



Exploiting microbial cell factories to secure the future of industrial enzymatic biocatalysis

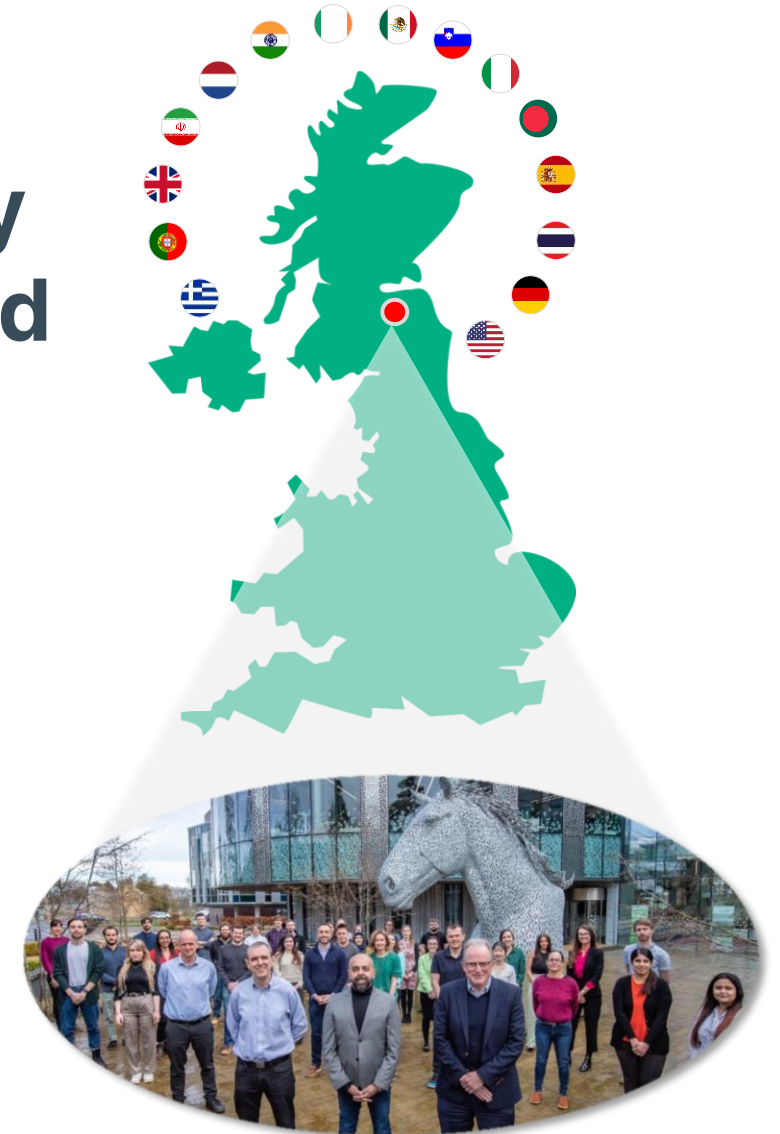
Rita Cruz – Section Head in Strain Development

26th October 2023

Human
Health

We accelerate
Engineering Biology
to benefit people and
our planet

Sustainable
Planet



Who are our customers?



Human Health

Pharmaceutical companies outsourcing development of biologics manufacturing processes, enzyme evolution, optimization of pathways to small molecules

Academic spin-outs and start-ups advancing early-stage research for proof-of-concept, funding/investment towards first in man

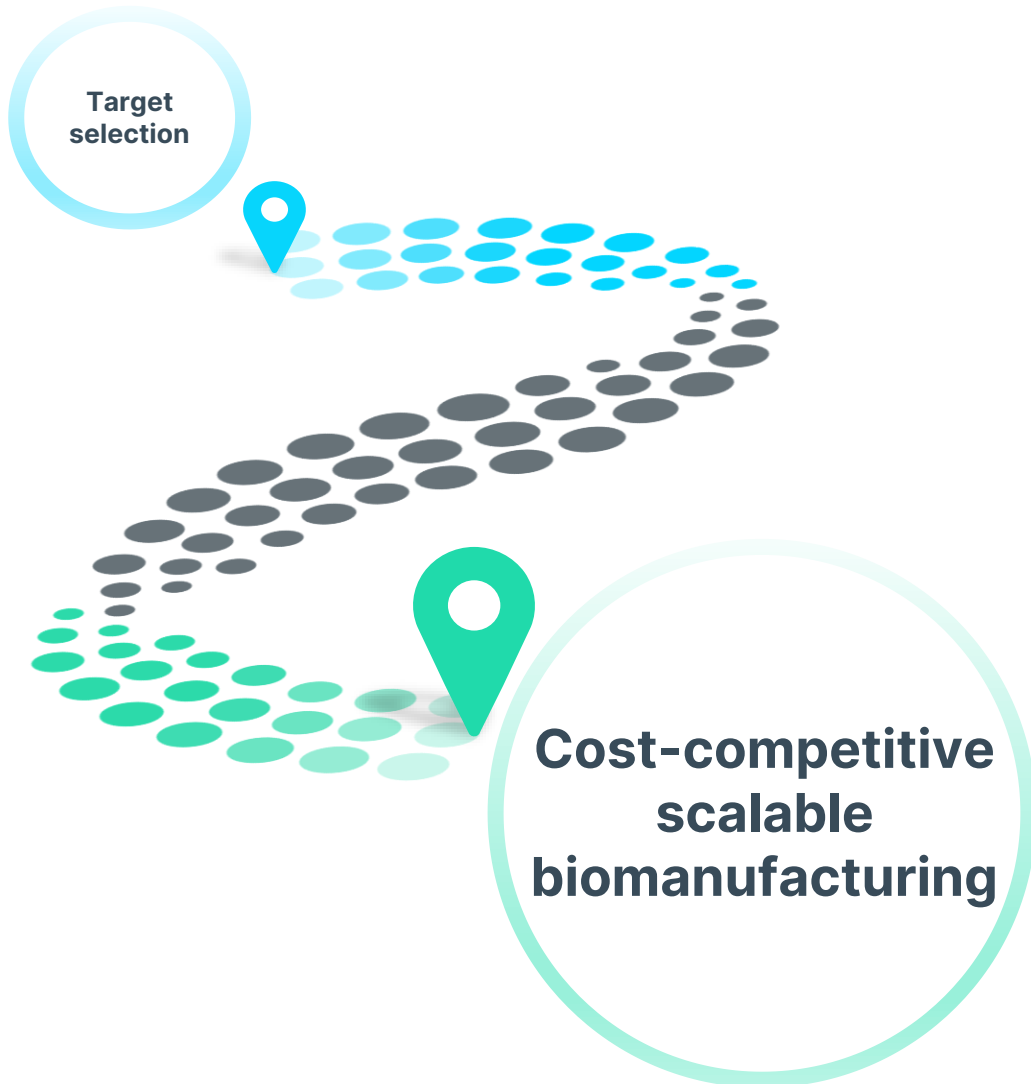


Sustainable Planet

Chemical companies/ Consumer Goods/Ag companies developing scalable biobased processes with competitive COGs, new IP, sustainability



Your trusted partner to sustainable biomanufacturing



Our *inGenius*[®] platform accelerates and de-risks your journey to sustainable biomanufacturing

12

expertise in 12 different
biomanufacturing hosts

>20x

yield enhancement
through innovative cell line
and USP development

2.6 M

successful bioprocess scale
up to 2.6 M litre scale

20

years of successful
delivery for our customers

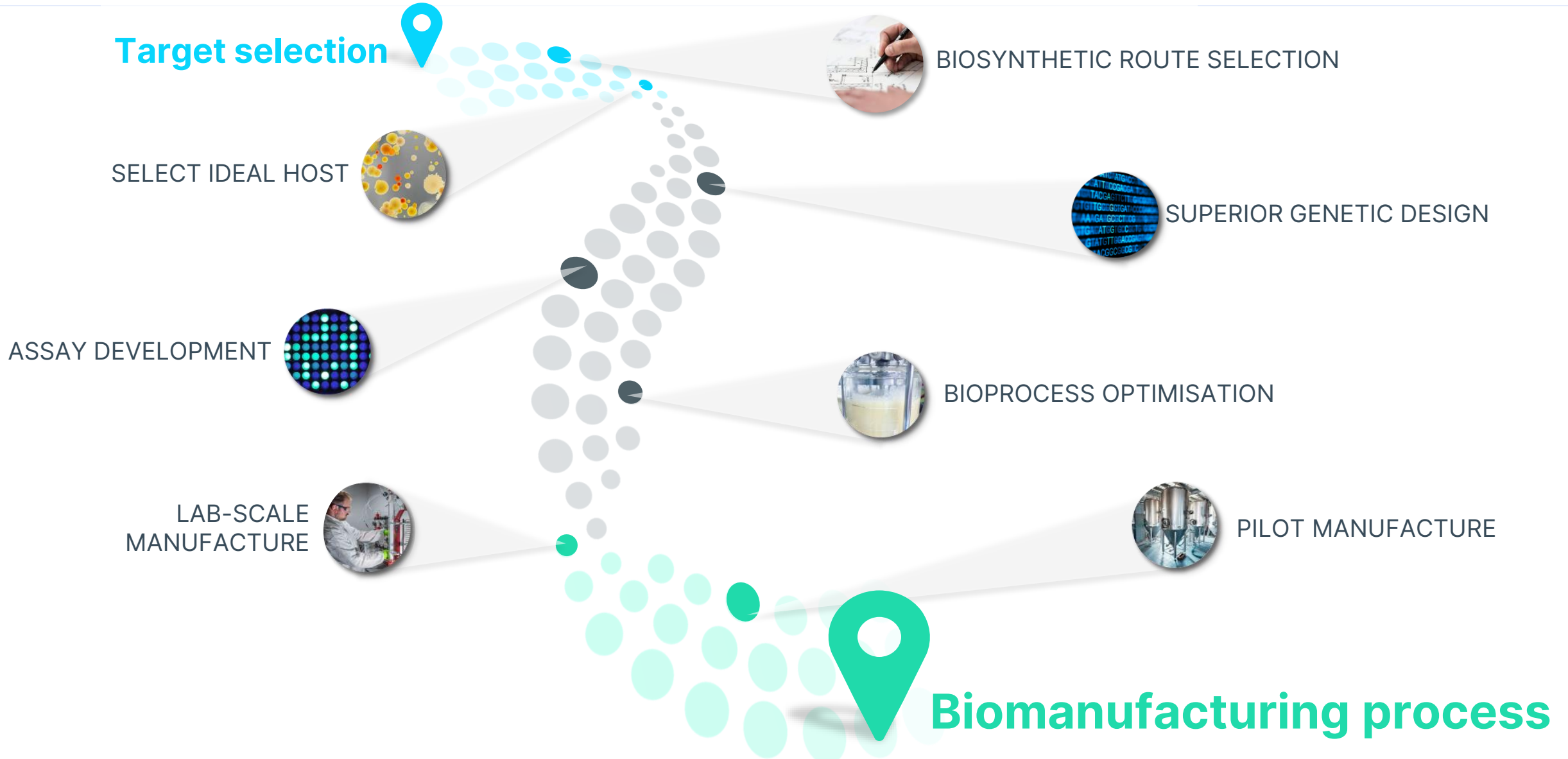
100%

success rate in delivery
into multi-sector
applications using
one of our hosts

>70

USP and DSP parameters
monitored and optimised
during process
development and
manufacture

The *inGenius* way

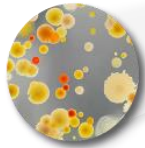


The *inGenius* way

Target selection



SELECT IDEAL HOST



12 biomanufacturing hosts including:

- Bacterial, yeast or mammalian
- Protease deficient strains
- Glycoengineered strains
- Product secretion
- GRAS regulatory status

BIOSYNTHETIC ROUTE SELECTION



Chemo-enzymatic design

- Uniquely integrated chemistry and biocatalysis for optimal route design
- Create new value, novel IP accessible routes that keeps customers competitive in their market

Biocatalyst adaptation

- Bespoke *in vivo/in vitro* high-throughput (HTP) screens for biocatalyst selection, screening and engineering

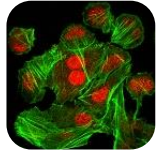


Biomufacturing process

Custom Host

Human health

Sustainable Planet



Mammalian cell lines



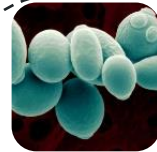
Streptomyces sp.



Escherichia coli



Corynebacterium glutamicum



Saccharomyces cerevisiae



Bacillus sp.



Pichia pastoris



Yarrowia lipolytica

Protein secretion/GRAS



Pseudomonas putida



Aspergillus niger



Vibrio natrigens

The *inGenius* way

Target selection



SUPERIOR GENETIC DESIGN

- Unique library of genetic elements
- Proprietary gene design algorithm "*codABLE*"
- Combinatorial HTP DNA assembly
- Antibiotic-free gene maintenance
- Genome editing with freedom to operate

ASSAY DEVELOPMENT



- Innovative custom assay development integrating chemistry and biology
- FACS-compatible ultra-HTP "*visABLE*®" screening
- DoE-based performance optimisation

Biomanufacturing process

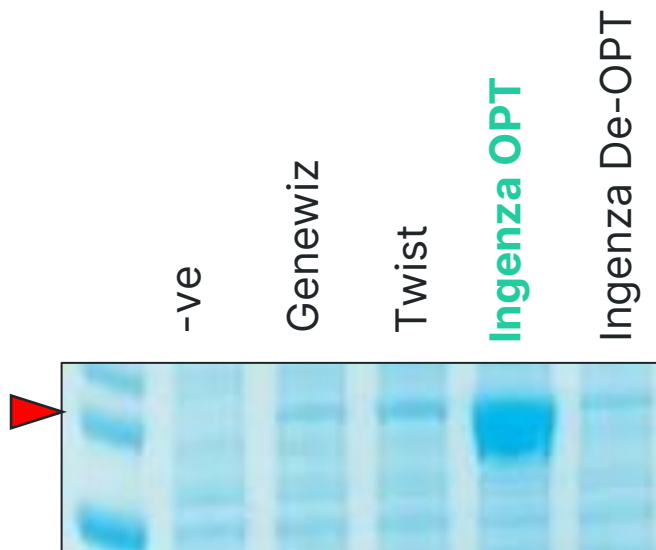


Custom Genetic elements

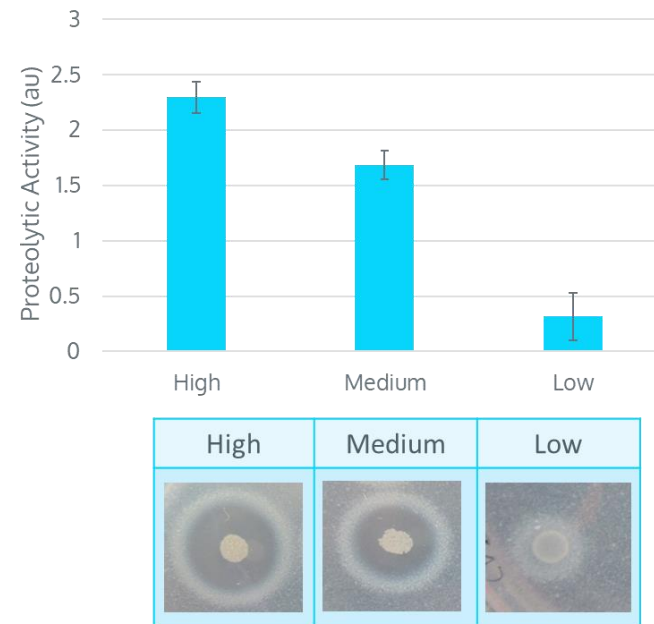
Enhanced recombinant protein expression with **codABLE®**

- > Recombinant expression of endotoxin-free enzymes in *B. subtilis*
- > Cost-effective and scalable biomanufacturing process free of endotoxins

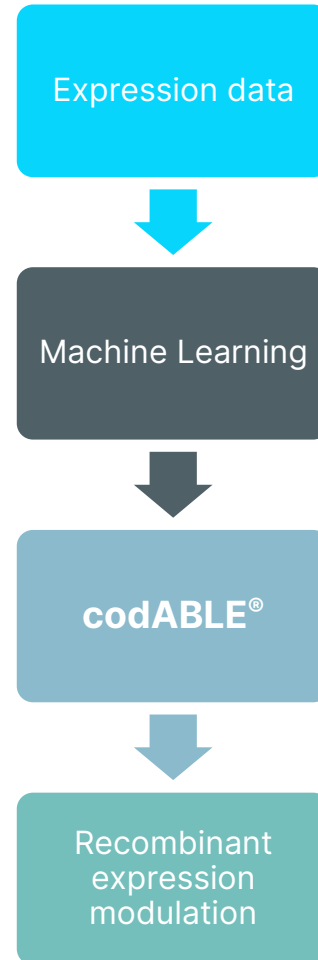
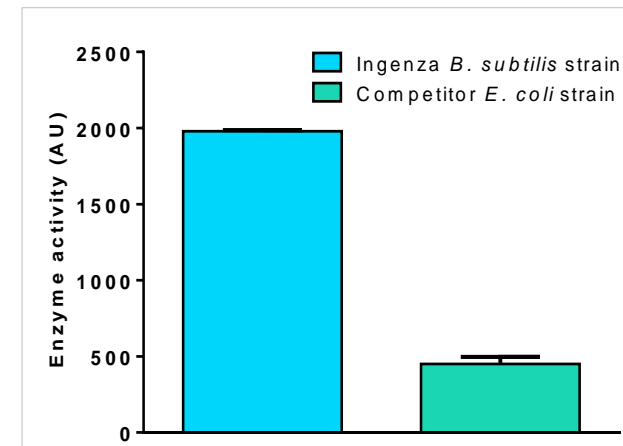
Intracellular Protein Expression



Extracellular Protein Activity

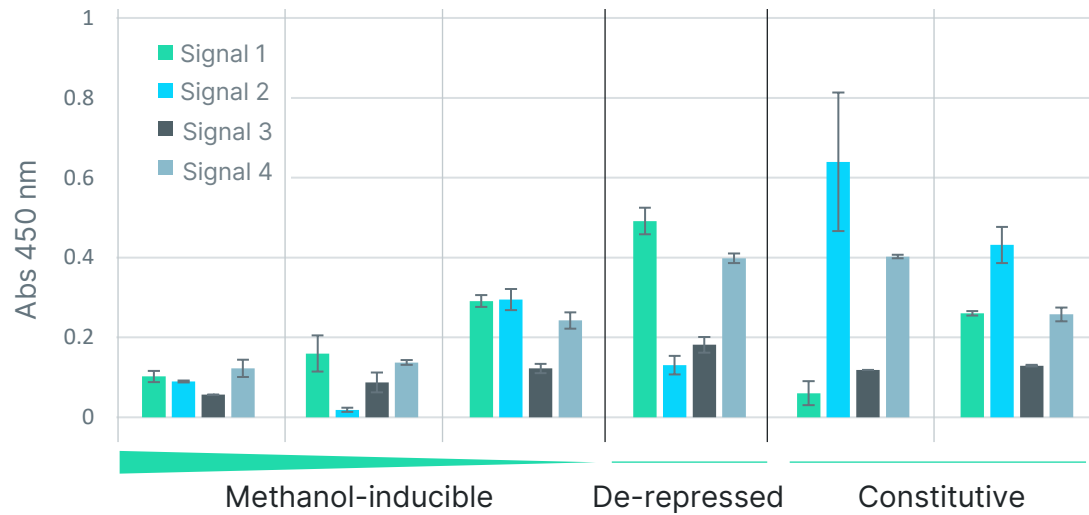


Intracellular Protein Activity

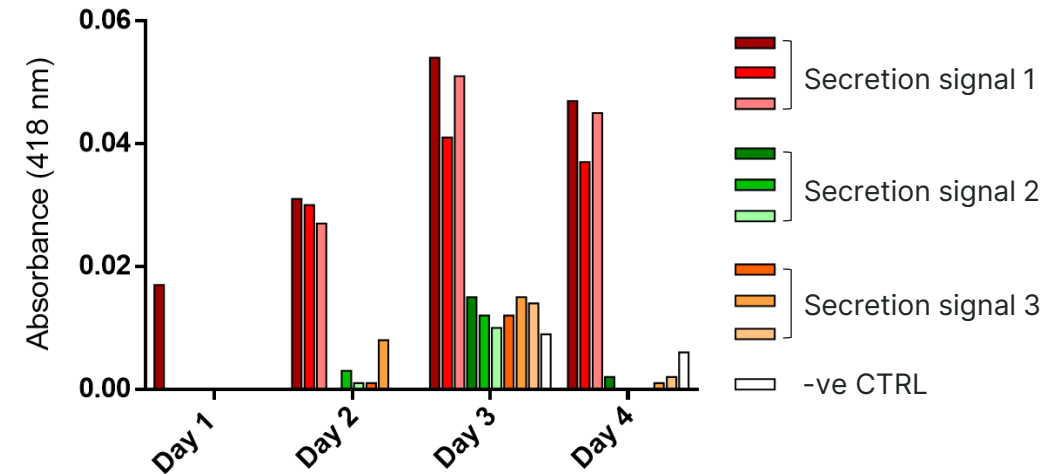


Custom Genetic elements

Library of promoters, secretion signals, plasmid backbones and integration sites with FTO for multiple hosts



P. pastoris: optimal protein expression and secretion



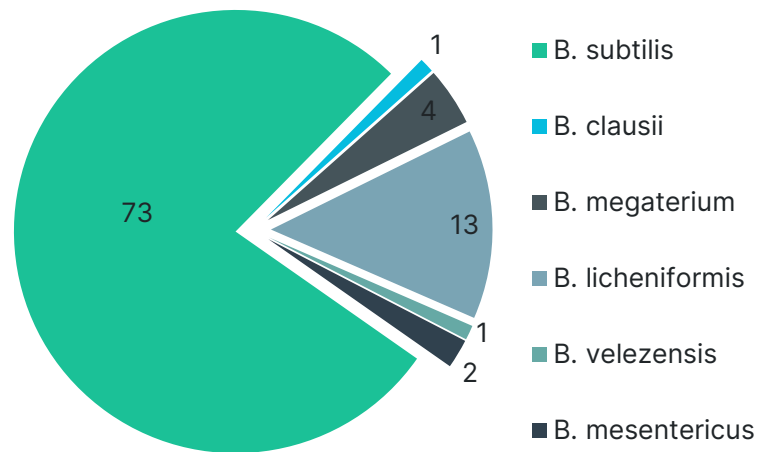
P. pastoris: secretion of industrial peroxygenase

- > Successful secretion of customer target protein and enzyme for use in industrial biocatalysis
- > Process economics meeting target COGs, reduced purification costs

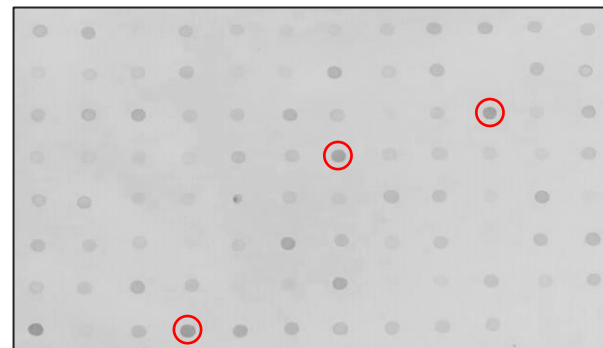
Custom Genetic elements

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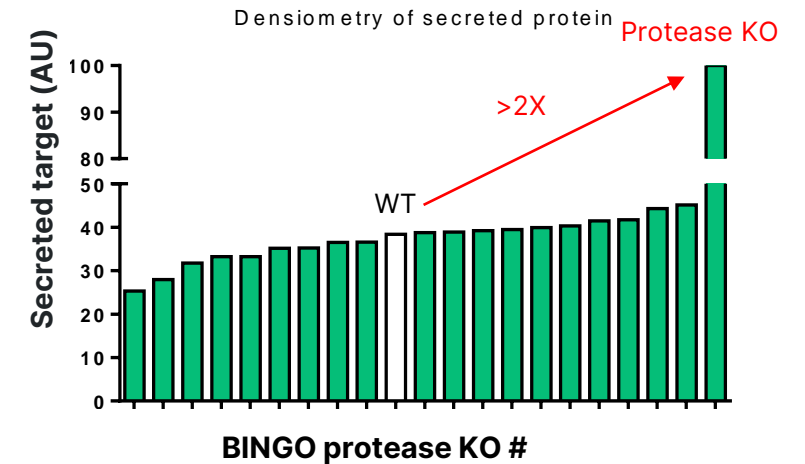
Library of secretion signal peptides



HTP screen of signal peptide library



Protease knockout screen

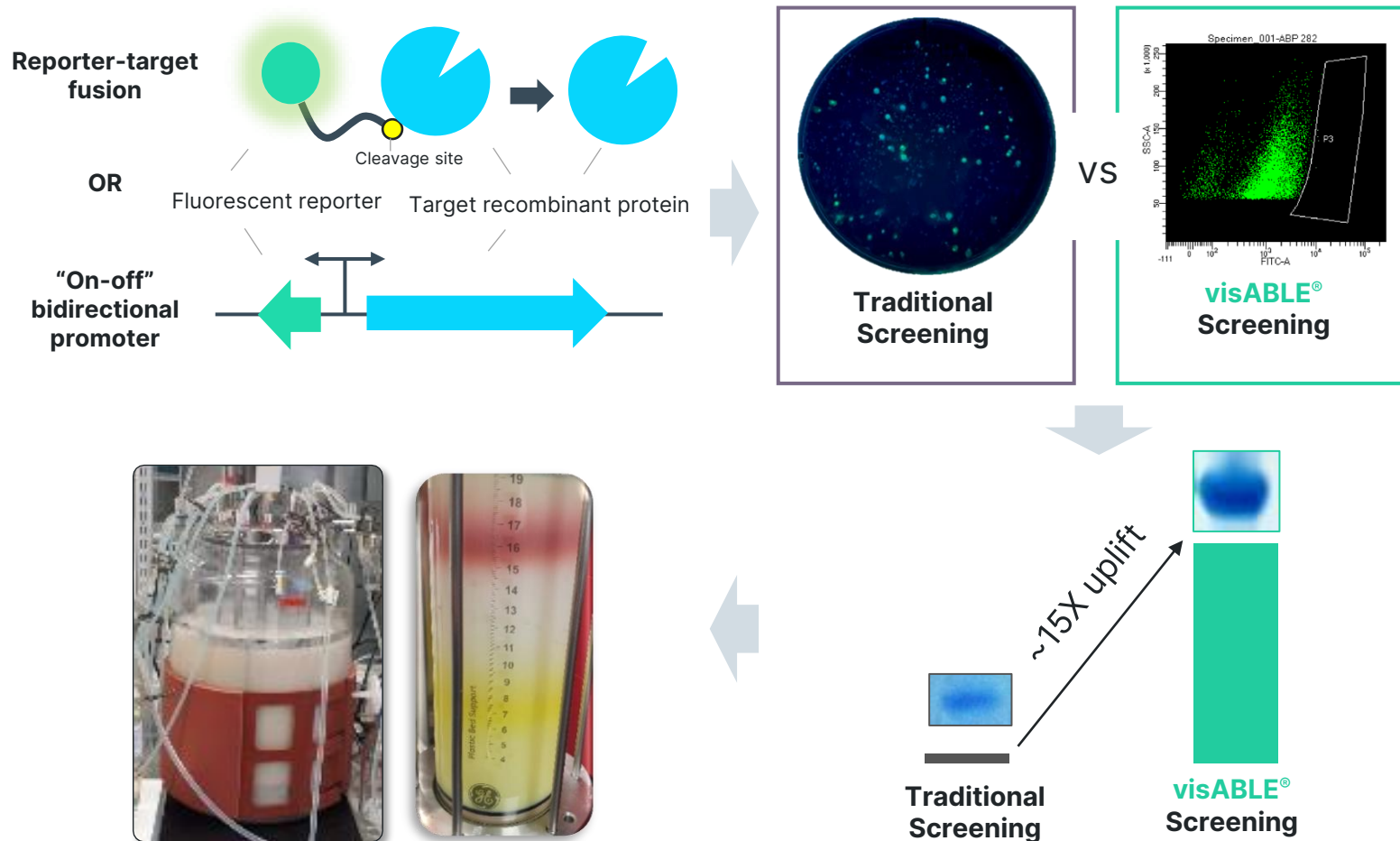


B. subtilis: optimal secretion of heterologous proteins

> Predictability in recombinant protein expression enabled secretion of customer target >0.5 g/L, at anticipated scale of 3,000 L

High throughput screening of recombinants

visABLE®: Ultra HTP detection and monitoring of jackpot clones



Outcomes

- > Ultra HTP identification & monitoring of jackpot clones (FACS)
- > Optimised USP/DSP developed
- > Titre increased from $\mu\text{g}'\text{s}$ to $\text{g}'\text{s}$, highly competitive COGS
- > Visual monitoring of upstream and downstream productivity/stability
- > Scarless low-cost reporter removal

The *inGenius* way

Target selection



BIOPROCESS OPTIMISATION

- Scalable upstream and downstream bioprocesses
- Batch, fed-batch, perfusion and continuous culture modes
- DoE optimisation of key process indicators
- Full analytical suite



LAB-SCALE MANUFACTURE

- Quality-controlled research Master Banks
- Product manufacture for activity, formulation and performance testing



PILOT MANUFACTURE

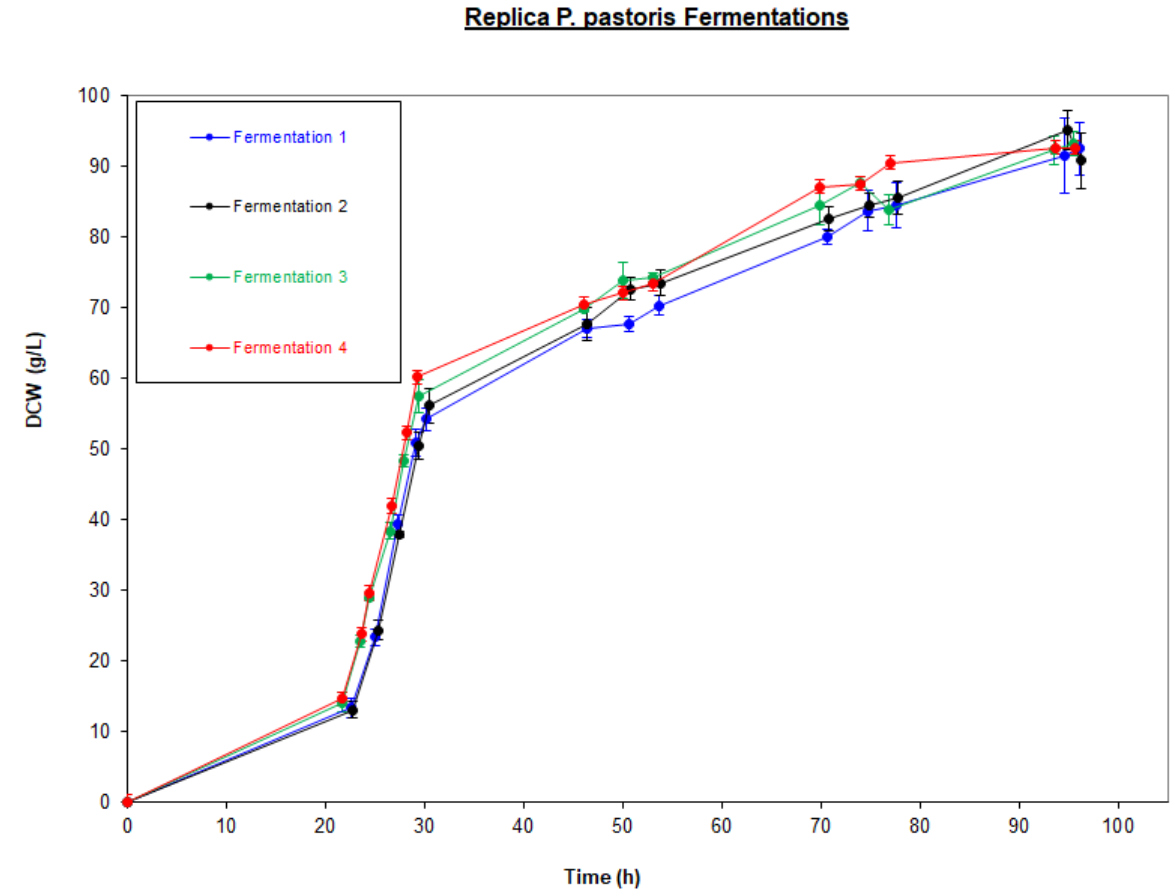
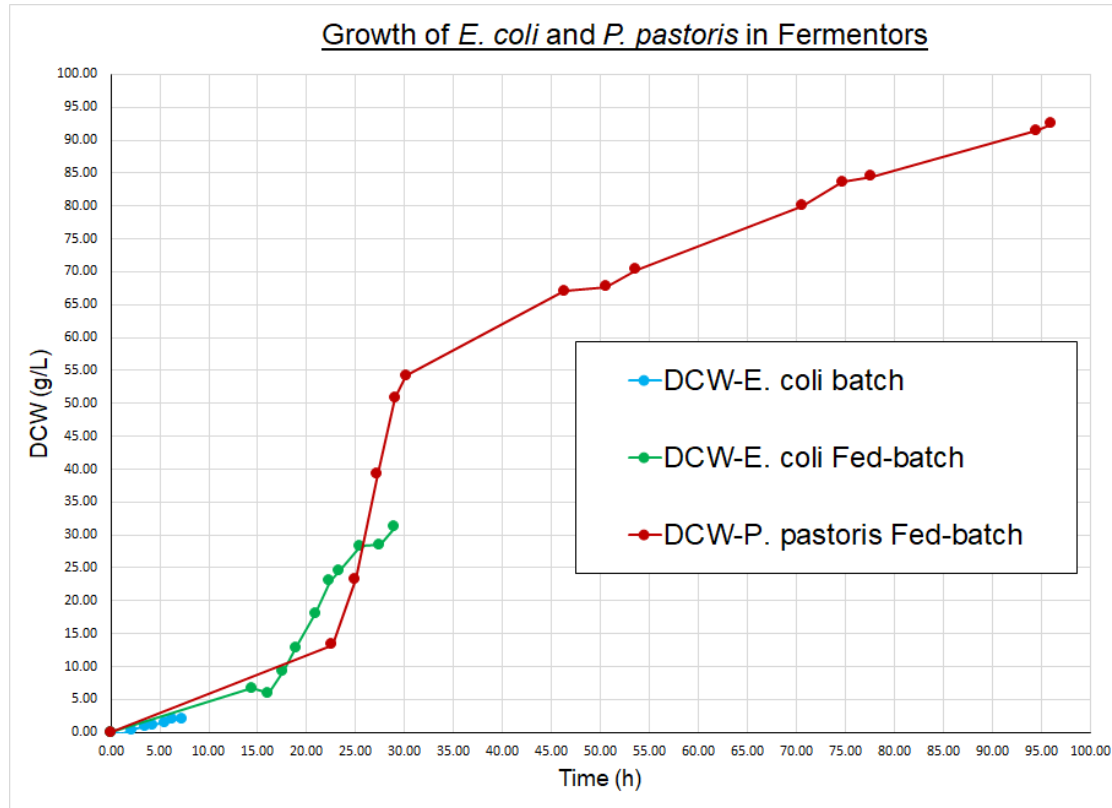
- History of successful technology transfer for bioprocess scale up
- Generation of material at customer facilities or in collaboration with trusted partners



Biomanufacturing process

Custom Scale-up

Examples of developed bioprocesses



Successful bioprocess Scale-up

Molecular Biology

Fermentation

Chemistry/DSP

USA - Customer Project
Pilot: 10 L
Production: 50,000 L

USA – Ingenza Process
Production: 100 L

USA – Ingenza Process
Pilot: 6,000 L
Production: 2.6 m L

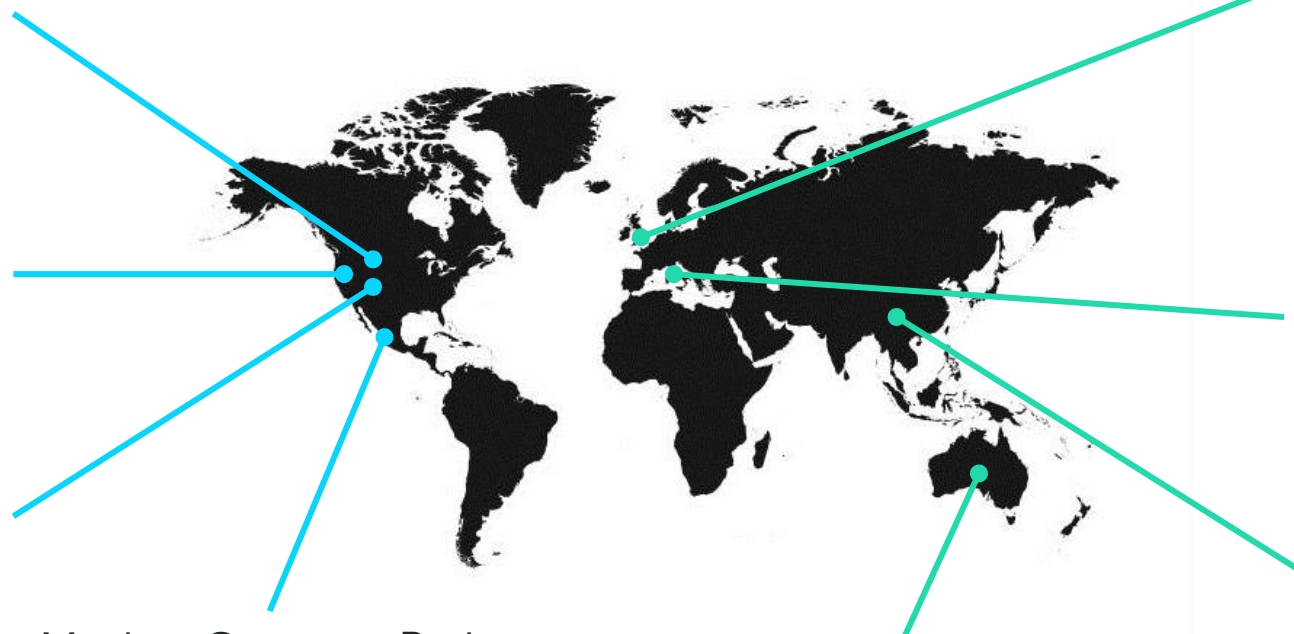
Mexico- Customer Project
Production

Australia- Customer Project
Pilot

UK
Customer Project: 100 L
Ingenza Process: 1,000 L

Europe - Customer Project
Pilot: 300L
Production: 22,000 L

Asia - Ingenza Process
Production: 5,000 L



Summary

Exploring alternative microbial cell factories can:

- Overcome biocatalyst toxicity and target molecule-host compatibility
- Improve biocatalyst functionality and recovery
- Reduce scale up costs

Ingenza's inGenius™ platform rapidly demonstrates bioprocess feasibility and brings increased predictability to ensure scalable and economically-viable biomanufacturing of biocatalysts.







<https://www.ingenza.com/resources>

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