A Pseudomonas fluorescens based platform for robust biotherapeutics manufacturing

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PFEnex
Innovative Solutions for Global Health

Safe Harbor Statement

This presentation, including the accompanying oral presentation (the "Presentation"), includes forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, which are based on current expectations, estimates and projections based on information currently available to management. These forward-looking statements include, among others, statements regarding the future potential of PF708 and Pfenex's product candidates, including future plans to advance, develop, manufacture and commercialize PF708 and its other product candidates, including the expected commercial strategy for PF708; potential market opportunities for PF708; the potential commercial US launch of PF708 upon an FDA decision on the therapeutic equivalence rating; Pfenex's beliefs regarding PF708's noninferiority to Forteo®; Pfenex's belief that the human factors study completes the data package required by the FDA to evaluate therapeutic equivalence; Pfenex's strategy to commercialize its product candidates and expand its development pipeline; the expected patent expiration timelines and strategies for Forteo and other branded reference drugs; developments and projections relating to competitors and the industry, including that, if launched, there may be limited competition for PF708; expectations with regard to future milestone, royalty, and other payments from Pfenex's collaborations with Jazz Pharmaceuticals, Alvogen, NT Pharma, Merck, SII, Arcellx and other third parties, including amounts and potential timing of receipt; Pfenex's expectations with respect to the advancement of PF743 and PF745 with Jazz Pharmaceuticals; Pfenex's expectations regarding regulatory submissions and responses; Pfenex's expectations regarding the timing and advancement of clinical trials and the types of future clinical trials for its and its collaboration partner's product candidates; and Pfenex's expectations regarding its IP strategy. Forward-looking statements are typically identified by words like "believe," "anticipate," "could," "should," "estimate," "expect," "intend," "plan," "project," "will," "forecast," "budget," "pro forma," and similar terms. Factors that could cause Pfenex's results and expectations to differ materially from those expressed in forward-looking statements include, without limitation, Pfenex's need for additional funds to support its operations; its success being dependent on PF708; Pfenex's reliance on its collaboration partners' performance over which Pfenex does not have control; the FDA may disagree that the human factors study report completes the information package and is sufficient to evaluate therapeutic equivalence for PF708; failure to achieve favorable results in clinical trials for its product candidates or receive regulatory approvals, including whether Pfenex obtains an "A" therapeutic equivalent designation for PF708; delays in its clinical trials or in enrollment of patients in its clinical trials; failure to market PF708, or its other product candidates due to the existence of intellectual property protection owned or controlled by a third party and directed to PF708, or its other product candidates; PF708, and its other product candidates may cause serious adverse side effects or have properties that delay or prevent future regulatory approvals or limit their commercial profile; risks associated with market acceptance, including pricing and reimbursement; Pfenex's ability to enforce its intellectual property rights; adverse market conditions; and changes to laws and government regulations involving the labelling, approval process, funding and other matters affecting biosimilars, therapeutic equivalents to branded products and vaccines. Pfenex has not launched any products, and there is no certainty that PF708 will be launched or as to the timelines on which launch will occur. Further, Pfenex may be subject to direct legal challenges by the manufacturers of reference products, and Pfenex could be delayed or prevented from launching PF708 or its product candidates as a result of court orders or as a result of the time necessary to resolve such challenges. Unless otherwise indicated, forward-looking statements represent Pfenex's management's beliefs and assumptions only as of October 14, 2019. You should read Pfenex's Quarterly Report on Form 10-Q for the quarter ended June 30, 2019, and Pfenex's Annual Report on Form 10-K for the year ended December 31, 2018, including the Risk Factors set forth therein, and its subsequent reports filed with the SEC, including the Risk Factors set forth therein, completely and with the understanding that Pfenex's actual future results may be materially different from what Pfenex expects. Except as required by law, Pfenex assumes no obligation to update these forward-looking statements publicly, or to update the reasons why actual results could differ materially from those anticipated in the forward-looking statements, even if new information becomes available in the future.

Agenda

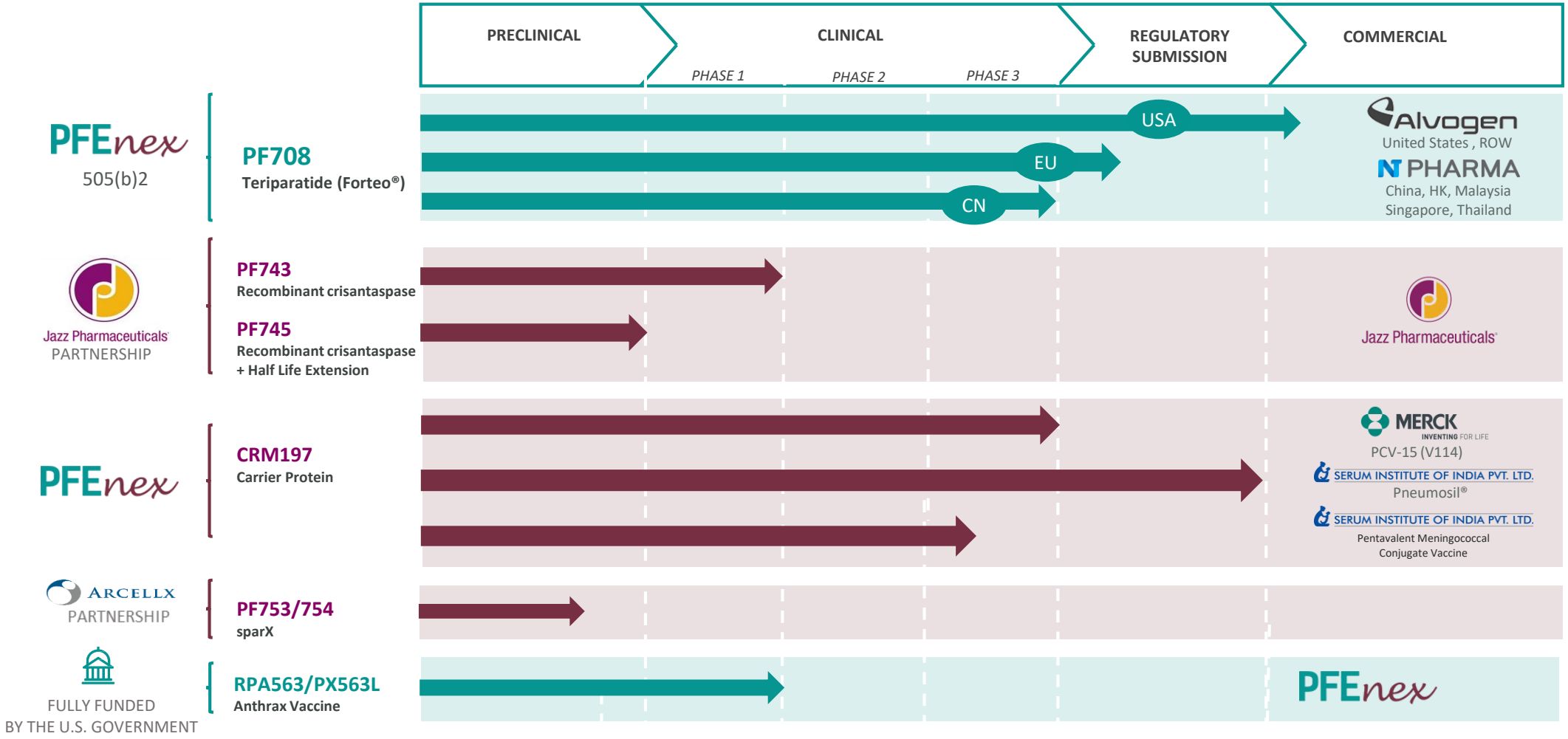
- Pfenex Company/Pipeline Overview
- The Pfenex Platform Technology
  - Toolbox for strain engineering
  - Parallel screening approach
- Case Studies

Pfenex Overview

- Located in San Diego, California USA
- Publicly traded (NYSE American:PFNX) product development and licensing company focused on developing therapies for unmet patient needs. Recently received our first US Marketing Authorization for PF708, a therapeutic equivalent candidate to Eli Lilly and Company's Forteo®
- Our proprietary Pfenex Expression Technology™ is leveraged to enable:
  - Advanced pipeline of potential therapeutic equivalents, biologics and vaccines including an FDA licensed product and clinical stage programs
  - Co-development partnerships with late clinical stage program
  - Supply of cGMP CRM197, a diphtheria toxoid carrier protein used in conjugate vaccines with three licensee programs in late stage clinical development
- Actively seeking platform partnerships, co-development and in-licensing opportunities that can be enabled by the Pfenex platform

Products in Development and Pipeline Products (includes licensed and partnered products)

Pipeline highlights



Pfenex Expression Technology

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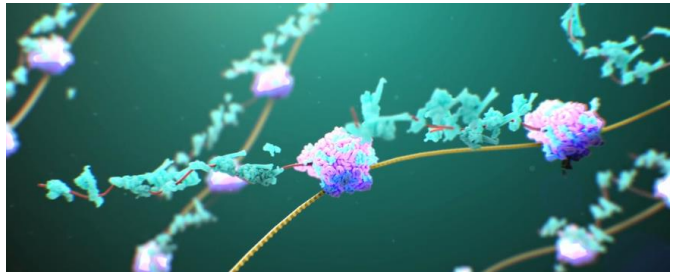
Pfenex Platform Technology

Our approach for microbial strain development:

- Discard the traditional, linear and iterative approach
- Implement a high-throughput, parallel strain screening technology

CELL BIOLOGY ⟶ EXPRESSION STRAIN

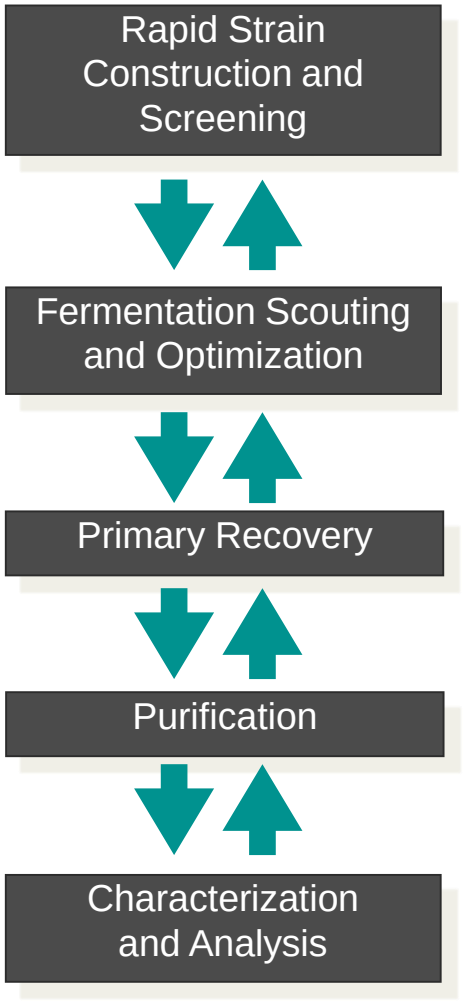
- *Let biology tell us what is the best strategy for production of any particular recombinant protein*



Pfenex – Parallel Development Approach

Protein production in *Pseudomonas fluorescens*

- Integrated development approach provides acceleration
- Goal: high titers of soluble, active protein



Automation-enabled process analytical

Strain Engineering
Thousands of strains- rapid cloning, periplasmic expression, 96-well screening

Process Analytical
Robotic sample processing, microchip SDS-CGE analysis and biolayer interferometry binding assays

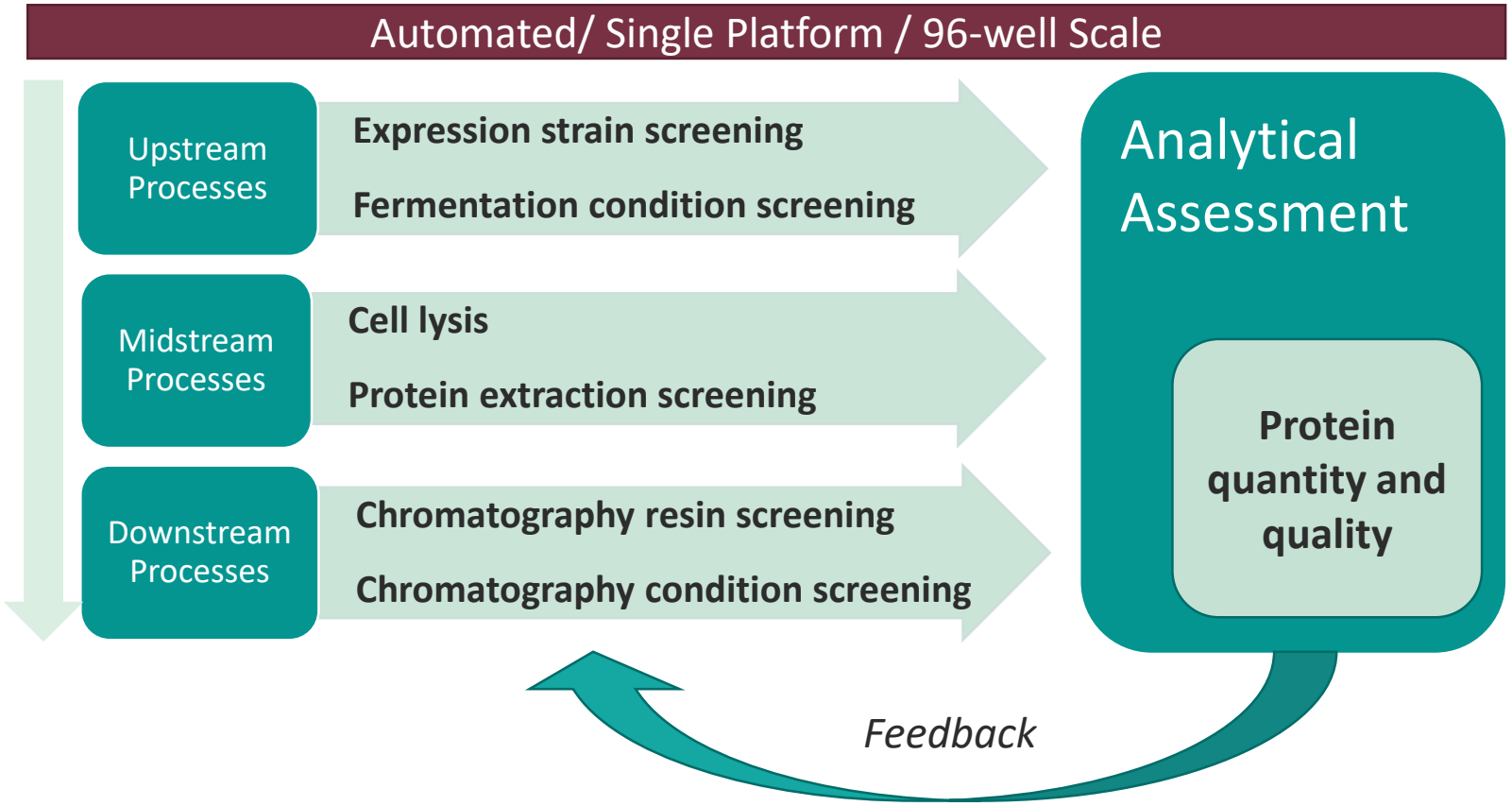
Fermentation Development
Multiple strains each evaluated in multiple scalable fermentation processes

Protein Purification
Primary recovery and chromatography options evaluated in parallel microtiter plate format

Product Quality Analysis
Detailed characterization (QTOF MS, RP-HPLC, SEC, fluorescence); impurity analysis (HCP, DNA, LPS)

Automated Sample Processing and Analysis

- Automated workflows: a key component of our parallel processing approach
  - Combining multiple process steps as uninterrupted, high throughput workflows
  - Rapidly providing feedback for the next round of experimentation

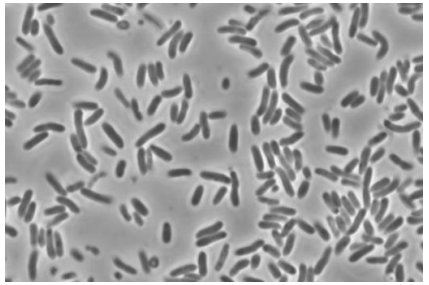


Pfenex Automation System

Pfenex Platform Technology

Pseudomonas fluorescens

Cultivation of *P. fluorescens* biovar I (MB101) & derivatives



P. fluorescens MB101 cells

P. fluorescens cultures, 2L DASGIPS

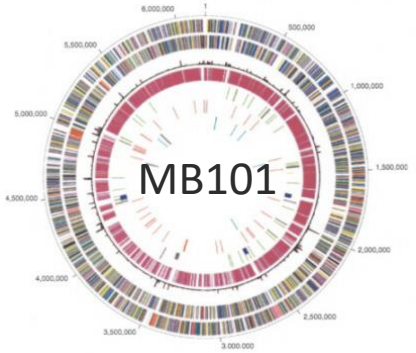
- Gram-negative, non-pathogenic bacilli
- Optimum growth temperature → 30-32C
- Obligate aerobe: requires adequate oxygen transfer to grow
  - Non-fermenting, minimal accumulation of inhibitory acids
- Defined mineral salts medium sup w/ inorganic nitrogen and a carbon source
  - Pfenex media: free of animal-derived components
- Non-antibiotic plasmid maintenance

Pfenex Platform Technology

Expression Toolbox

Strain and Expression Vector Libraries to Enhance Protein Expression

- *P. fluorescens* strain MB101 genome sequenced
  - 6.5 mega base genome > 6,300 genes (1.4 X *E. coli*)
- Targets for strain engineering identified
  - Functional annotations
  - RNA-seq, transcription array, proteome analysis
- Toolbox components generated for building expression strains
  - Targeted chromosomal gene deletions/insertions
  - Over-expression of helper proteins
  - Multiple inducible promoters
  - Multiple rates of translation initiation (RBS sequences)
  - Multiple signal peptides (recombinant protein translocation)
  - Fusion Partners



Pfenex Expression Technology®

Gene Expression Screening Maximizes Combination of Tools

Toolbox components combined to produce thousands of unique expression strains constructed and screened in parallel

- Codon Optimized for *P. fluorescens* → ***Genes***
- X
- 2 Plasmids
- 4 Transcriptional Promoters
- 3 RBS Sites/ Translation Initiation Strengths
- 53 *P. f.* Native Signal Peptides

Expression Vectors

- X
- 60 Folding Helper Overexpression Host Strains
- 150 Protease Deletion Host Strains

Host Strain Phenotypes

Pfenex Platform Technology

Expression Toolbox

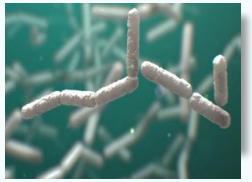
- Toolbox components combined to produce thousands of unique expression strains constructed and screened in parallel using automated workflows
- Speed, Quality and Yield create significant advantages in real opportunity costs

Automation-enabled HTP Strain Construction, Expression and Analysis

HTP STRAIN CONSTRUCTION

plasmids

+



host strains

+

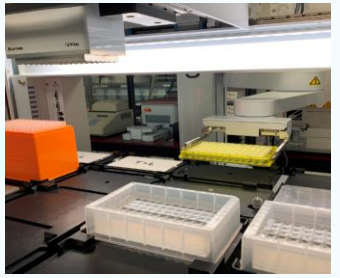
electroporate and grow transformants in selective media (Seed Cultures)

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

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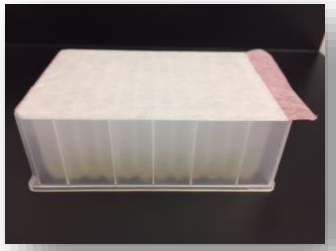
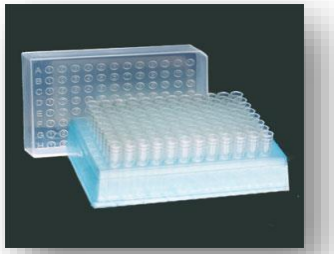
High Throughput Analytical Workflow

Challenge: Accurately analyze target in culture lysate



HTP growth plate
2 ml, 96 deep well



Sonicate sample plate
2 ml, 96-well

QUANTITY ADDRESSED

1st-tier analysis

SAMPLES: 1000s

Measure target mass yield

HTP SDS-CGE
30 min per plate

HTP BLI
90 min per plate

Requires binding partner

BLI allows both quantity and quality measurements in crude cell extracts

QUALITY ADDRESSED

2nd-tier analysis

SAMPLES: 10-100s

Measure Fragments, Aggregates, etc.

MS

HPLC

+ Western blot and other methods

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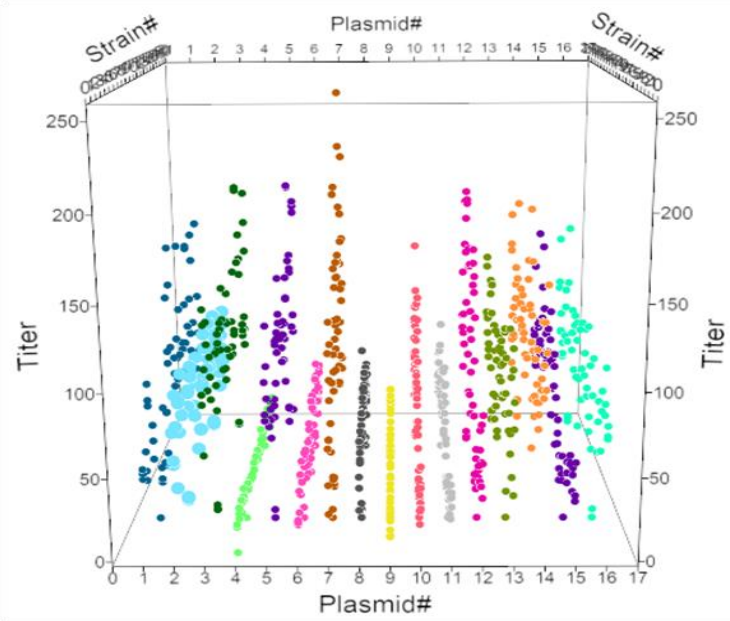
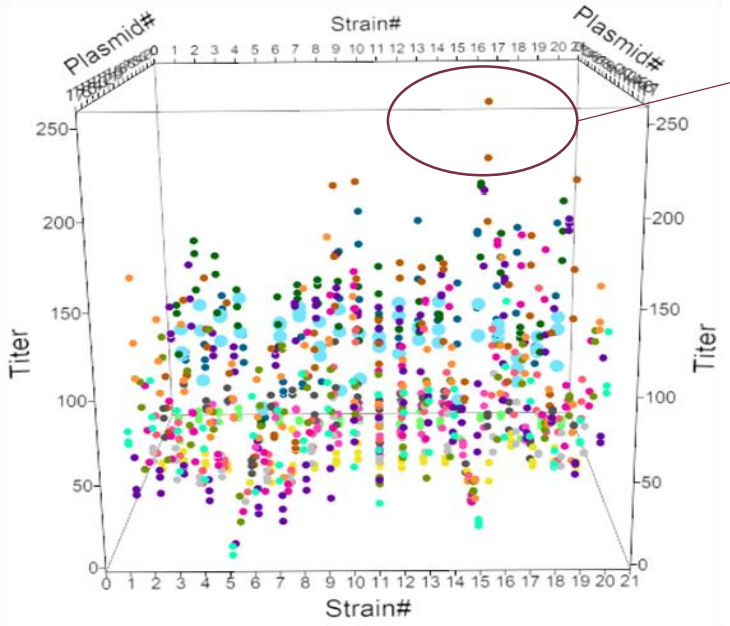
Expression Space – Plasmid & Host Strain Interaction

Plot of SDS-CGE estimated titers of Fab fragment produced in Pfenex 96-well HTP cultures





Goal: high titers of soluble, active protein

Pfenex explores a much larger experimental design space, defined by interactions between expression strategy and host phenotype

- Showing the optimal plasmid expression strategy
- Showing the optimal host strain phenotypes

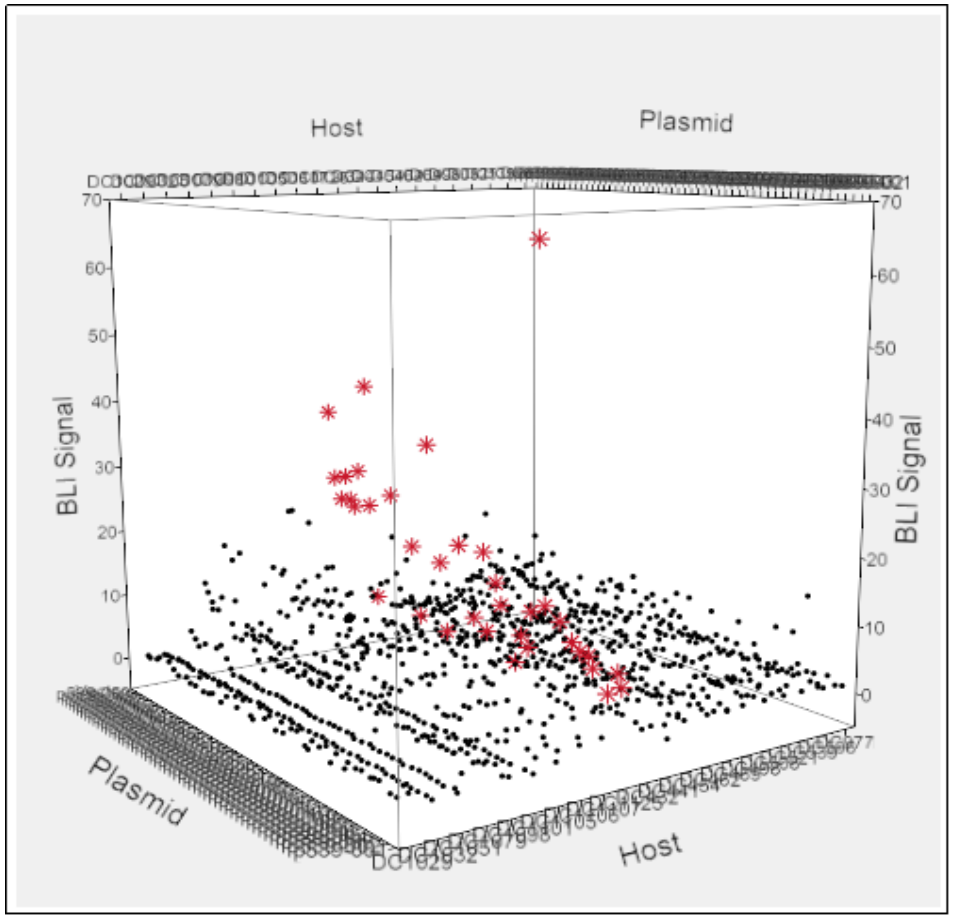
Pfenex Platform Technology

Expression Space – Plasmid & Host Strain Interaction

Power of the Platform:
Identifying the Best Expression Strategy

- In the majority of cases the parallel processing approach yields numerous strains producing at least some protein
- In the illustrated case, significant expression was achieved:
  - *Host strain: over-expression of a particular protein folding modulator
  - Plasmid: significant titer with one particular promoter, RBS and secretion leader combination

Strains vs. Active Binding Signal, BLI



1,000 strain screen

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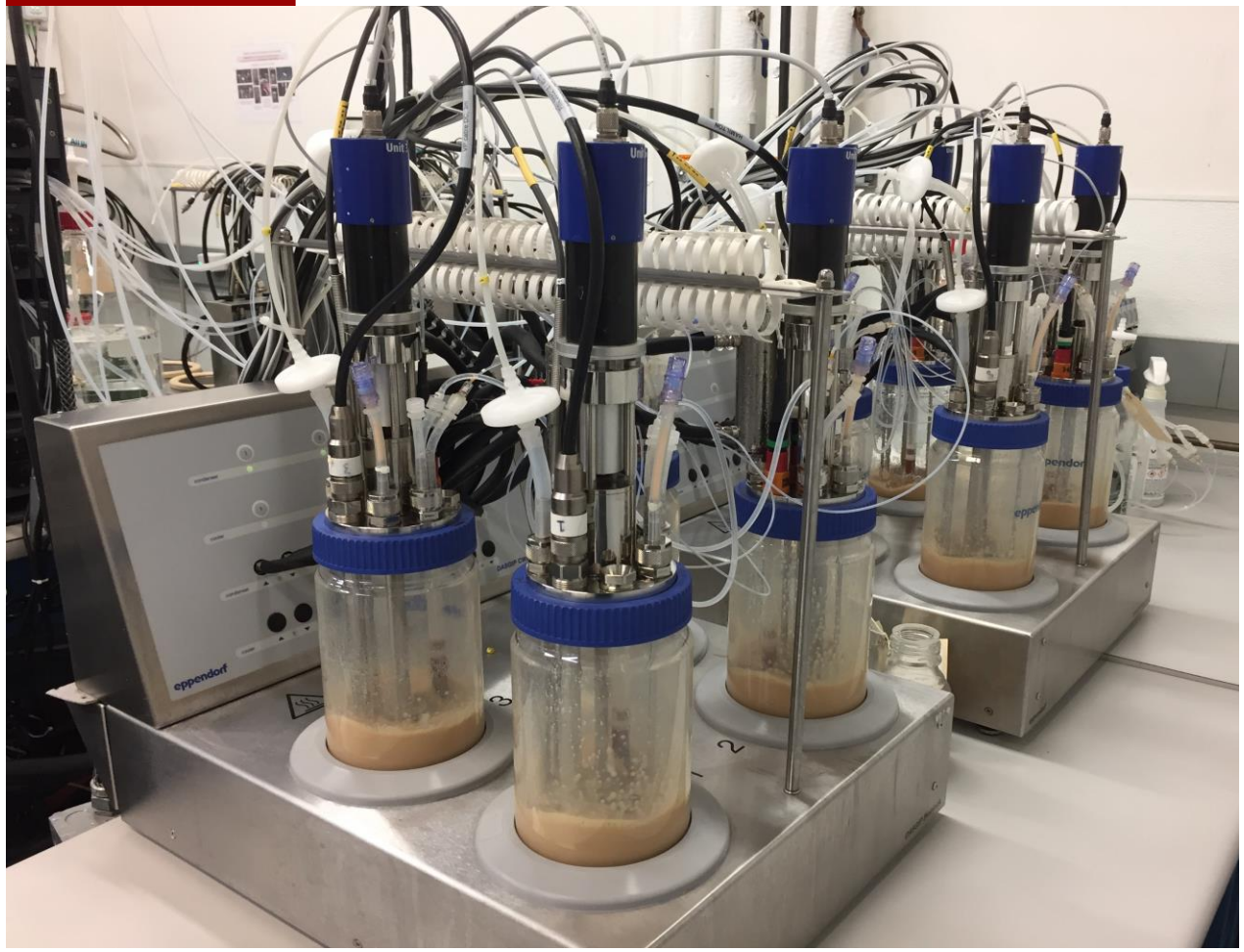
Small-scale Fermentation Assessment

- 5-10 strains advanced
- Up to 9 variable induction conditions screened using DoE approach

  Typical factors tested
  - Temp.
  - pH
  - Inducer level
  - Wet cell weight
- Selection of robust production strain for further development

24 x 2L units



Case Study:
Fusion Partners for Peptide Expression

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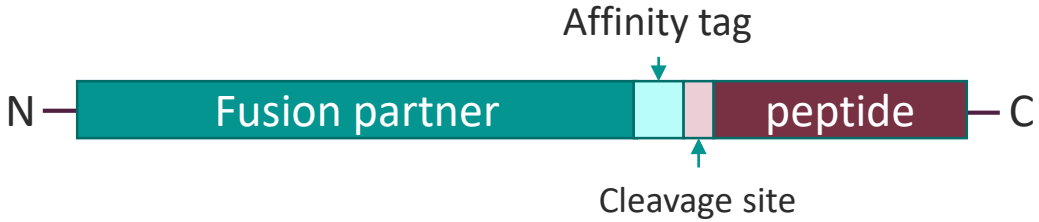
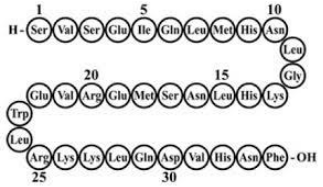
Fusion Partner for Peptide Expression

Small highly expressed proteins (native to *P. fluorescens*) identified and screened as fusion partners to enable peptide production

- DnaJ-like chaperone 9.2 kD
- FklB PPIase: 21.8 kD
- FrnE PPIase: 23.9 kD
- EcpD chaperone: 28.5 kD

Typical Fusion Protein Construct

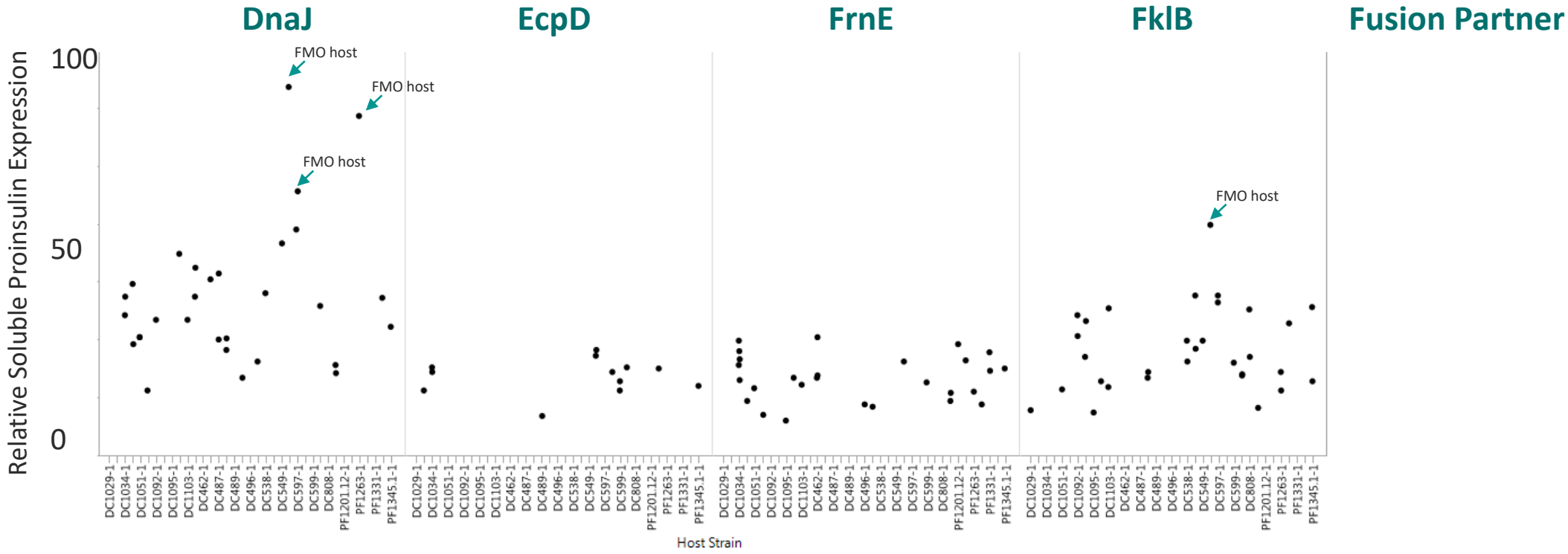


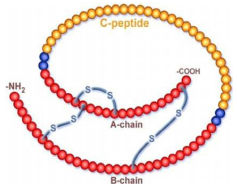


Example:
PTH 1-34 peptide

Fusion Partner Screen

Fusion Partner- Proinsulin Expression (0.5mL scale)



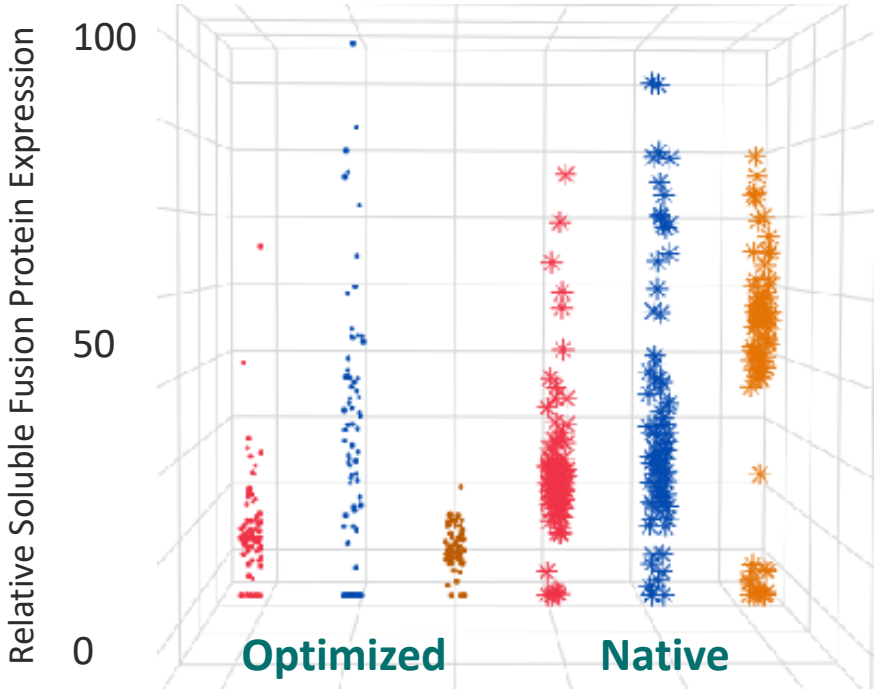


Pro-insulin

- Relative soluble titer (SDS-CGE) normalized for proinsulin expression
- Expression level influenced by fusion partner and host strain
- DNAJ-like fusion partner production strains exhibit highest soluble titer
- Selected folding modulators enhance soluble expression

Fusion Partner Screen

Teriparatide Expression



Each dot represents a unique production strain construct

Red- DNAJ-like fusion partner
Blue: FklB fusion partner
Orange: FrnE fusion partner

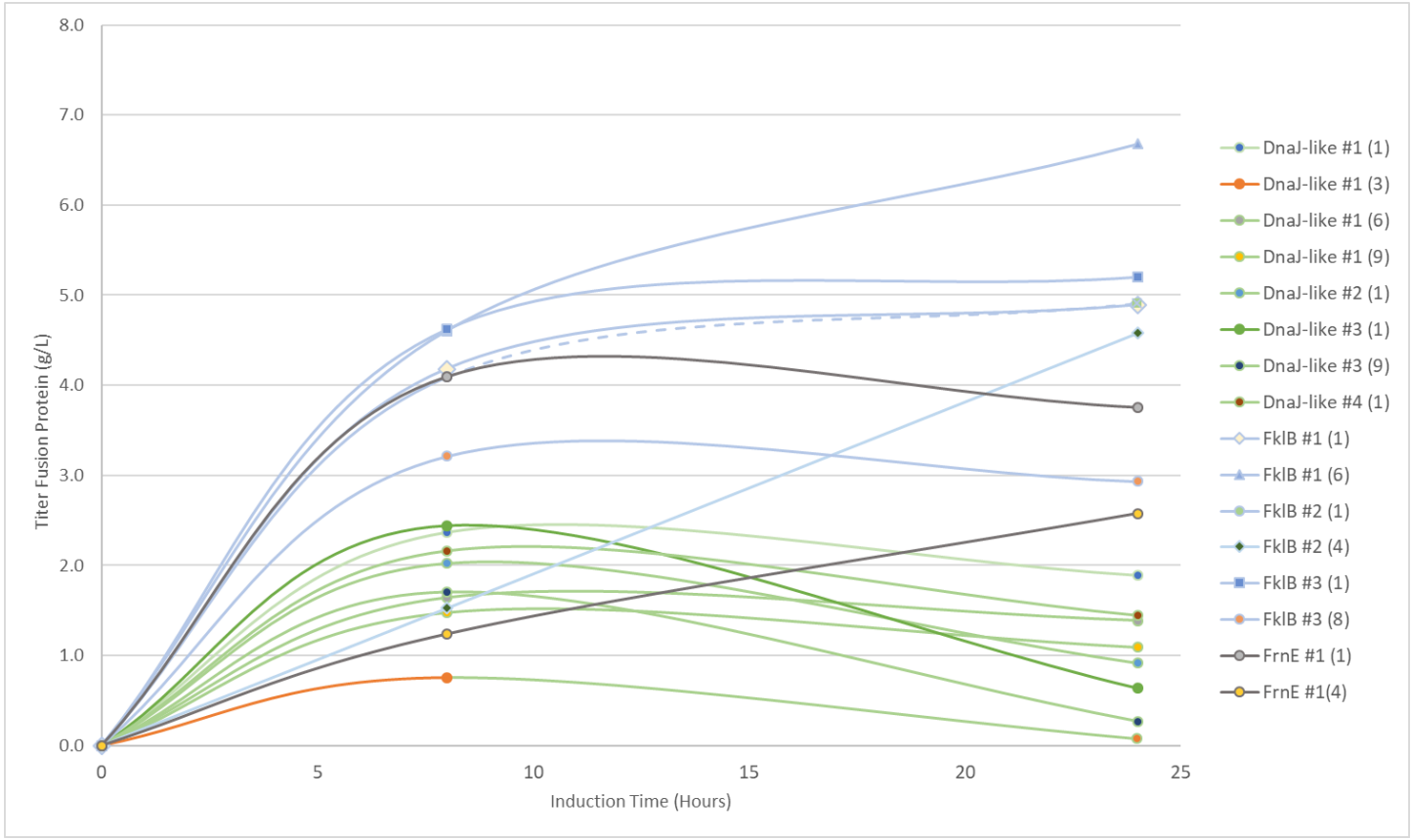
- Expression from constructs with native fusion partner sequence work as well as or better than sequences optimized with peptide fused
- Host strains influence fusion protein expression at 0.5mL scale
- Each fusion partner (native DNA sequence) shows potential for high level expression
- DNAJ-like protein favored due to smaller fusion partner size

Fusion Partner Screen

Teriparatide Expression

10 strains advanced to fermentation assessment (2L scale) under variable fermentation conditions

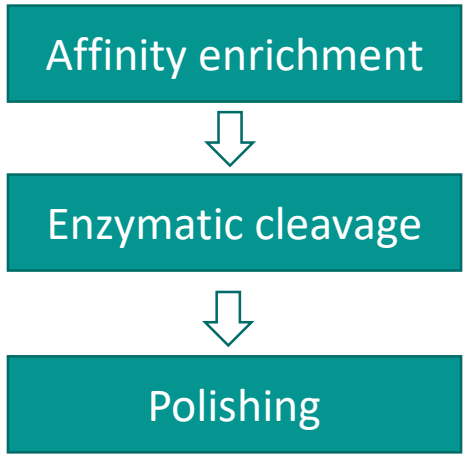
- DNAJ-like protein fusion strains (green lines) appear to undergo degradation between 8-24 hrs. post induction
- Highest titers observed from FlkB fusion protein production strains (blue lines) at > 6g/L (non-optimized fermentation process)



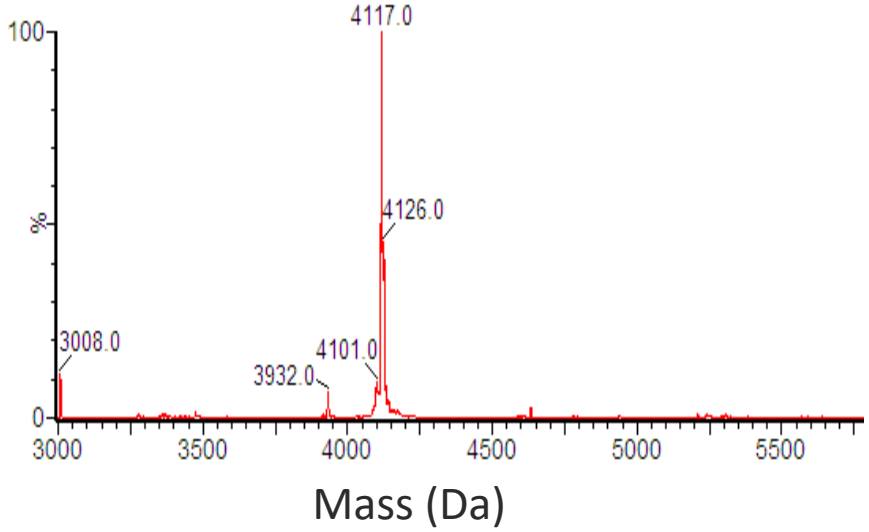
Legend = Strain (fermentation condition)

Fusion Partner Screen

Peptide Recovered from Expression Fusion Protein



Intact Mass Analysis- Enriched Teriparatide

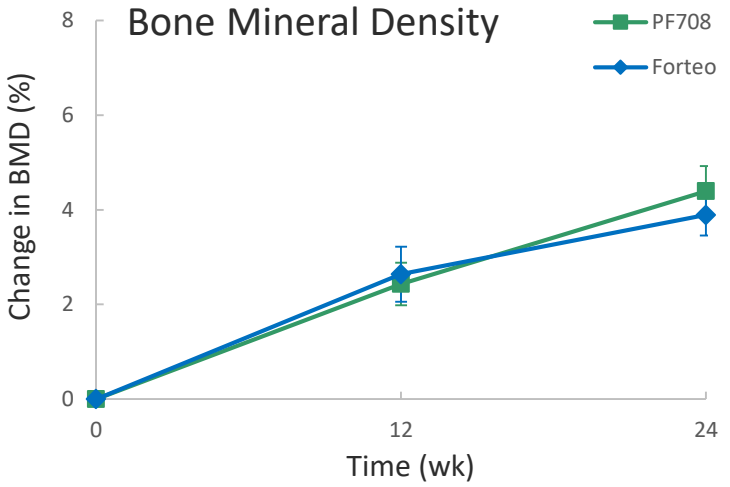


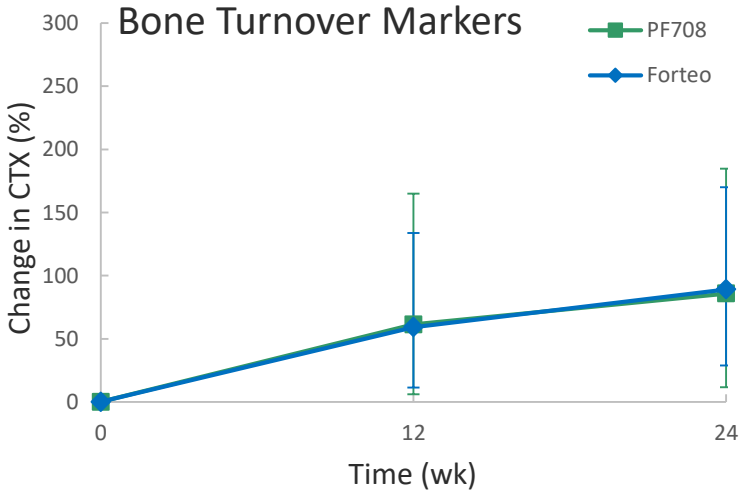
- ✓ Peptide easily recovered from expressed fusion protein
- ✓ FklB fusion protein production strain advanced to process development

PF708 (teriparatide) Clinical

Positive Clinical Results

- Phase 3 (PF708-301) study comparing PF708 and Forteo in 181 osteoporosis patients demonstrated comparable overall profiles across multiple endpoints:
  - No imbalances in severity or incidence of adverse events
  - No clinically or statistically significant differences in immunogenicity, bone mineral density and bone turnover markers
- Received FDA marketing authorization Oct 2019
- Positive Human Factors testing showed the non-inferiority (no worse than) of the user interface of PF708 to Eli Lilly's Forteo





Case Study: Crisantaspase Expression

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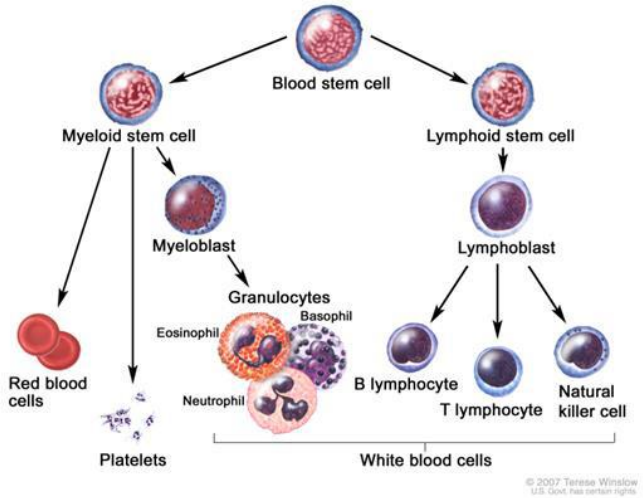
Crisantaspase Expression

Jazz Pharmaceuticals/Pfenex partnership for treatment of Acute Lymphoblastic Leukemia (ALL)

- Production of Crisantaspase (*Erwinia* derived L-asparaginase) in *P.f.* – a key drug for ALL patients with hypersensitivity to Oncaspar® – to alleviate chronic supply shortage of currently commercialized product
- In 2016 Pfenex granted Jazz worldwide rights to develop and commercialize multiple hematology/oncology products
  - PF743 recombinant crisantaspase
    - Jazz successfully completed Phase 1 study
    - Pivotal single arm P2/3 study to be initiated by Jazz in H2 2019
  - PF745 recombinant crisantaspase with a half-life extension technology
- Initial crisantaspase expression feasibility study targeted titer > 250 mg/L
  - Completion of feasibility delivered > 20 g/L titer using an unoptimized process at 2L scale



Acute Lymphoblastic Leukemia (ALL) is a malignant cancer of the bone marrow and blood resulting in an abnormal number of immature white blood cells

- 6,000 patients diagnosed each year in the US, half of which are children under 14 years of age
- Most common form of pediatric cancer
- L-asparaginase depletes asparagine in blood

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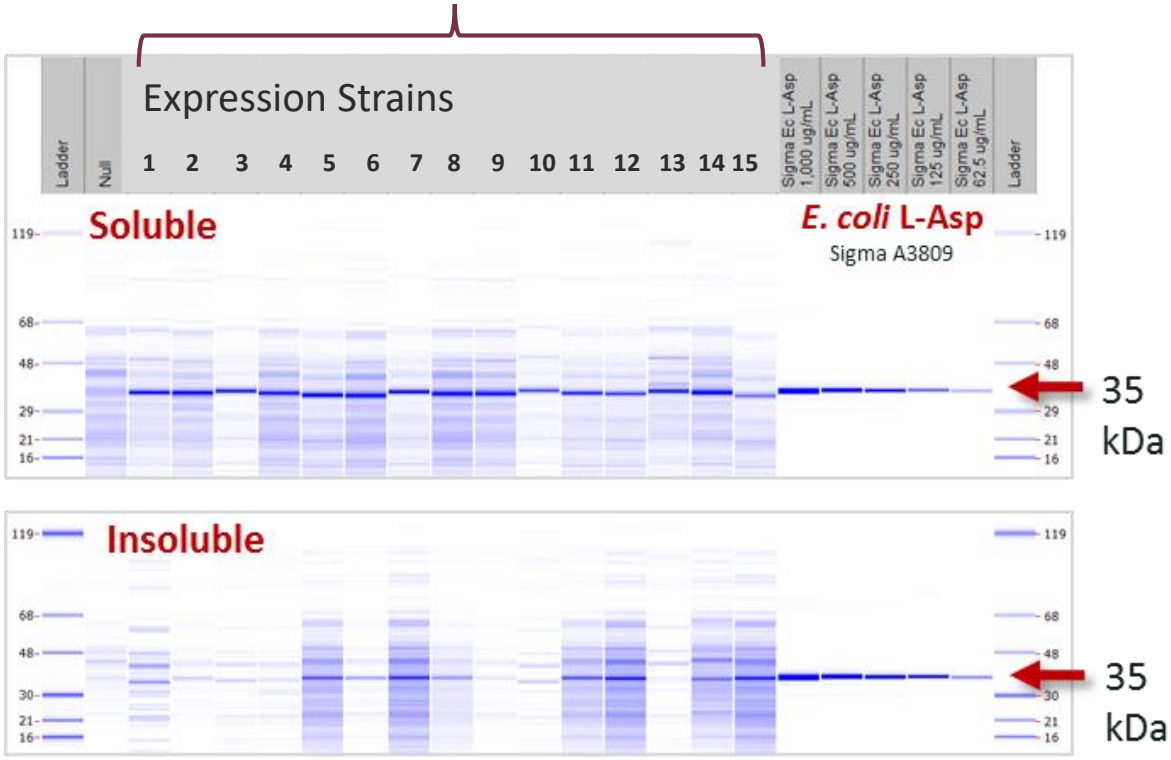
Crisantaspase Expression

- HTP 96-well strain screening identified strains producing active crisantaspase (or L-asparaginase) greater than 1 g/L soluble monomer was observed
- Construction of *P. fluorescens* host strains deficient in native L-asparaginase completed (chromosomal KOs)
- 5 total strains, representing a diversity of expression strategies, scaled to 2L fermentations for evaluation under multiple induction conditions
  - 4 periplasmic and 1 cytoplasmic localization strains

15 unique crisantaspase expression strains identified at 96-well scale screening



SDS-CGE Gel-like images, 3X diluted cultures

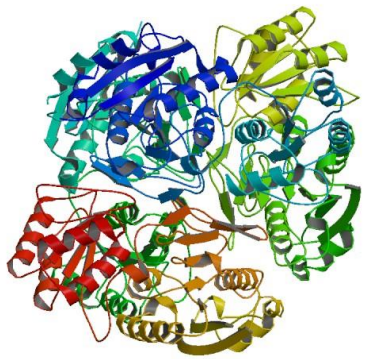


Image Source: Drugbank.ca

Tetrameric asparaginase *Erwinia chrysanthemi*

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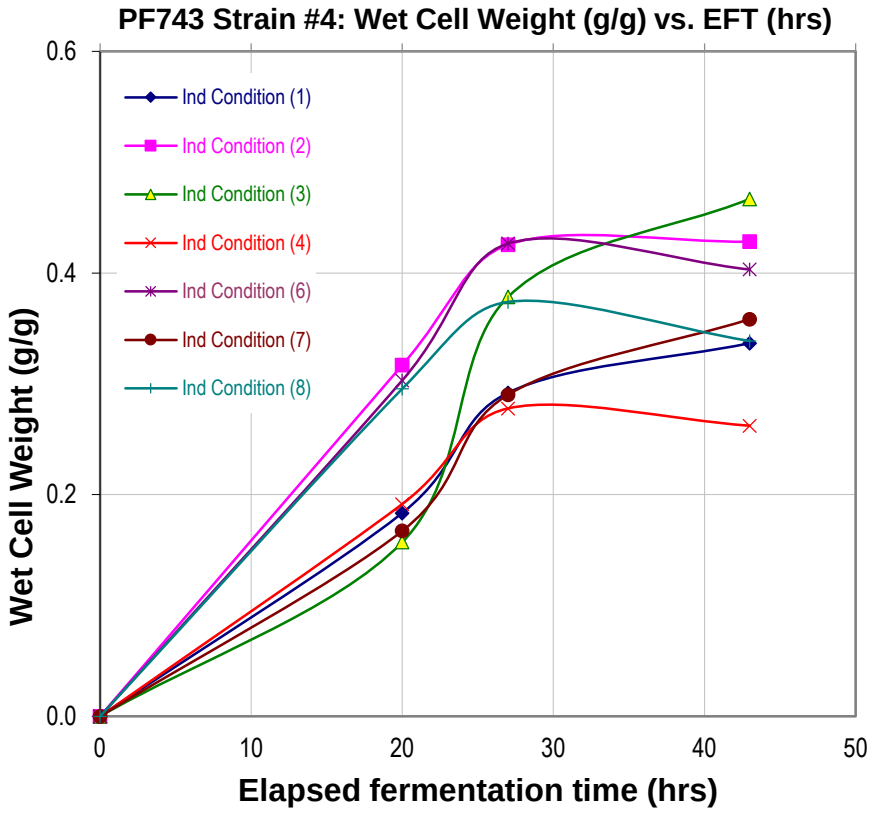
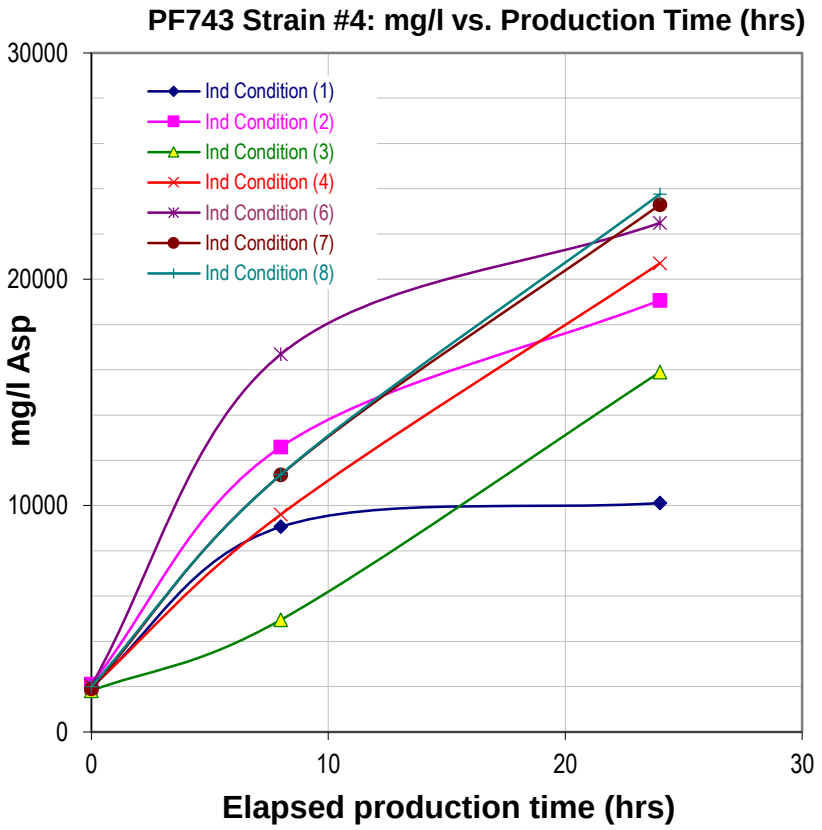
Crisantaspase Expression

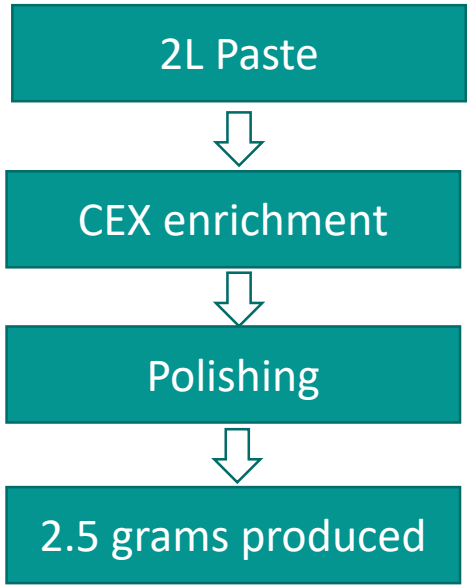
- Cytoplasmically localized PF743 (Strain #4) showed highest overall soluble titers of monomer ranging from 19 to 23 mg/L
- PF743 Strain #4 was cultivated under 8 different induction conditions (factors: wet cell weight at induction, IPTG concentration, pH, temperature)





Pfenex Expression Technology

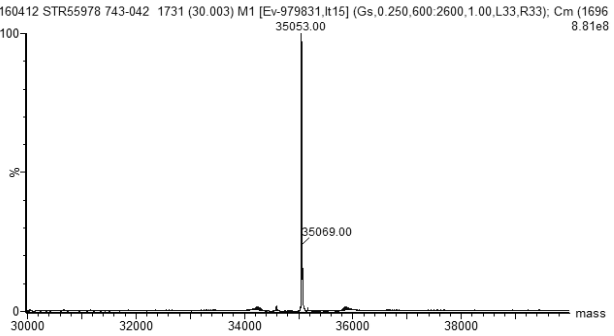
Crisantaspase Expression



Methods used for evaluation:

I. Activity using Nessler-based method
II. Tetramer by SEC
III. Western/SDS-PAGE
IV. Expected mass by LC-MS
V. Purity by RP-HPLC
VI. CD/IF

LC-MS: Intact Mass



- ✓ Small-scale expression and purification of crisantaspase using the Pfenex Expression Technology successfully demonstrated
- ✓ Greater than 2.5 grams of *P. fluorescens* produced crisantaspase meeting initial purity and potency targets was produced
- ✓ Chosen production strain moved forward into process development

Pfenex Expression Technology™ Value Proposition

Speed of Development – Opportunity Cost Avoidance

- Extensive expression toolbox: expression strategies and host strains
- Robust high throughput methods developed to rapidly identify optimal strains
- Rapid fermentation optimization and reliable scale-up to thousands of liters
- More leads through development, allowing more and faster "go, no go" decisions
- Success rates >80%, after previous failure in other expression systems on over 174 lead proteins

Product Quality

- Correct disulfide bonding
- Secretion leader processing fidelity
- Very low product heterogeneity
- No use of antibiotics or animal derived components
- Scalability of production- small scale to commercial quantities

Cost of Goods

- High specific and volumetric expression of target protein
- High cell densities with defined mineral salts medium
- Two-day fermentation process
- Simplified and scalable downstream processing methods, periplasmic release; no unique processing equipment required

Thank you

See the Pfenex platform explainer video at

https://www.pfenex.com/technology/

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