Fusion partners for robust peptide production in *Pseudomonas fluorescens*

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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 Pfenex's product candidates, including future plans to advance, develop, manufacture and commercialize its product candidates, including the expected commercial strategy for PF708 depending on type of FDA approval; potential market opportunities for Pfenex's product candidates including PF708, PF582, PF529, PF690, and Px563L/RPA563; the potential FDA approval of the NDA for PF708, and the earliest potential commercial US launch of PF708 in the fourth quarter of 2019; Pfenex's 2019 focus; the expected patent expiration timelines and strategies for Forteo, Lucentis, 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; expected milestones for Px563L/RPA563, including the next option periods potentially triggered for cGMP manufacturing and Phase 1/2b study; the potential size of the market and potential government demand for a procurement contract for Px563L/RPA563; expectations with regard to future milestone, royalty, and other payments from Pfenex's collaborations with Jazz Pharmaceuticals, Alvogen, NT Pharma, Merck, SII and other third parties; Pfenex's expectations to continue advancement of PF743 and PF745 with Jazz Pharmaceuticals; expectations with respect to Pfenex's ability to receive future payments under its government contracts; Pfenex's expectations with respect to the initiation of the World Health Organization prequalification process for certain of its collaborations with Merck and SII; Pfenex's expectations regarding the use of abbreviated regulatory pathways for the approval of its product candidates, including use of the 505(b)(2) regulatory pathway for PF708 and the 351(k) pathway for PF529; Pfenex's expectations regarding the timing and advancement of clinical trials and the types of future clinical trials for its product candidates; Pfenex's expectations regarding it's well defined IP strategy to support the potential launch of PF708 at market formation; and Pfenex's expectation for potential strategic partnership opportunities to maximize value for advancement of PF582, PF529, and its other product candidates. 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, Px563L/RPA563, and its other product candidates; Pfenex's reliance on its collaboration partners' performance over which Pfenex does not have control; failure to achieve favorable results in clinical trials its product candidates or receive regulatory approval; delays in its clinical trials or in enrollment of patients in its clinical trials; failure to market PF708, Px563L/RPA563, or its other product candidates due to the existence of intellectual property protection owned or controlled by a third party and directed to PF708, Px563L/RPA563, or its other product candidates; PF708, Px563L/RPA563 and its other product candidates may cause serious adverse side effects or have properties that delay or prevent regulatory approval or limit their commercial profile; if approved, 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 received marketing approval for any product candidates, nor has Pfenex launched any products, and there is no certainty that any marketing approvals will be obtained, products launched, or as to the timelines on which they will occur. Further, even if Pfenex obtains marketing approval, Pfenex may be subject to direct legal challenges by the manufacturers of reference products, including Eli Lilly and Co., and Pfenex could be delayed or prevented from launching its product candidates, including PF708, 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 its March 11, 2019 press release announcing results for the year ended December 31, 2018. You should read 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

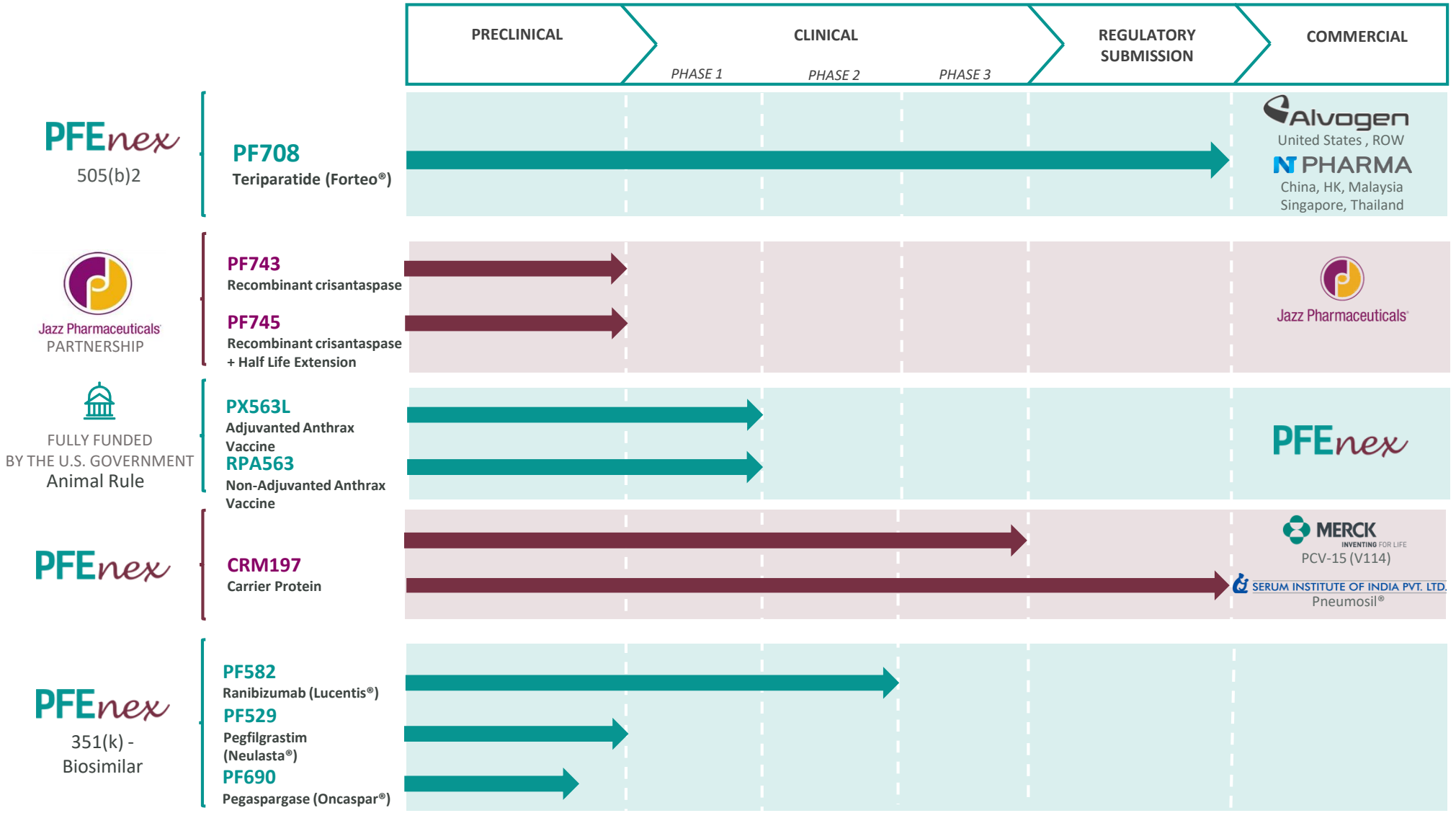
- Pfenex Company/Pipeline Overview
- The Pfenex Platform Technology
  - Toolbox for strain engineering
  - Parallel screening approach
- Case Studies

Pfenex Overview

- Located in San Diego, California USA
- Publicly traded (NYSE American:PFNX) clinical-stage development and licensing biotechnology company focused on leveraging our Pfenex Expression Technology™ to develop and improve protein therapies for unmet patient needs.
- Our proprietary Pfenex Expression Technology™ is leveraged to enable:
  - Robust pipeline of biosimilar and therapeutic equivalent products
  - Product development partnership with Jazz Pharmaceuticals to develop hematology/oncology products
  - Medical counter-measure vaccine collaborations with the US government
  - Supply of cGMP CRM197, a diphtheria toxoid carrier protein used in conjugate vaccines

Products in Development and Pipeline Products (includes partnered products)

Pipeline highlights



Pfenex Expression Technology®

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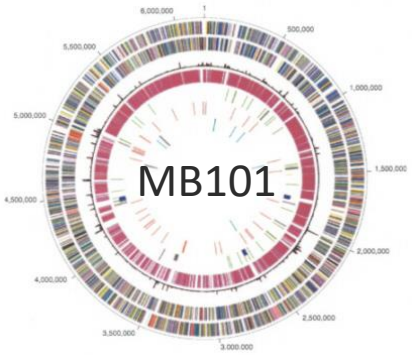
Pfenex Platform Technology

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*)
  - Ongoing bioinformatics annotation and curation
- Targets for strain engineering identified
  - Functional annotations
  - RNA-seq, transcription array, proteome analysis – applied to cultures undergoing target protein expression
- 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 Platform Technology

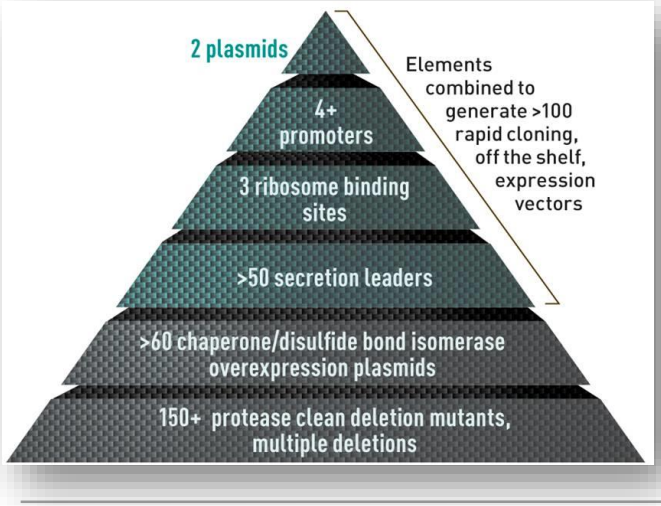
Our approach for microbial strain development:

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

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

Pfenex Platform Technology

Expression Toolbox

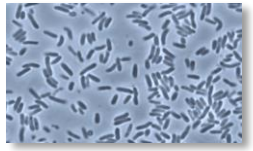


- Toolbox components combined to produced thousands of unique expression strains constructed and screened in parallel using automated workflows
- Speed, Quality and Yield create significant advantages in real opportunity costs

STRAIN CONSTRUCTION

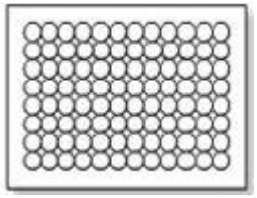
plasmids

+



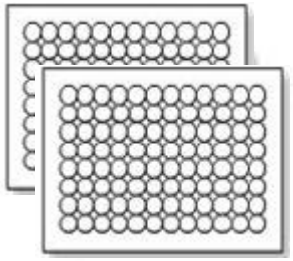
host strains

SEED



electroporate and grow transformants in selective media

HTP EXPRESSION



inoculate seed culture into HTP medium

Replicate cultures grown for 24h prior to induction

Cultures induced and incubated for 24h

HARVEST & ANALYSIS

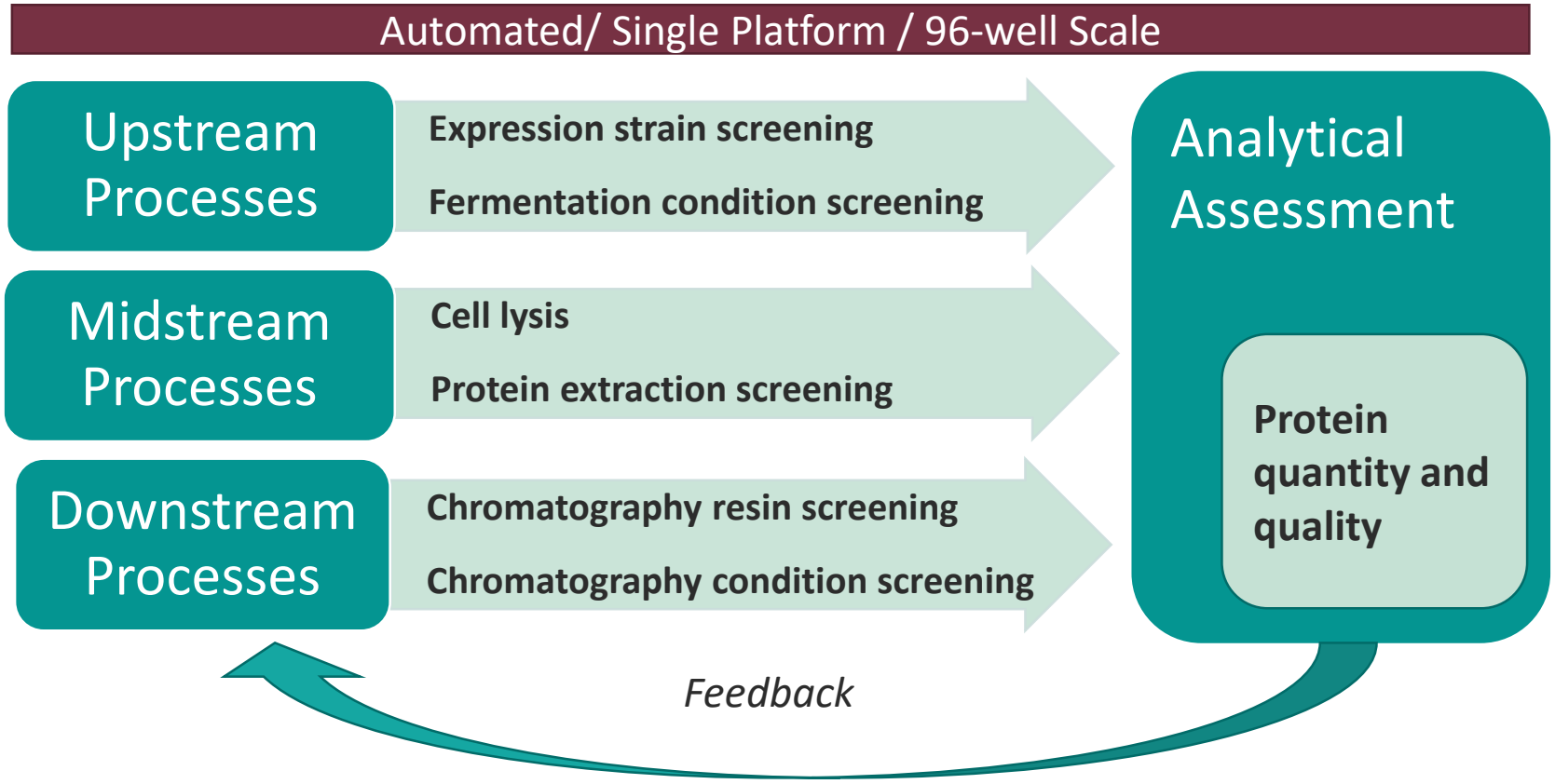
OD_{600} 30-50 typical

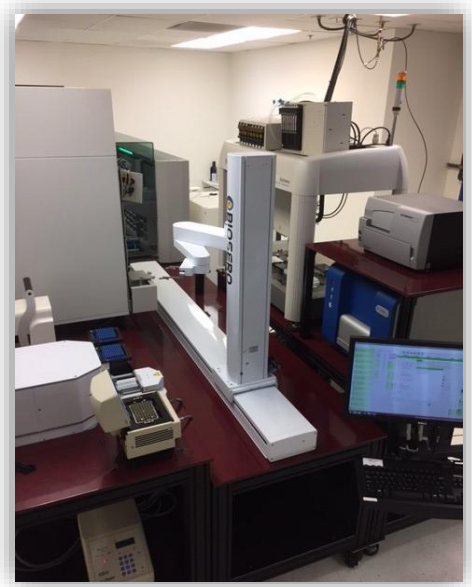
96-well growth and expression

0.5 ml cultures per well

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

Automated Sample Processing and Analysis

Upstream Processes (96-well)

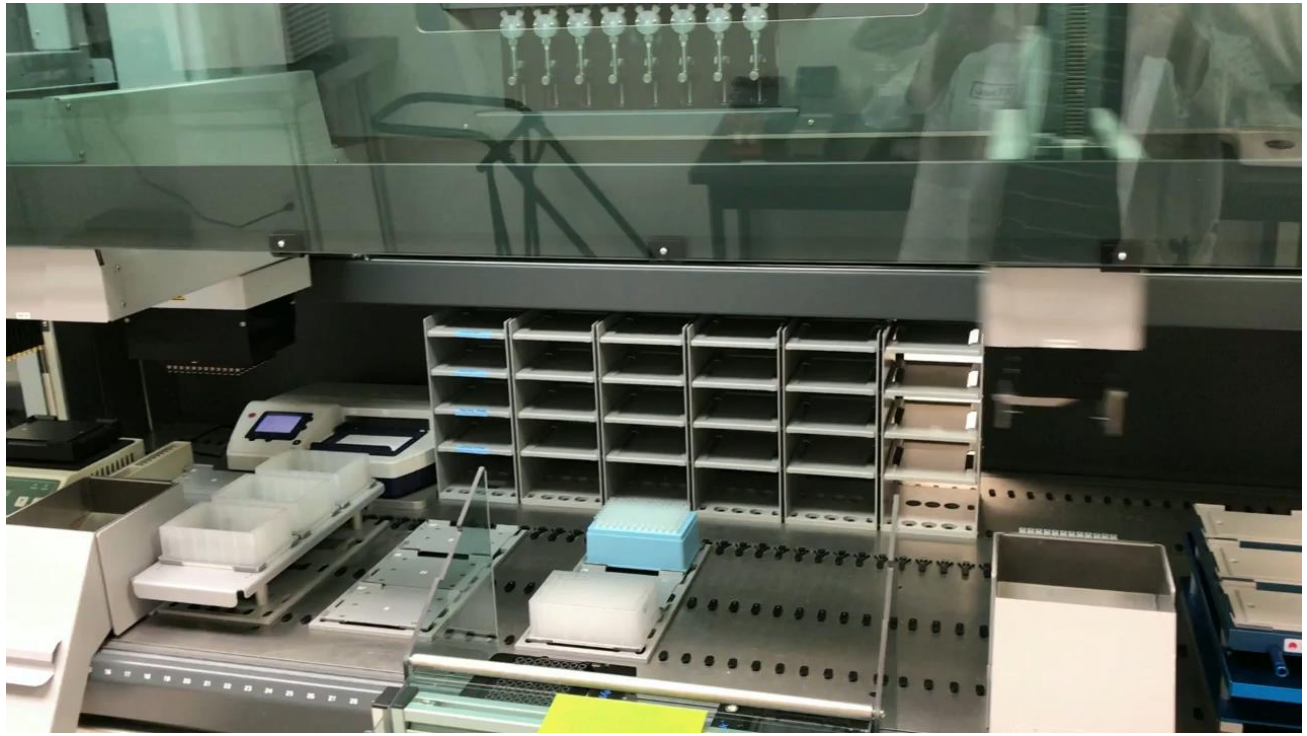
- Electroporation
- Competent cell prep
- Culture liquid transfers
- Glycerol stock prep

Analytical Processes (96-well)

- SDS-CGE
- ELISA assays
- Protein A_{280}
- Culture OD_{600}

Downstream Processes (96-well)

- Resin screening
- Osmotic shock lysis
- Buffer screening and exchange



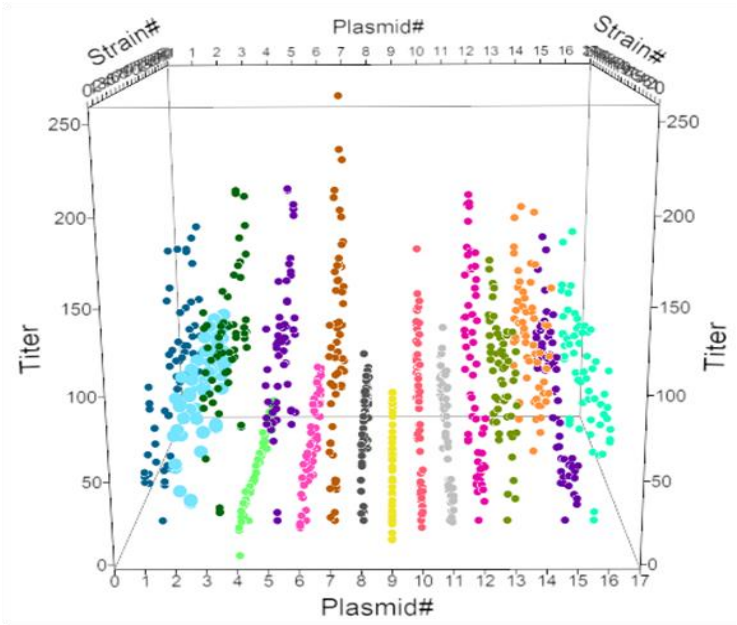
Pfenex Biosero Automation System

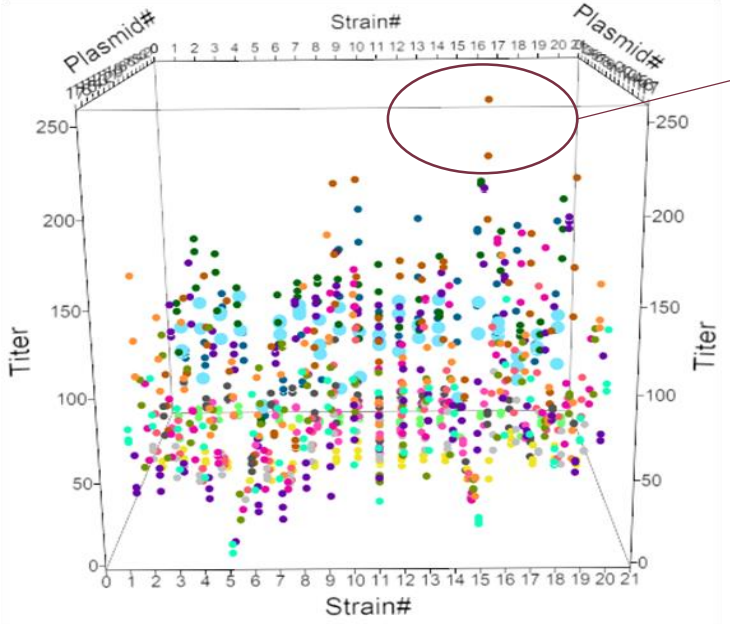
- Green Button Go Control Software (Biosero)
- Flex Robotic Arm (Precise)

Pfenex Platform Technology

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

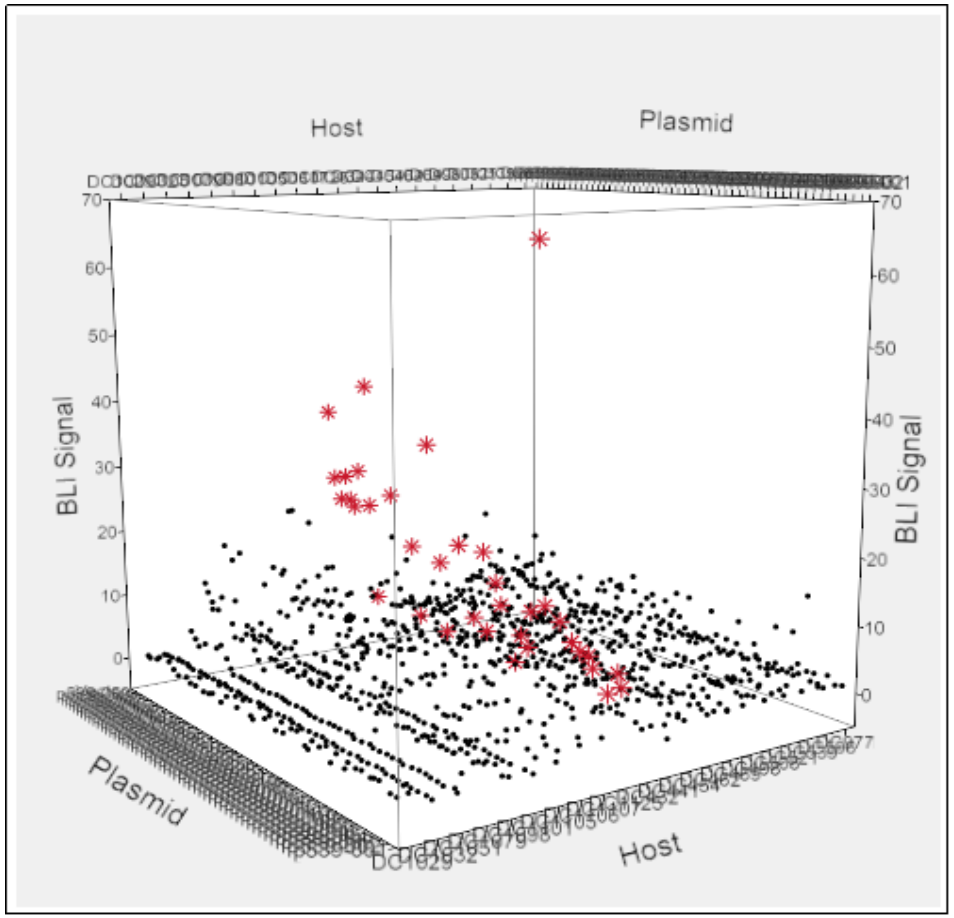
Pfenex Platform Technology

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

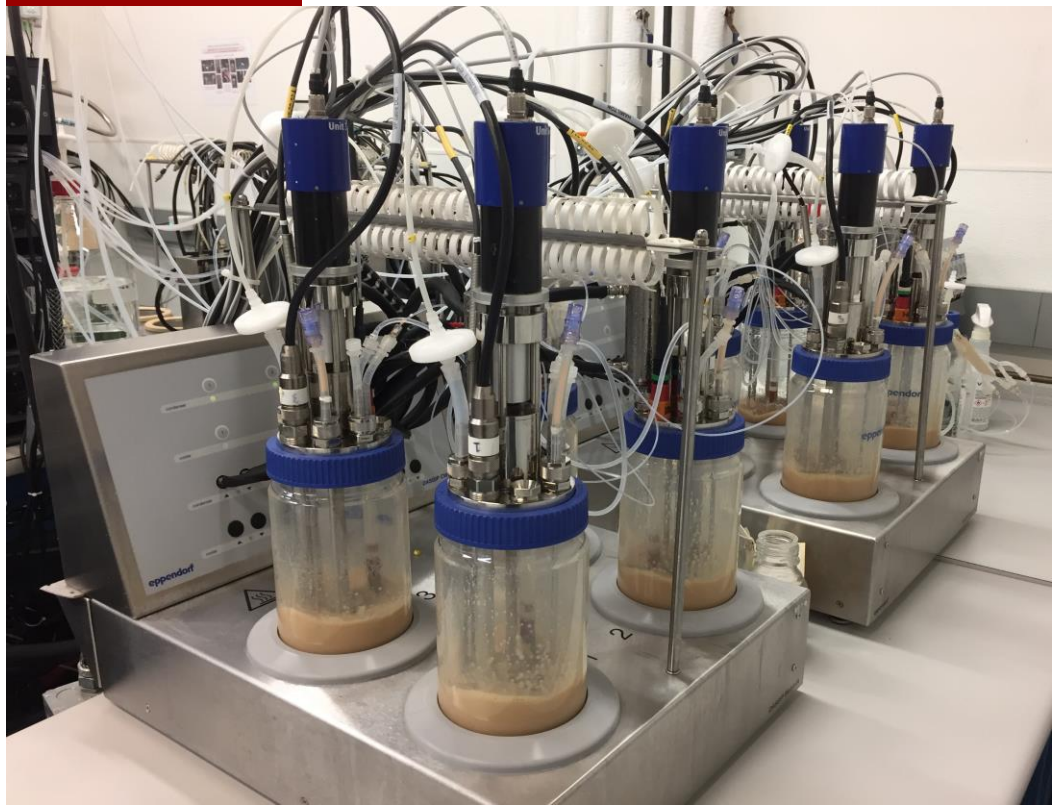
Pfenex Platform Technology

Small-scale Fermentation Assessment

- 5-10 strains advanced
- Variable induction conditions screened using DoE approach

  Typical factors tested
  - Temperature
  - pH
  - Inducer level
  - Cell density
- Selection of robust production strain for further development

24 x 2L units



Case Study: Peptide Production

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Fusion Partner for Peptide expression

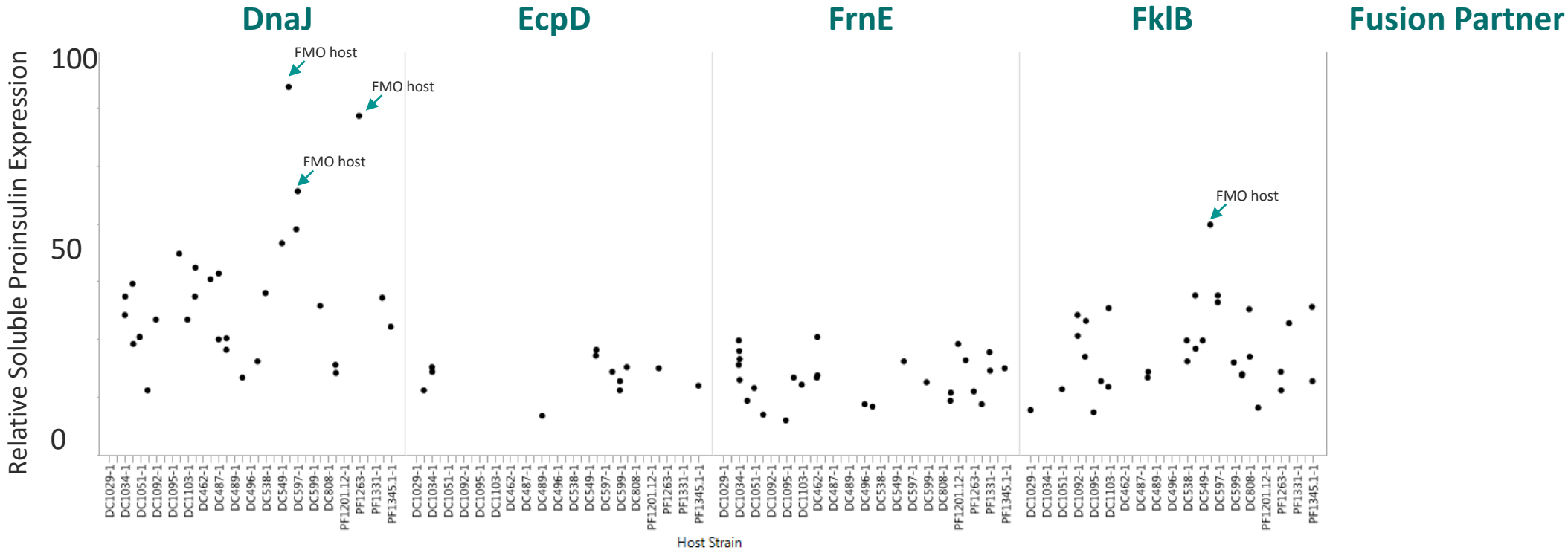
Small proteins (native to *P. fluorescens*) identified and screened as fusion partners to enable peptide production

- 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

Fusion Partner Screen

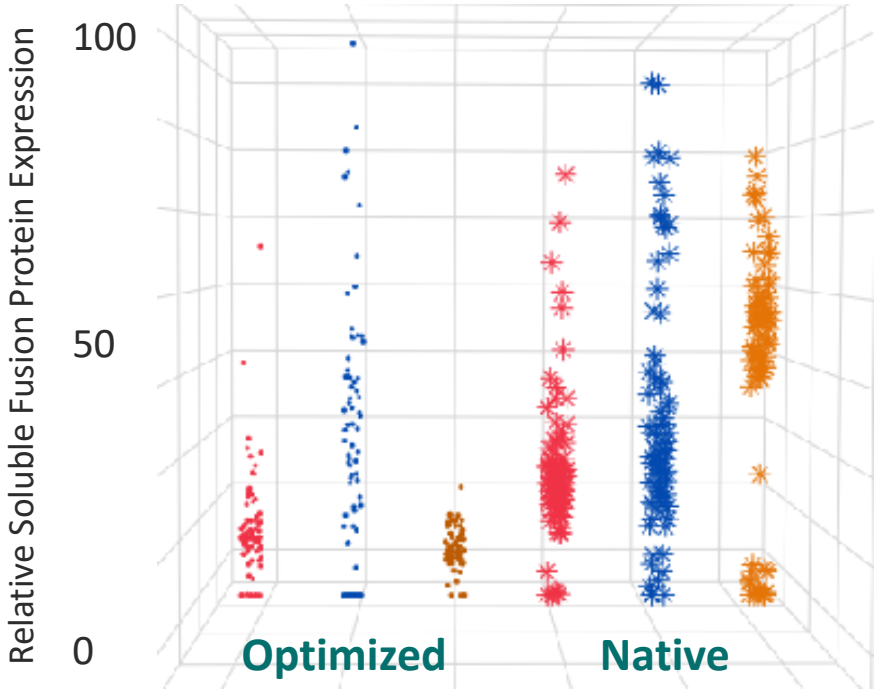
Fusion Partner- Proinsulin Expression (0.5mL scale)



- 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

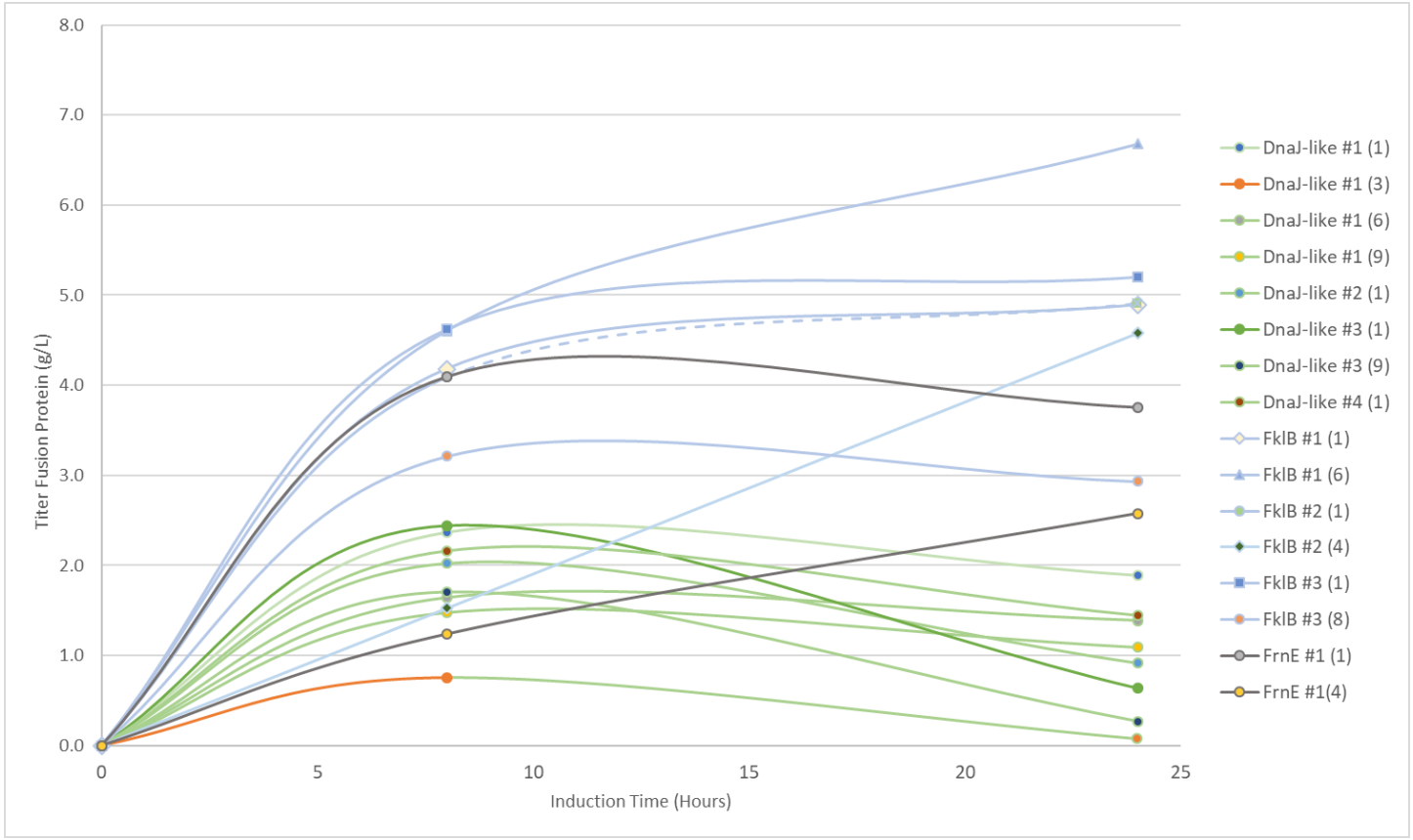
- Expression from constructs with native fusion partner sequence work as well as or better than sequences optimized with peptide fused
- Host strains influence fusion protein expression at 0.5mL scale
- Each fusion partner (native DNA sequence) shows potential for high level expression
- DNAJ-like protein 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 in each of the 4 production strains advanced, under a variety of fermentation conditions
- Highest titers observed from FlkB fusion protein production strains (blue lines) a >6g/L (non-optimized fermentation process)



Legend = Strain (fermentation condition)

Fusion Partner Screen

Peptide Recovered from Expression Fusion Protein

Affinity enrichment

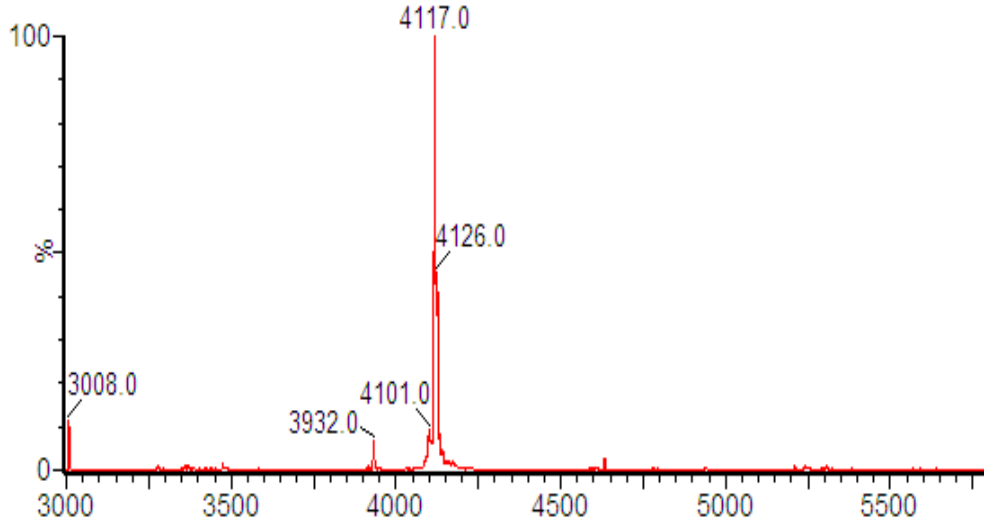
⇩

Enzymatic cleavage

⇩

Polishing

Intact Mass Analysis- Enriched Teriparatide

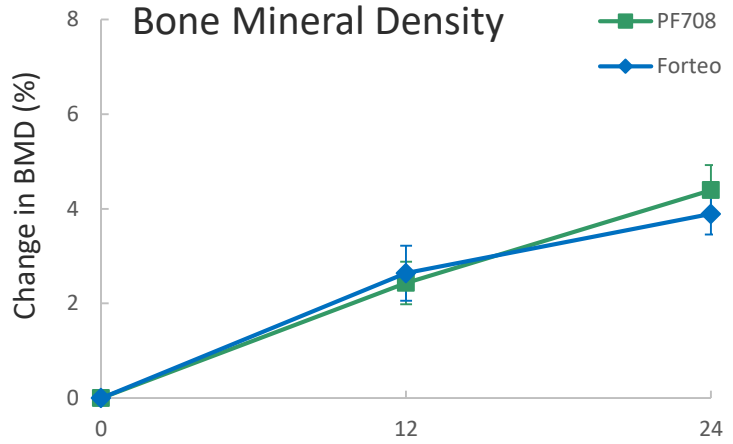


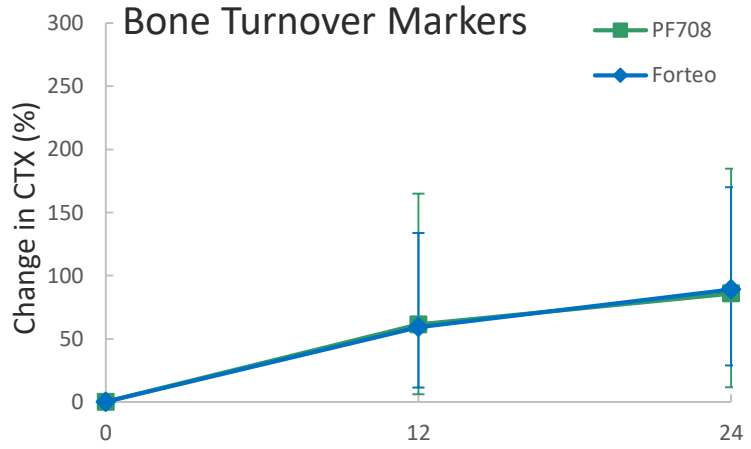
Peptide easily recovered from expressed fusion protein
FklB fusion protein production strain advanced to process development

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
- Phase 1 (PF708-101) study in healthy subjects demonstrated bioequivalence





Summary

- Small *P. fluorescens* proteins (and truncations) serve as robust fusion partners for peptide production
- Optimization of fusion partner gene with peptide coding sequence not needed, which facilitates construction of rapid cloning vectors for fusion partner screening
- Highly productive strains identified through screening fusion partners in combinations with host strain array
- Use of engineered affinity tag and specific cleavage site enable process development to support clinical and commercial production of active peptide

Acknowledgements

Fusion Partner Development Team

Hongfan Jin

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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 120 programs

Product Quality

- Correct disulfide bonding
- Secretion leader and fusion partner 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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