

SynBio A to Z: integrated bioengineering platforms for efficient biomanufacturing in prokaryotic and eukaryotic production systems

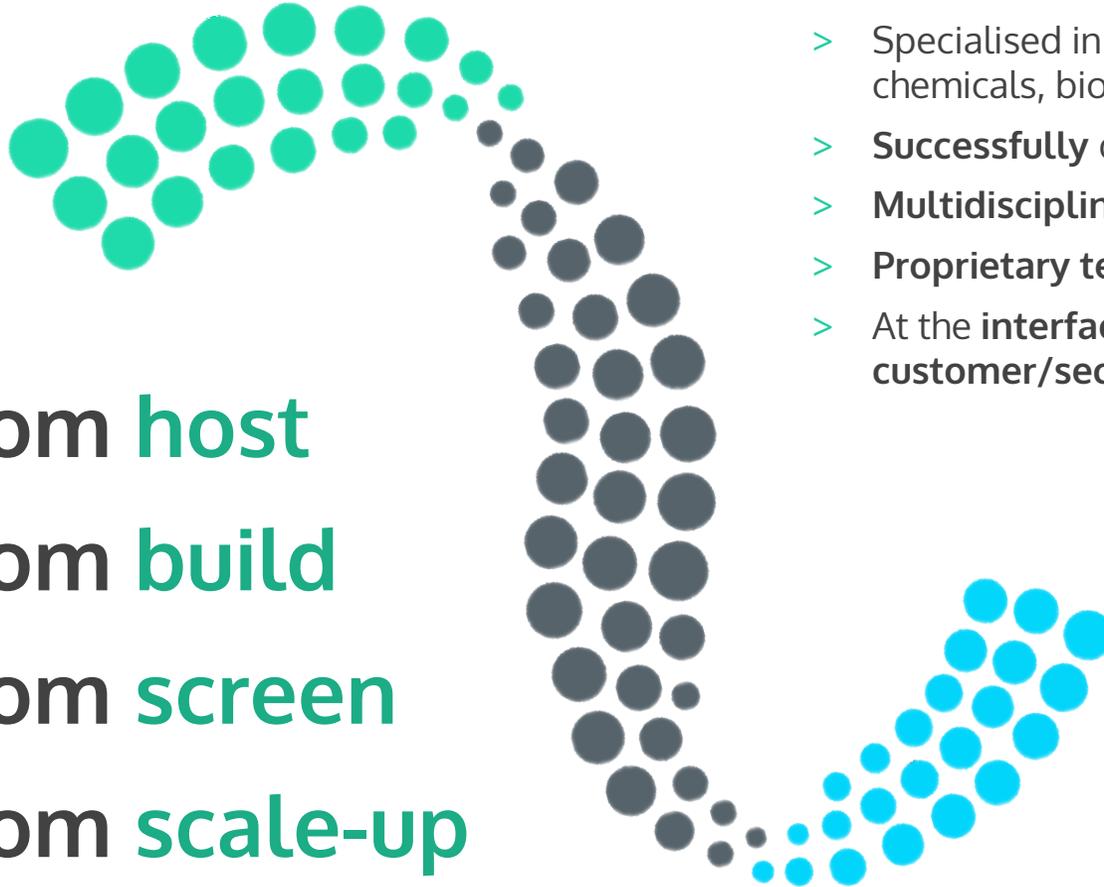
Cell Factories for Industrial Bioproduction (CFIB)

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29th March 2022



Ingenza: Advanced by Nature[®]



Custom **host**
Custom **build**
Custom **screen**
Custom **scale-up**

- > World leader in **applied Engineering Biology**
- > Specialised in the **design, development and biomanufacture** of chemicals, biologics, pharmaceuticals and biofuel products
- > **Successfully** delivering for our customers since 2003
- > **Multidisciplinary** team
- > **Proprietary technologies**
- > At the **interface between Engineering Biology tools and customer/sector needs**



Successful customer applications



Multi-Disciplinary Expertise



Molecular Biology

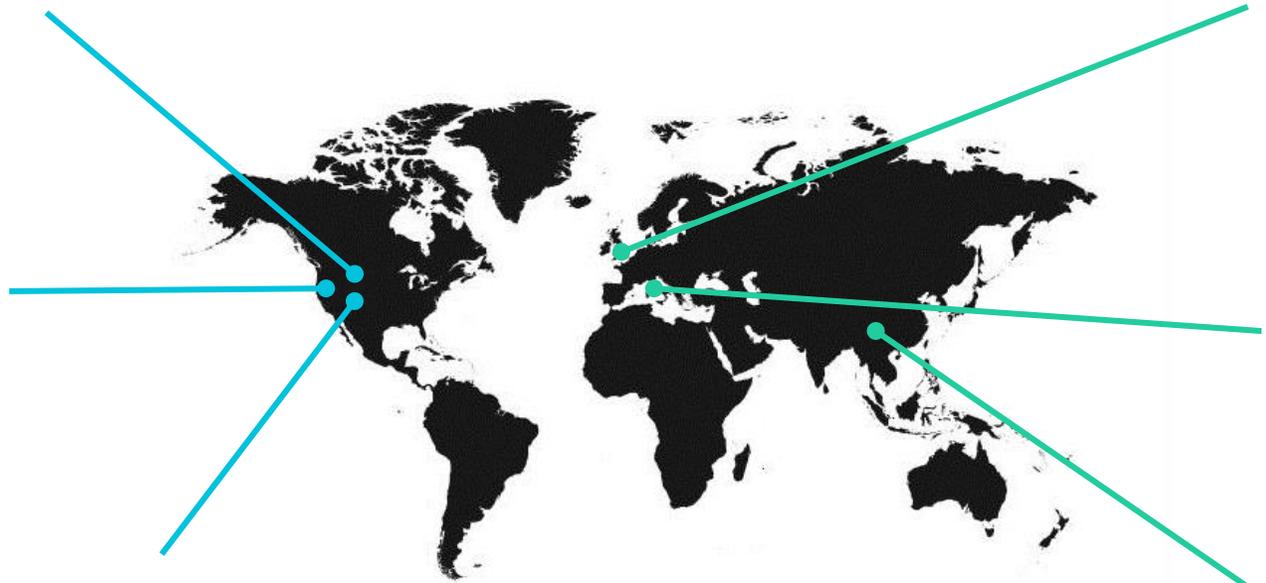
Fermentation

Chemistry

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

USA - Ingenza process
Production: 100 L

USA - Ingenza Process
Pilot: 6,000 L
Production: 30,000 L & 2.6m L

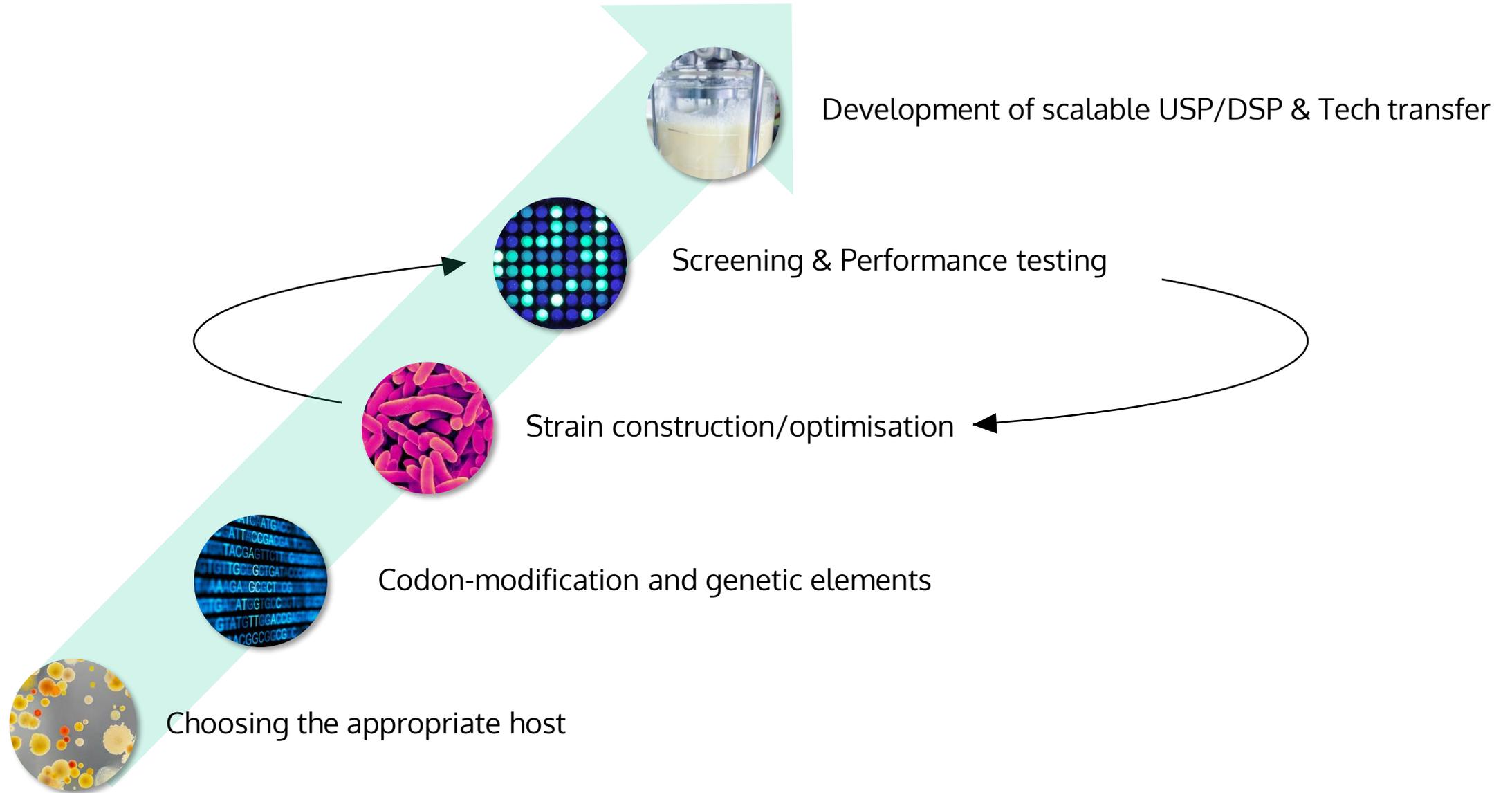


UK
Customer Project: 100 L
Ingenza Process: 1,000 L

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

Asia - Ingenza Process
Pilot: 3 L
Production: 5,000 L

Engineering Biology Stages



Custom: Host



E. coli

- Model organism
- Years of experience
- Large synthetic biology toolbox



Bacillus sp.

- Secretion of heterologous proteins
- Long history of industrial exploitation



P. putida

- High tolerance to solvents
- Diverse metabolism
- Well defined genetic toolbox



S. cerevisiae

- GRAS
- Large toolbox available
- Highly scalable



P. pastoris

- GMP compliant for pharma
- Production of secreted recombinant proteins
- Engineered glycosylation



C. glutamicum

- Heterologous protein and small molecule secretion
- High cell-specific productivity



Streptomyces sp.

- Complex secondary metabolism
- Producer of numerous medical compounds



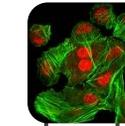
Vibrio natriegens

- Fast-growing molecular biology host
- Doubling time <10 min



Aspergillus niger

- Capacity to secrete large amounts of enzymes
- Production of citric acid
- Various metabolites

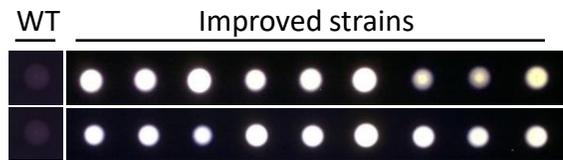


CHO cells

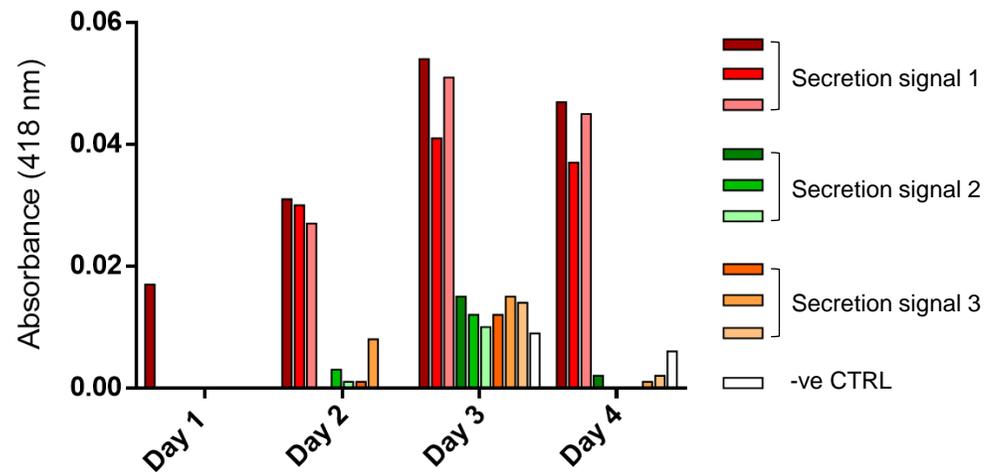
- Mammalian cell line
- Human post-translational modifications
- Targeted gene integration

Custom: Genetic elements

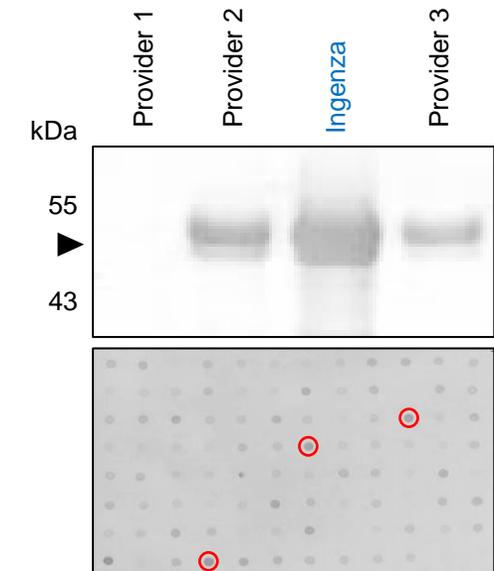
- > Library of promoters, secretion signals, plasmid backbones and integration sites with FTO for multiple hosts
- > inABLE[®] combinatorial DNA assembly platform
- > Superior codon modification algorithm for *Bacillus* spp



S. cerevisiae: optimal expression of a starch degrading enzyme



P. pastoris: secretion of industrial enzymes

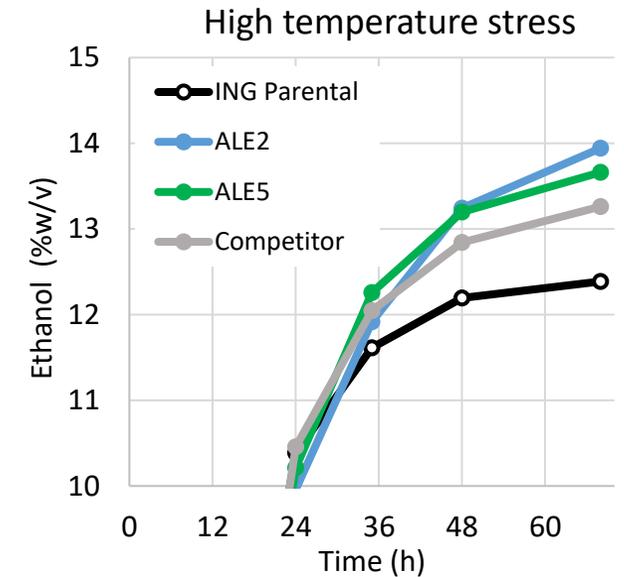
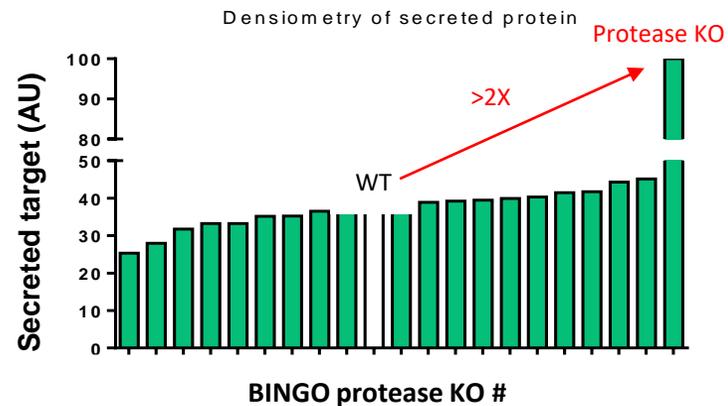
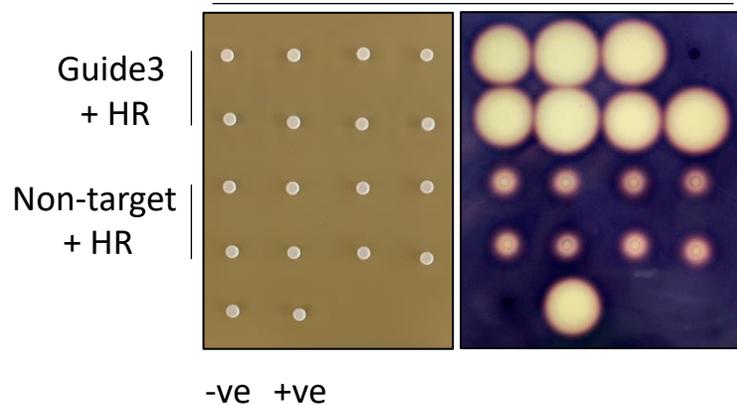


B. subtilis: optimal expression & secretion

Custom: Strain optimisation

- > Collection of genetic backgrounds, including protease/glycosylation-deficient, super-secreting, asporogenic and chaperone-overexpressing strains
- > CRISPR/MAD7 genome editing with FTO for *Bacillus* (BINGO) and industrial (i.e. diploid) *S. cerevisiae* strains
- > Strain mutagenesis and Adaptive Laboratory Evolution (ALE) programmes in house & with partners

Soluble starch plate



S. cerevisiae: marker-free cassette integration and gene deletion



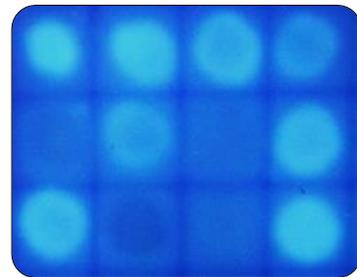
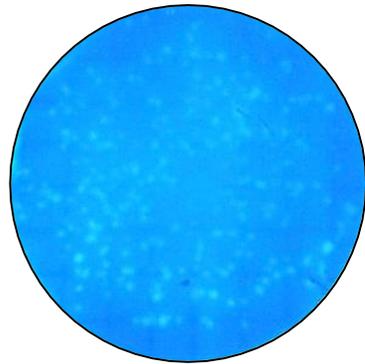
B. subtilis: enhanced stability of secreted protein targets



S. cerevisiae: improvement of thermotolerance via ALE

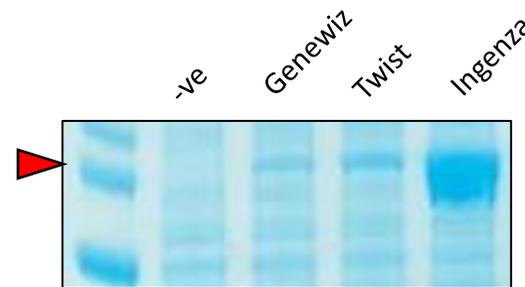
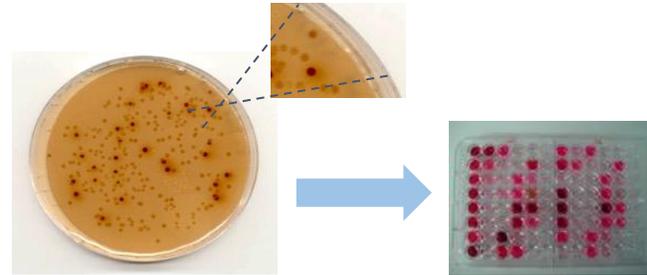
Custom: Screening

Mitsubishi Chemical



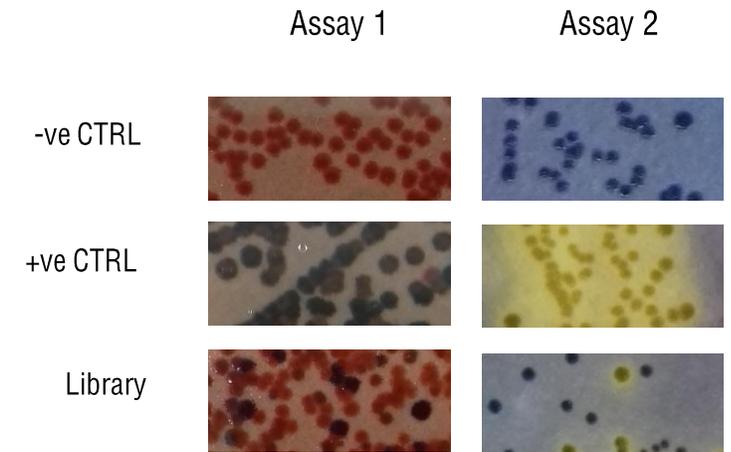
Biosensors for durable bioplastics

Allergan



Improved protein expression

Unilever (Enzyme evolution)



Assays tailored to target fatty acid modifying proteins

Custom: Scale-up

Lab scale – in house

Scale up – with partners



Aquila
biolabs CGQ
0.1-0.5 L



Ambr®250 single-use
modular
0.1-0.25 L



BioBLU®
1.5-10 L



Glass Autoclavable
Vessels
0.3-20 L



CPI (UK): 50 L – 10,000 L
Also in Asia, Europe and USA:
Pilot scale: 10 L – 6,000 L
Production: 100 L – 2.6m L



Supporting manufacture
(inc. GMP)

Scalable bioprocesses developed under the principles of GMP

Successful **Technology Transfer**

Custom: Process Development



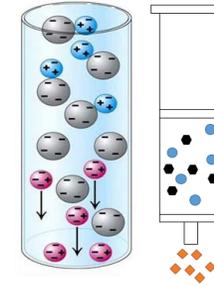
DSP development and optimisation



GE AKTA systems
(0.1-150 mL min⁻¹)



Continuous ATF/TFF
(2-40 L mL min⁻¹)



IEX SPE



Freeze drying

> Cost-effective, robust and scalable product purification and isolation

Bioprocess Analysis



HPLC UV/RID/FLD
LCMS



GCMS/FID



CEDEX



FLUOstar
plater reader

Access to local facilities:

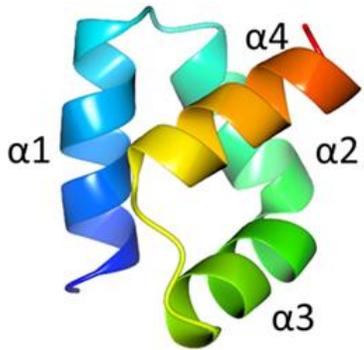
NMR
MALDI
Proteomics

> Identify/monitor KPIs and determine bioprocess rate limitations

Addressing Antibiotic resistance

Bringing the antibacterial peptide NI01 to market

- > Infections by MDR bacteria are a growing threat to healthcare, killing ~700,000 people p.a. globally
- > Bacterial resistance detected for all 90+ FDA approved antibiotics: deaths may reach 10 million by 2050



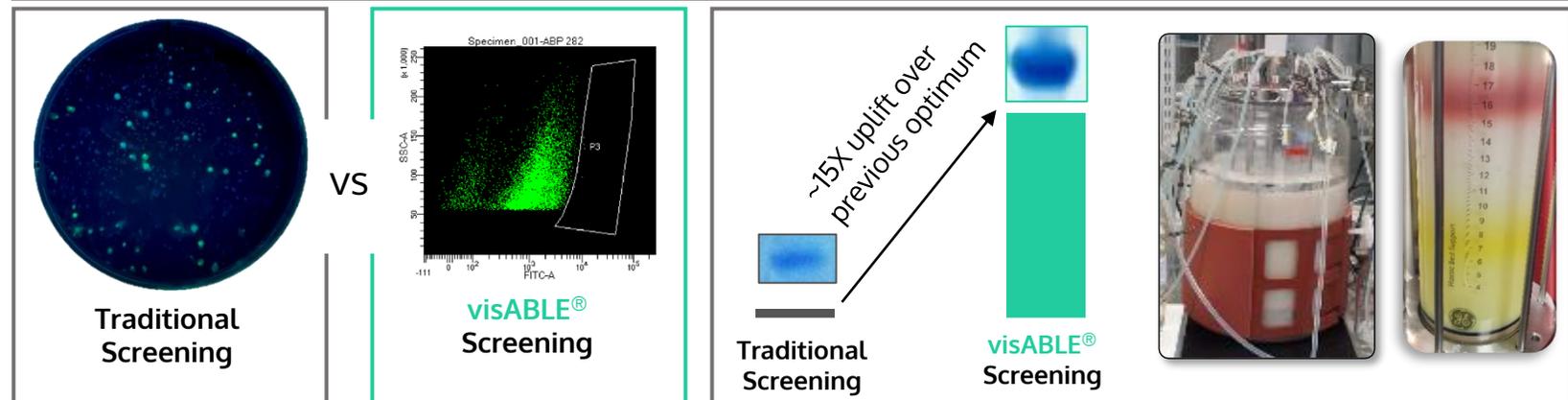
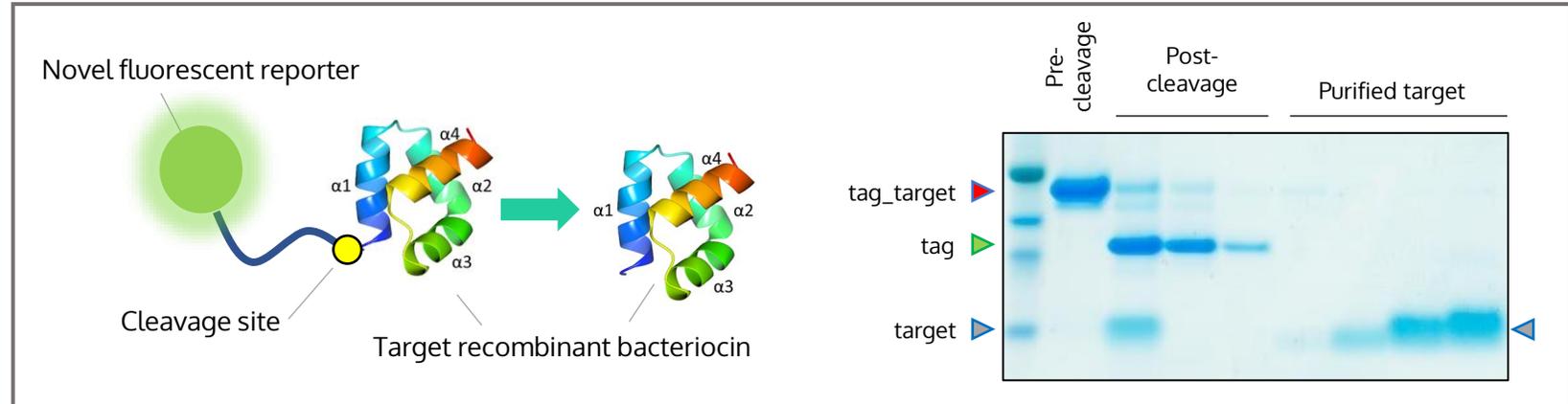
- Epidermicin NI01: active against *S. epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, biofilm forming strains
- Single dose efficacy, development of resistance not observed
- Low yield of recombinant peptide in prokaryotic expression hosts
- Ingenza has worked closely with Prof. Mathew Upton (University of Plymouth/Amprologix) to develop a viable GMP, bio-manufacturing process for Epidermicin NI01

Addressing Antibiotic resistance

visABLE[®]: AMP & Biologics production in *P. pastoris*

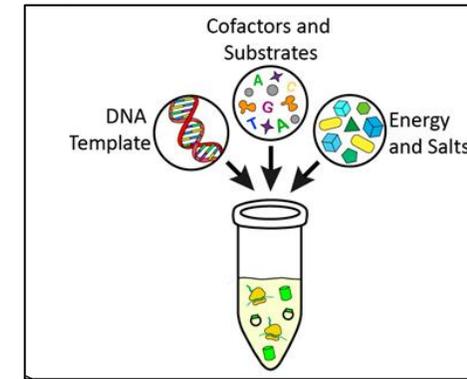
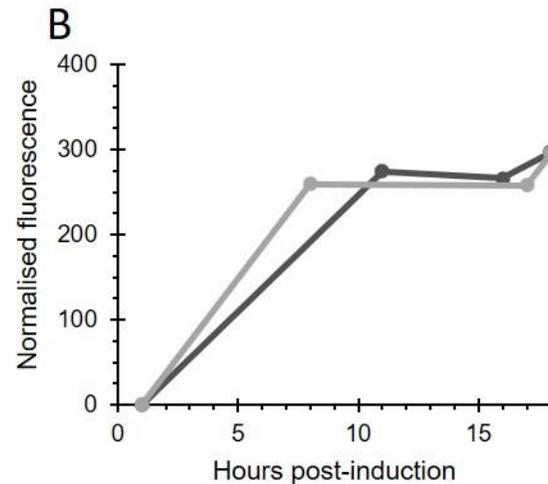
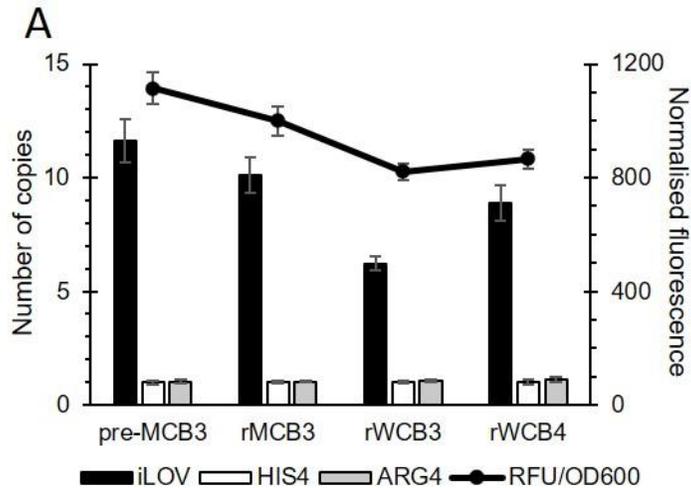


- > Visual monitoring of upstream and downstream productivity/stability
- > Free of 3rd party IP, Ultra HTP detection and monitoring of jackpot clones (FACS)
- > Scarless low-cost reporter removal
- > Customised target purification
- > "Masking" of target toxicity
- > AMP titre increased from µg's to g's, enabling highly competitive COGS
- > Ingenza management of product formulation and pre-clinical toxicology testing (3rd parties)

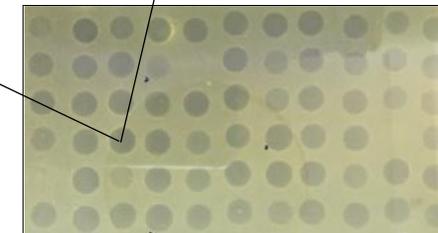


Addressing Antibiotic resistance

visABLE[®]: AMP & Biologics production in *P. pastoris*



AI-driven design, screening and learning allowed identification of NI01 variants with altered antimicrobial activity



visABLE[®] allows monitoring of strain stability and productivity in real time



Altered specificity from gram positive to gram negative (i.e. *S. aureus* to *Acinetobacter baumannii*)

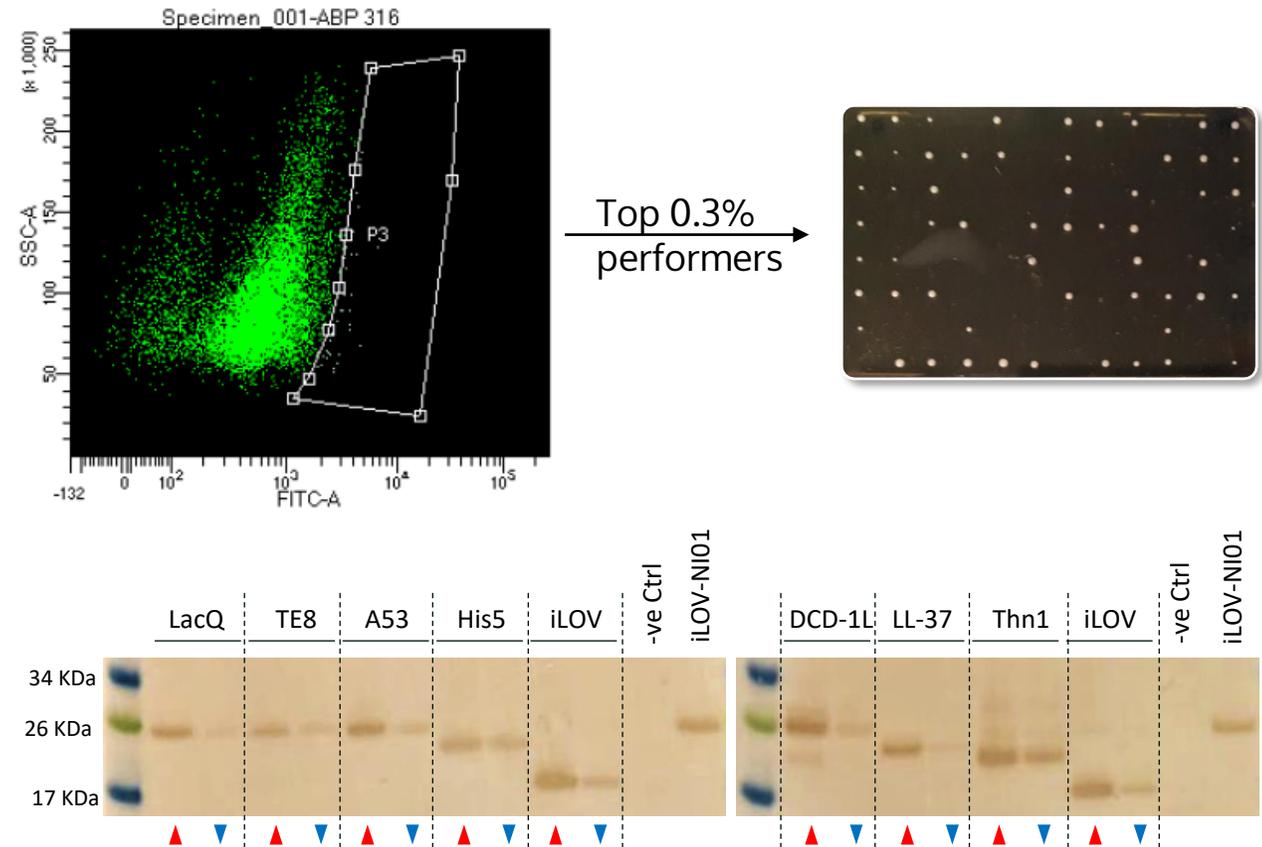
Addressing Antibiotic resistance

visABLE[®]: AMP & Biologics production in *P. pastoris*

Peptide	Origin	% Identity to NI01	Activity
TE8	<i>S. capitis</i>	97%	<i>Staphylococcus sp.</i>
LacQ	<i>L. lactis</i>	51%	<i>Staphylococcus sp.</i>
A53	<i>S. aureus</i>	36%	<i>Staphylococcus sp.</i>
LL-37	Human	21%	<i>E. coli, Staphylococcus, Klebsiella</i>
His5	Human	30%	<i>Candida</i>
DCD-1L	Human	17%	<i>Staphylococcus, Candida</i>
Thn1	<i>P. maculiventris</i> (insect)	16%	<i>E. coli, Klebsiella</i>

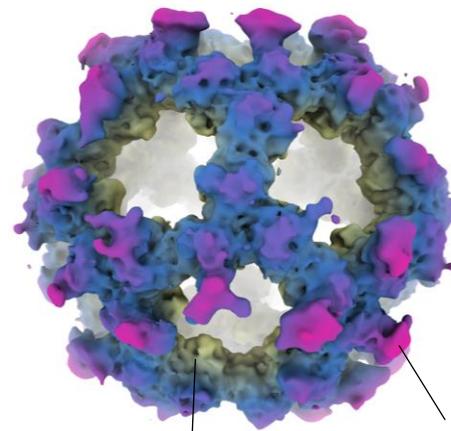
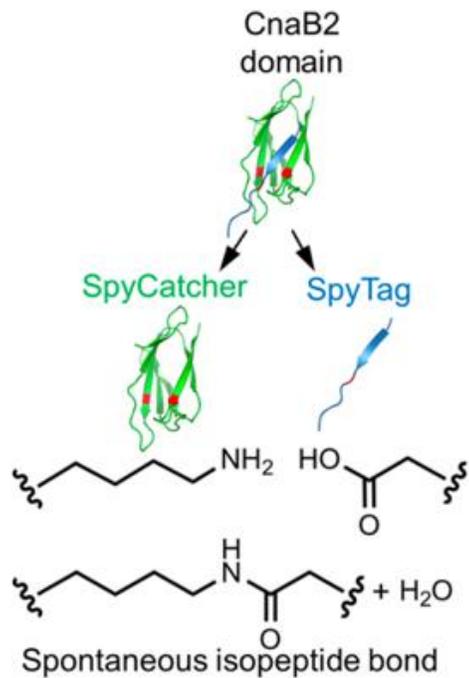


visABLE[®] allows identification of superior *P. pastoris* manufacturing strains



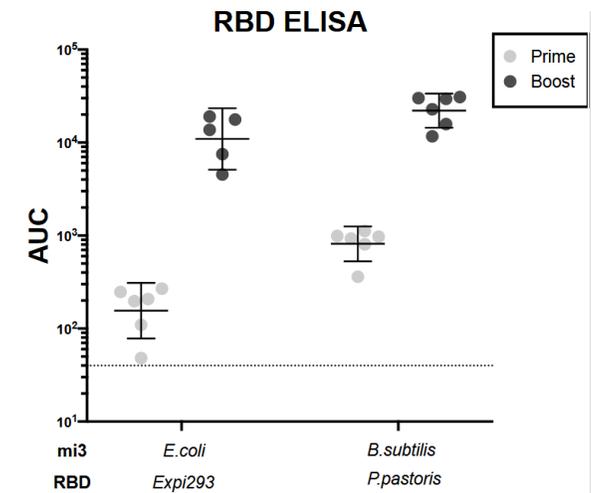
Addressing equitable global healthcare

- > Development of a fully-microbial and versatile recombinant subunit vaccine candidate with competitive COGs
- > Simplified DSP, no endotoxin removal required
- > Identical immunogenicity performance *in vitro* and *in vivo* to mammalian-derived product



SpyTag-RBD secreted from *P. pastoris*
vs. mammalian cells

SpyCatcher-VLP secreted from *B. subtilis*
vs. intracellular in *E. coli*

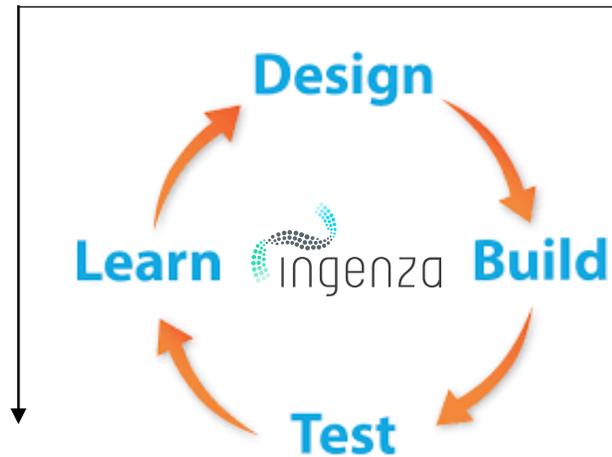


Success via Integrated Project Team



Project onboarding

Customer



- > Strain construction & testing
- > Recombinant material QC
- > Scalable USP/DSP development
- > Cell banking to pre-GMP
- > QMS & project management

Tech transfer



3rd party CRO/CMO

Why work with us?



Successfully delivering for our customers since 2003

- Project definition steps through close engagement with customers
- Multiple bio-products scaled and commercially manufactured

Multidisciplinary team

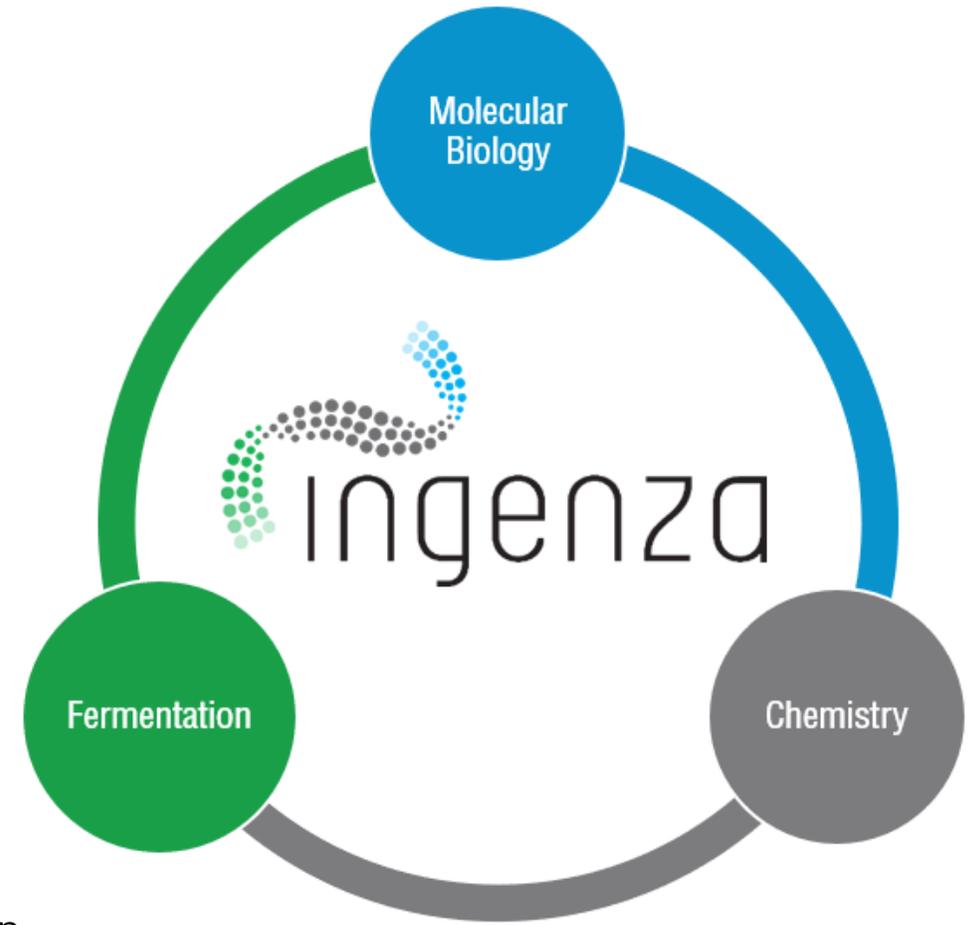
- Product concept through to pre-commercial scale production
- Synthetic chemistry team synergistic with biotech capabilities

Proprietary technologies

- inABLE[®] and visABLE[®] suites, expression hosts free of 3rd party IP
- Scalable fermentation modes/protocols

Flexible collaboration/business models

- Risk-sharing, technology licensing, fee-for-service, equity partnership



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