A *PSEUDOMONAS FLUORESCENS*-BASED PLATFORM VALIDATED FOR RAPID CANDIDATE SELECTION AND ACCELERATED CMC DEVELOPMENT

www.pelicanexpression.com
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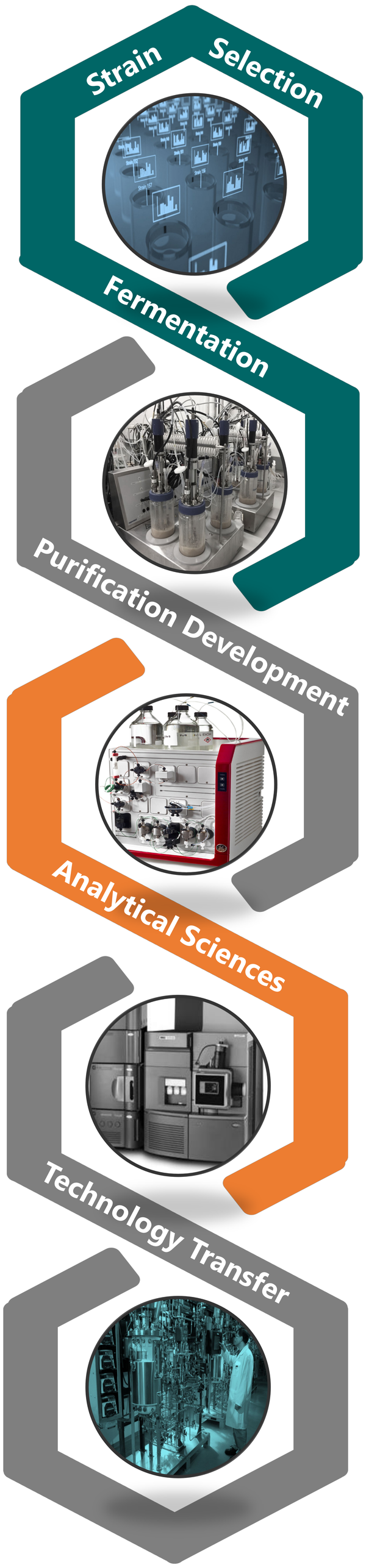
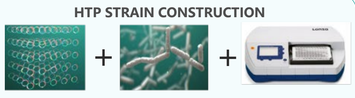


The Pelican Expression Technology™ platform is a validated and versatile expression system for developing protein therapeutics from concept-to-clinic. With over 20 years of experience and 4 commercially approved products, the proven platform enables rapid candidate selection and accelerated CMC development for a wide range of complex modalities. Over 175 lead proteins, including difficult-to-produce proteins that failed to express in *E.coli*, yeast, and mammalian cells such as CHO, have been successfully produced using Pelican Expression Technology™. The combination of high-throughput strain screening, advanced analytics, state-of-the-art fermentation capabilities between 1 and 100L production in-house, complementary purification development capabilities from HTP to bench and pilot-scale, and proven technology transfer experience renders the platform capable of progressing from gene to production scale in under 12 weeks.

Platform Development and Integrated Process Development

Automation-enabled HTP Strain Construction, Expression, and Analysis

HTP STRAIN CONSTRUCTION



plasmids + host strains + Electroporate and grow transformants in selective media (seed cultures)

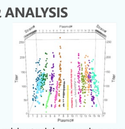
HTP EXPRESSION

96 deep well growth and expression

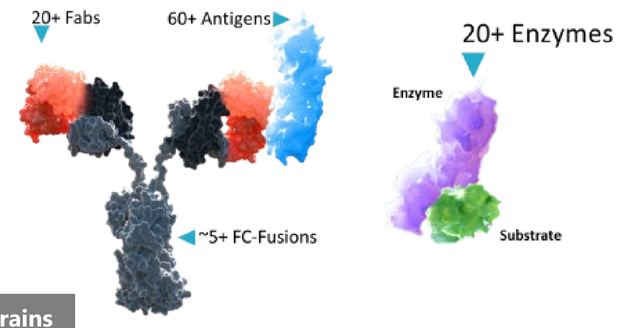
0.5 ml culture per well, induce 24 hrs.

HTP HARVEST & ANALYSIS

OD_{600} 30-50 at harvest



Combinatorial screening approach can assess thousands of unique expression strains in parallel



Product titer and quality assessed early in pipeline to down select to candidate production strains

QUANTITY ASSESSED
1st-tier analysis (Samples: 1000s)

HTP SDS-CGE
(20 sec/sample)
Measures titer

HTP BLI
(1 min/sample)
Allows both quantity and quality

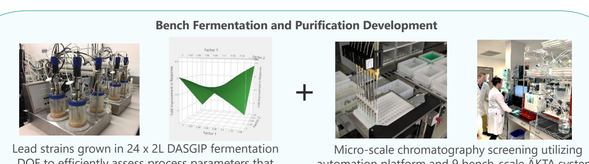
QUALITY ASSESSED
2nd-tier analysis (Samples: 10-100s)

Mass Spectrometry
including a Thermo Scientific Orbitrap LTQ fitted with microfluidic sample flow (ZipChip™), a Xevo QTof with HDX capability, and a Bruker MALDI Flex for analysis of PEGylated proteins

Ultra Performance Liquid Chromatography, CE, icIEF and more

Bench to pilot scale fermentation and purification for efficient process development and scale up

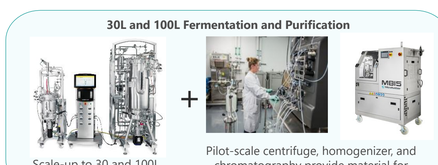
Bench Fermentation and Purification Development



Lead strains grown in 24 x 2L DASGIP fermentation DOE to efficiently assess process parameters that maximize titer and quality

Micro-scale chromatography screening utilizing automation platform and 9 bench-scale ÄKTA systems support accelerated development

30L and 100L Fermentation and Purification



Scale-up to 30 and 100L Sartorius fermenters

Pilot-scale centrifuge, homogenizer, and chromatography provide material for characterization

Technology Transfer

Tech Transfer Documentation and Support

Technology transfer to global CMOs, CROs and pharmaceutical companies.

Case Studies

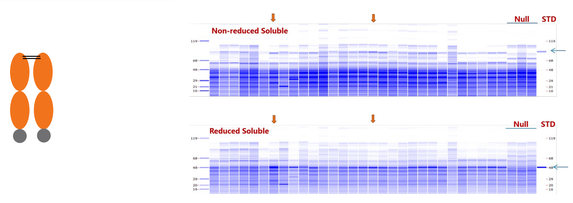
Fc fusion protein expression

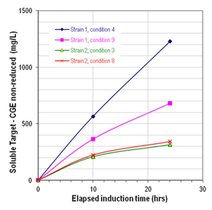
Challenge: Insoluble *E. coli* expression/refold but soluble expression desired

Background
- 400 unique strains evaluated
  - 10 expression plasmids
  - 40 host strains
- Screen for soluble expression of dimeric Fc fusion, protein A binding

Strain engineering (96-well plate)
- SDS-CGE detected induced bands (blue arrow) in non-reduced and reduced soluble samples
- Protein A binding (BLI) correlates with non-reduced SDS-CGE titer
- Red arrow: strains selected for fermentation assessment

Fermentation Assessment (2 L)
- Down-selected strains assessed at 2 L scale under multiple induction conditions
- SDS-CGE → >1g/L
- 3-10X improvement over 0.5mL scale





SOLUTION: **Production strain with highest soluble titer identified for further development**

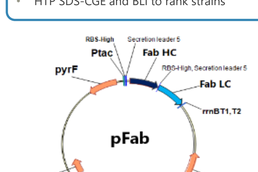
FAB antibody fragment titer improvement

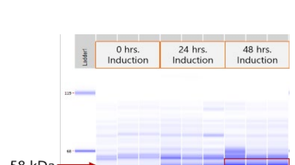
Challenge: Low titer with *E. coli* platform not sufficient for clinical supply | Cost-effective process requires higher titers

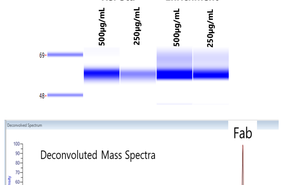
Strain Engineering (96-well plates)
- 800 unique strains
  - 160 expression plasmids
  - 40 combinations of secretion leaders, RBS, and codon optimization
- HTP SDS-CGE and BLI to rank strains

Fermentation Assessment (2 L)
- Down-selected strains assessed at 2 L scale under multiple induction conditions
- SDS-CGE and BLI

Quality Assessment
- 58 kDa Fab confirmed by LC-MS after enrichment culture
- Further development of product in progress







SOLUTION: **Production strain with highest quality and titer identified for further development**

Approved Products

Alvogen® Teriparatide Injection
20 mcg per dose. Each single-patient-use pen will deliver 28 subcutaneous doses.

Alvogen®

Vaxneuvance™ Pneumococcal 15-valent Conjugate Vaccine

MERCK

Pneumosil
Pneumococcal Polysaccharide Conjugate Vaccine (Adsorbed, 10-Valent)

SERUM INSTITUTE OF INDIA PVT. LTD.

RYLAZE™ asparaginase erwinia chrysanthemi (recombinant)-rywn for injection 10mg/0.5mL per vial

Jazz Pharmaceuticals

Summary

The Pelican Expression Technology™ can deliver significant competitive advantages in expressing lead protein candidates with its diverse toolbox and automation. With an array of robust high throughput methods, viable production strains can quickly be identified for further development and scale-up.

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PELICAN™
P. fluorescens expression technology