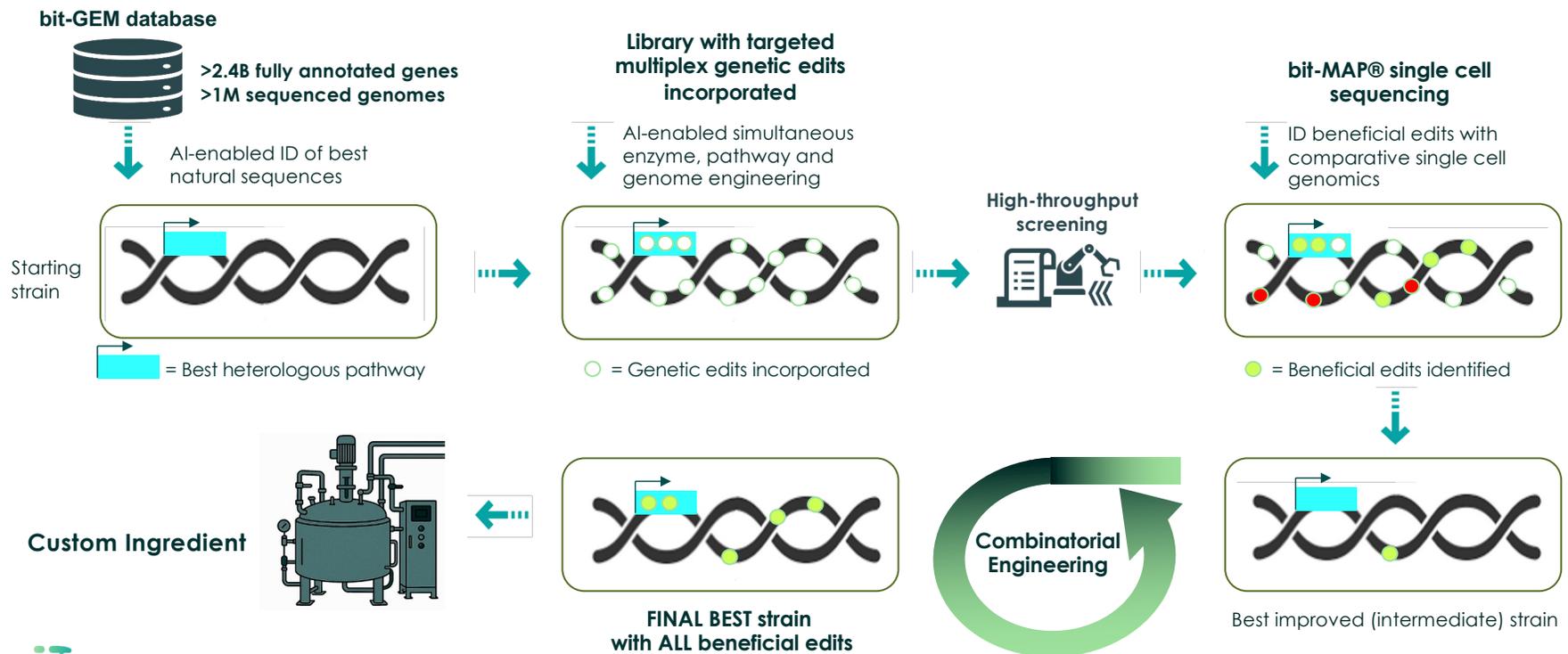


Making years long processes possible within weeks to months

Microbes power the industries that will shape the future of our planet.

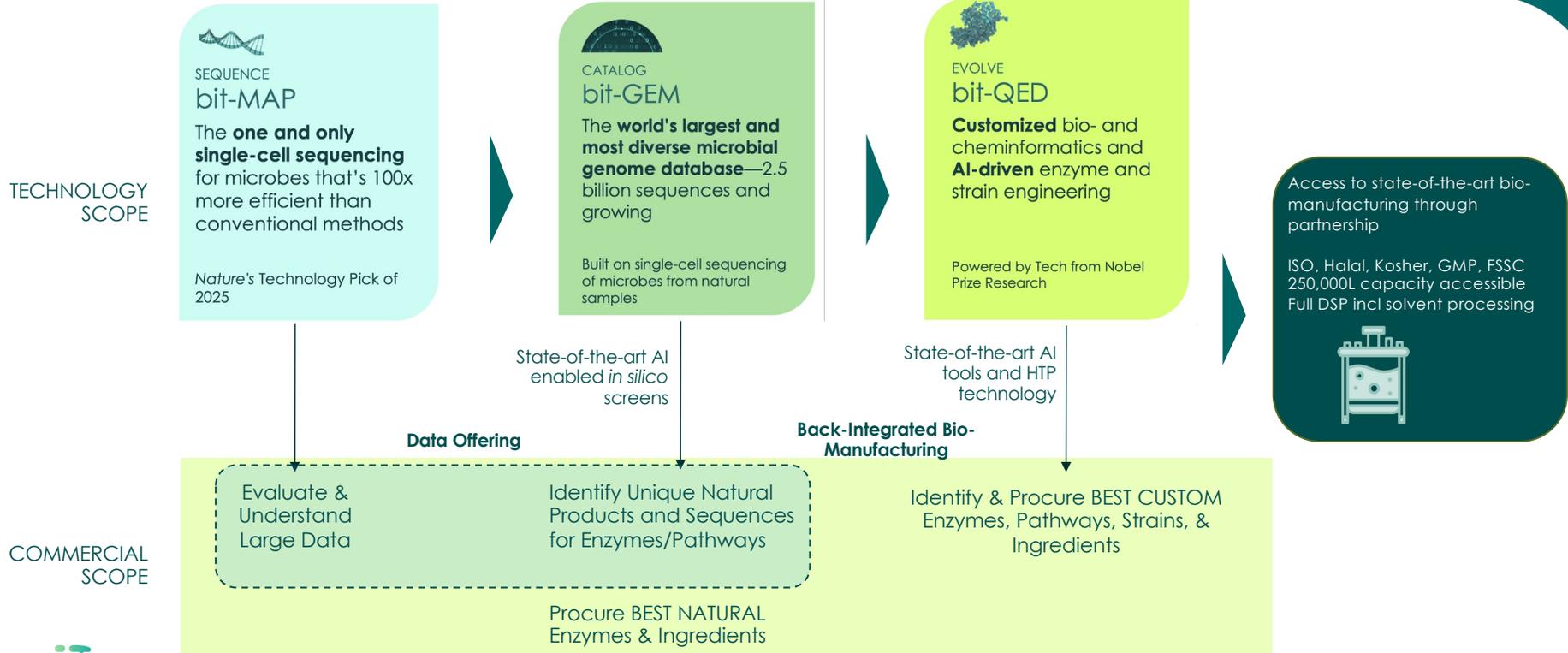
- Pharma/Life Science
- Food/Agriculture
- Energy
- Materials
- Chemicals
- Climate Resistance

Simultaneous AI-enabled strain engineering



bitBiome Technology Map

Broadest Application Scope



Case Studies

bitBiome projects have tackled a wide variety of functional parameters and scales

Identifying the best starting point possible

Discovery



Stability



Heat



Solvents



pH



Efficient *in silico* and *in vitro* protein improvement

Engineering



Cofactor swapping



Substrate expansion



Inhibitor resistance



Protein production at R&D scales and beyond

Production



Fermentation



Secretion



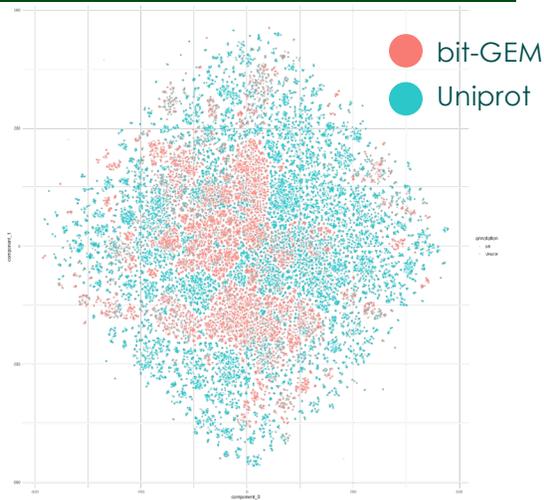
Host selection



bit-GEM identified multiple novel plastic degrading enzymes

CASE STUDY 1

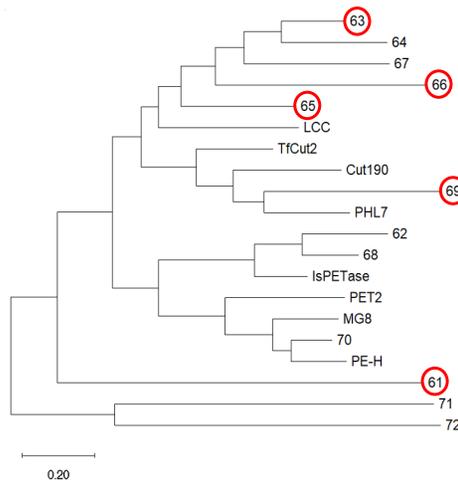
Many unique sequences in bit-GEM



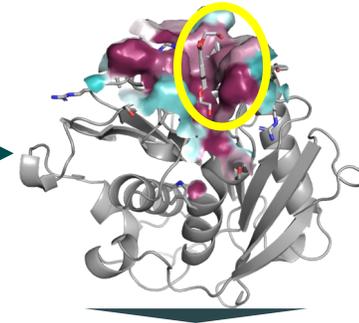
bit-GEM contains ~35,000 genes related to plastic degradation enzymes (~65,000 in Uniprot).



Selecting genes strongly related to the known PET hydrolases

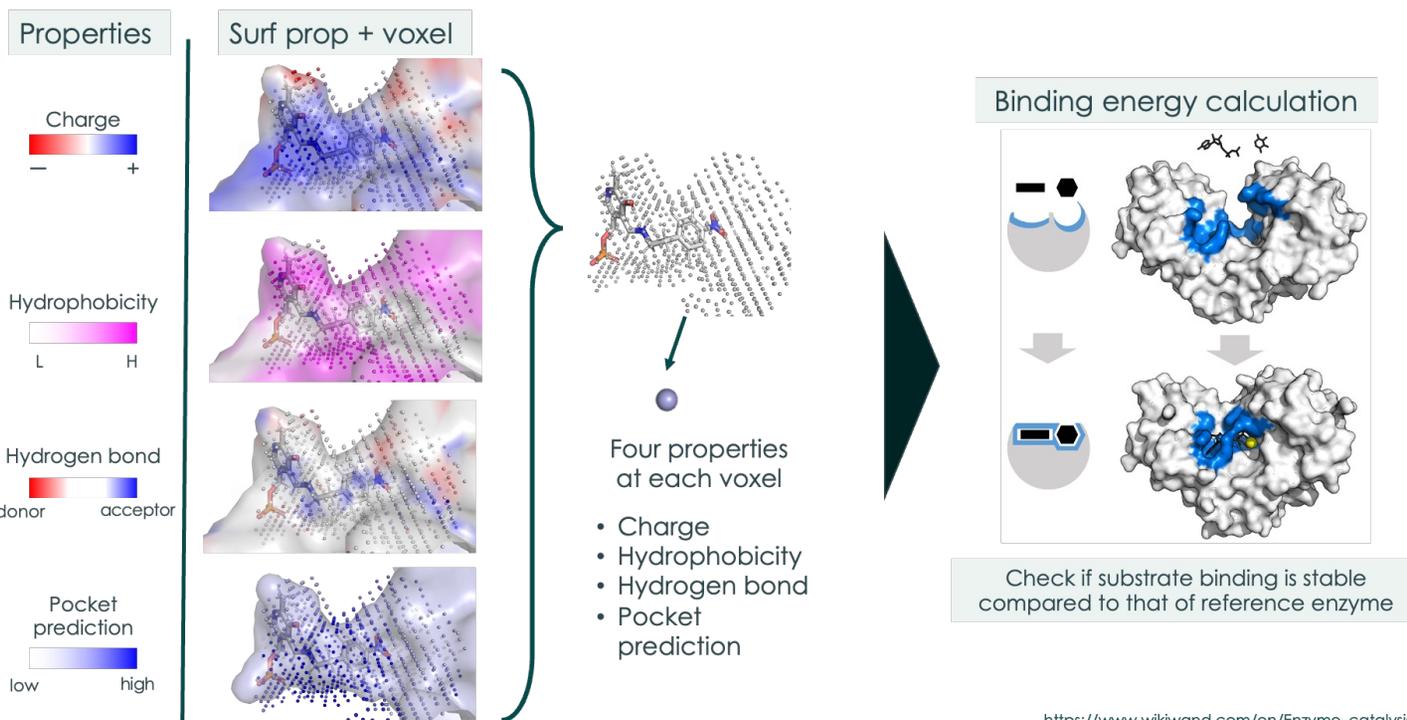


3D structural modeling to determine the target sites



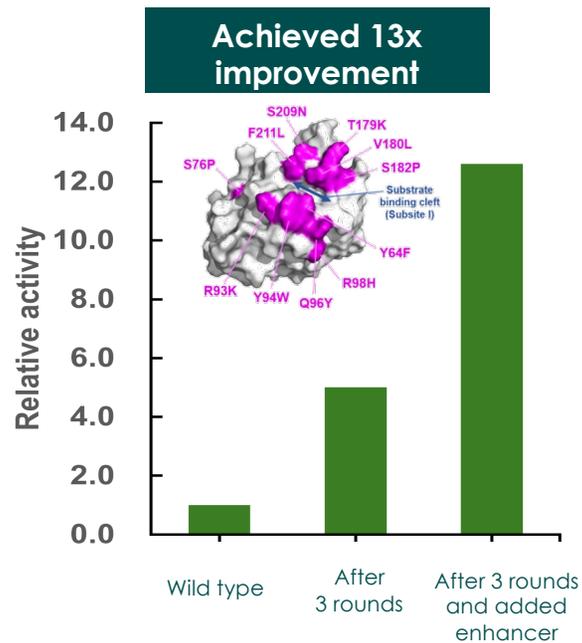
High throughput screening to identify strong mutants

3D structure-based enzyme discovery & selection using ML and our sequence database

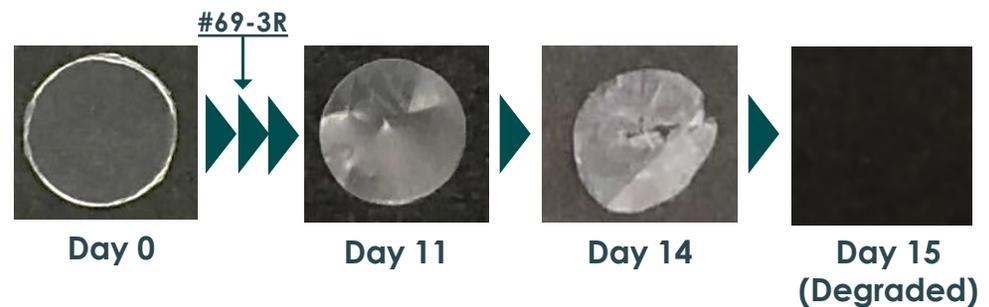


bitBiome identified and improved a novel PET degrading enzyme

ENZYME IDENTIFIED FROM SOIL-BASED MICROBIAL SEQUENCES



bitBiome's modified enzyme completely breaks down PET films



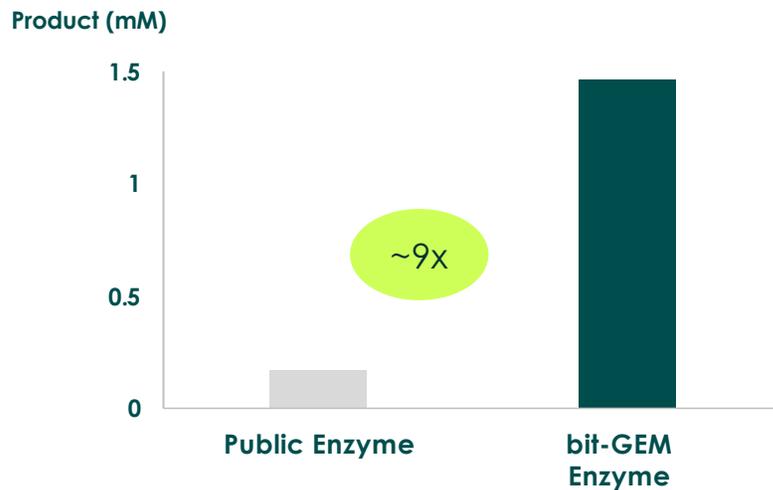
Key benefits

- High stability
- Active at 40°C, no heating required

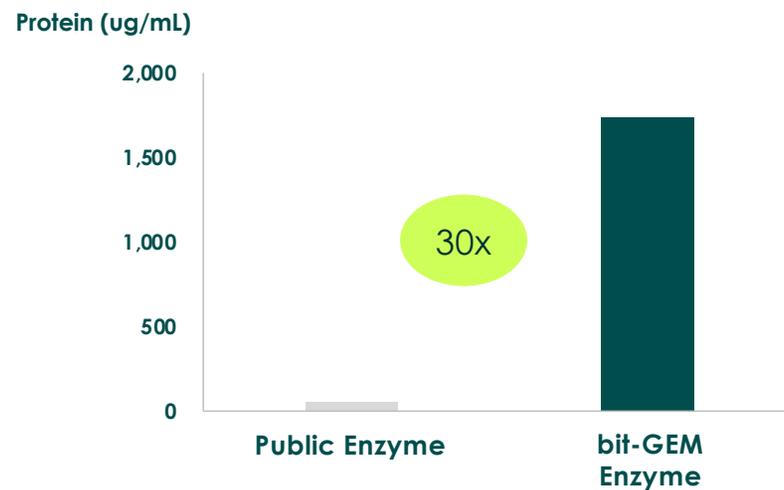
bit-GEM Identified a natural enzyme with 270x higher productivity than current best-in-class from environment

CASE STUDY 2

9-fold higher activity than publicly available enzyme



Significant improvement in expression in *E. coli*

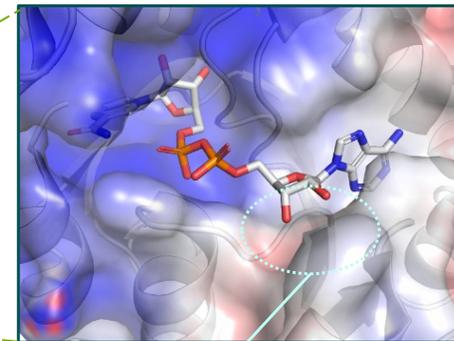
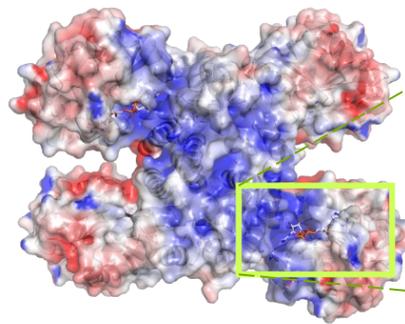


Cofactor Swapping

CASE STUDY 3

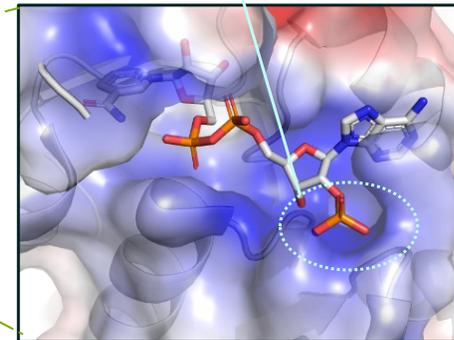
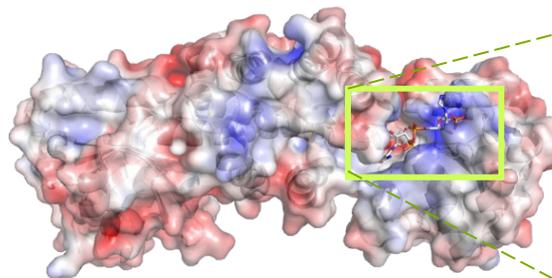
REFERENCE SEQUENCES

human
hydroxyisobutyrate
dehydrogenase
complexed with NAD⁺



Positive charge (blue) is necessary to bind to phosphate moiety of NADP

Streptomyces sp. GF3546
imine reductase
complexed with NADP⁺



Key clients and partners

Traditional biotech champions



Leading manufacturing companies coming to biotech



What they say about bit-GEM and bit-QED



"TOK is committed to achieving carbon neutrality and net zero emissions by 2025. As a result, we have decided to work with bitBiome due to their **extensive knowledge in enzyme discovery and unique natural enzyme sequence library**. Their speed of development and R&D knowledge was exceptional, and we have full confidence in their abilities to help us achieve our goals now and for the future."

TOK (Tokyo Ohka Kogyo)



"We are confident that by integrating bitBiome's **unique gene database and advanced in silico enzyme exploration** technologies, including surface feature analysis, with our AminoScience® technologies, we can unlock new value. This collaboration will **accelerate the development of innovative products** and solutions, delivering enhanced value to our customers and partners."

Shintaro Iwatani, PhD,
The head of Biosolutions Labs.,
Ajinomoto Co., Inc.



Thank you

laura@bitbiome.bio

