Engineered Fusion Partners Enabling High Yield Recombinant Peptide Production

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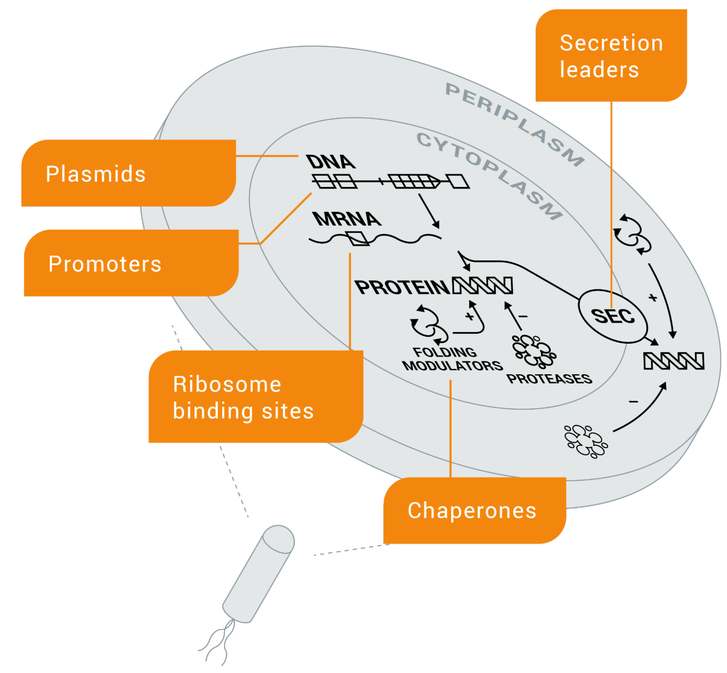
ABSTRACT

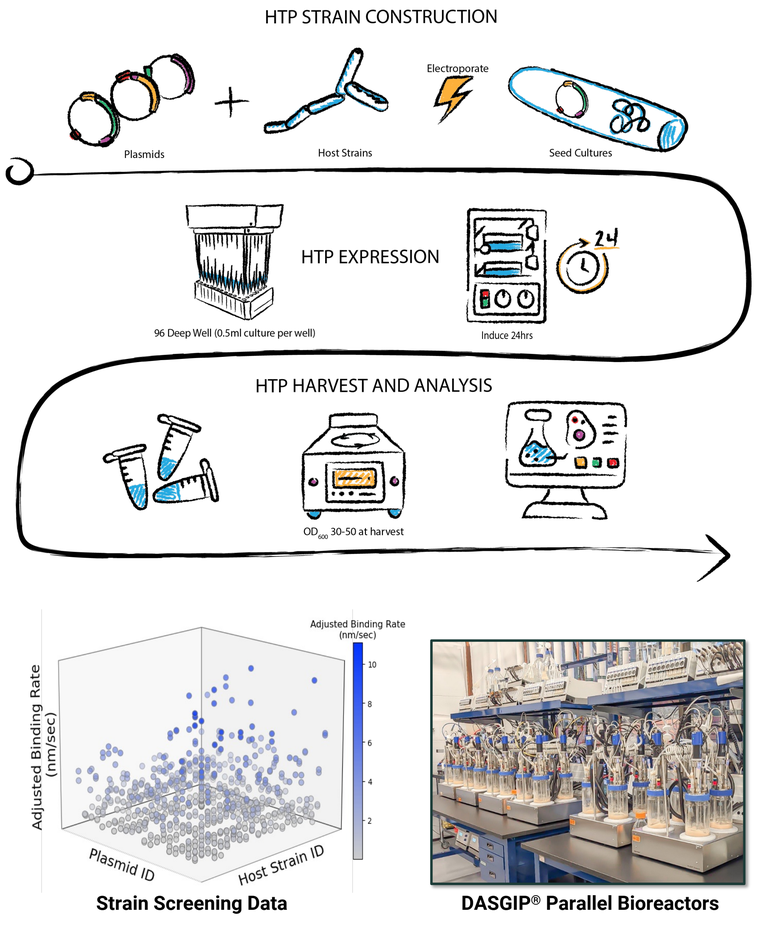
Peptide therapeutics are a fast-growing class of medicines, but traditional solid phase peptide synthesis (SPPS) has limitations in cost, scalability, and environmental impact. Pfenex Expression Technology® (Pfenex) provides an alternative by using a tailored collection of small, highly expressed Pseudomonas fluorescens proteins as fusion partners to enable efficient recombinant peptide production. Potential fusion constructs are evaluated using Pfenex's modular screening tools to maximize expression levels, followed by site specific enzymatic cleavage to liberate the desired peptide. This manufacturing strategy has been clinically proven and commercialized for teriparatide, demonstrating strong yields and regulatory approval. Collectively, the Pfenex platform establishes recombinant production as a viable, sustainable, and competitive approach for large scale peptide manufacturing.

BACKGROUND

High-Throughput Strain Construction, Expression and Analysis



- Bioinformatics and transcriptomics leveraged to develop an extensive toolbox of expression strains and plasmids
- PET toolbox components are combined to produce hundreds or thousands of unique expression strains.



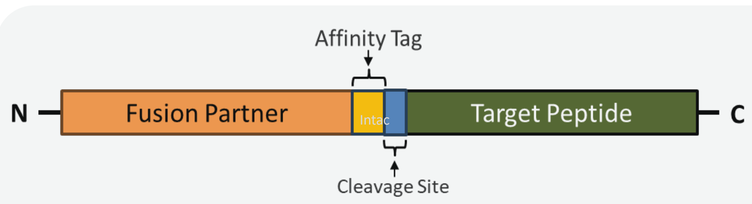
Strain Selection Workflow

- High-throughput screening (96-well, 0.5 mL scale) identified candidate strains producing high titers
- Automated screening evaluated manufacturing strains for titer and solubility prior to 2L scale-up

RESEARCH

Protein Fusion Proteins

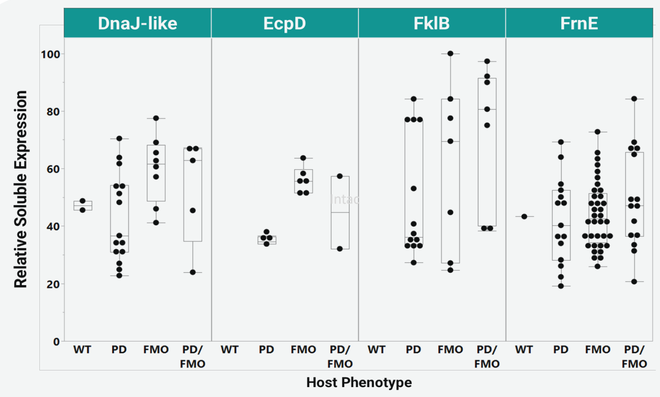
- Four highly expressed proteins from *P. fluorescens* characterized as fusion partners for peptide production
- Engineered linker with affinity tag and recognition site for site specific cleavage



Identified *P.fluorescens* Partners

- DnaJ-like chaperone: 9.2 kD
- EcpD chaperone: 28.5 kD
- FklB PPIase: 21.8 kD
- FrnE PPIase: 23.9 kD
- Additional undisclosed fusion partners

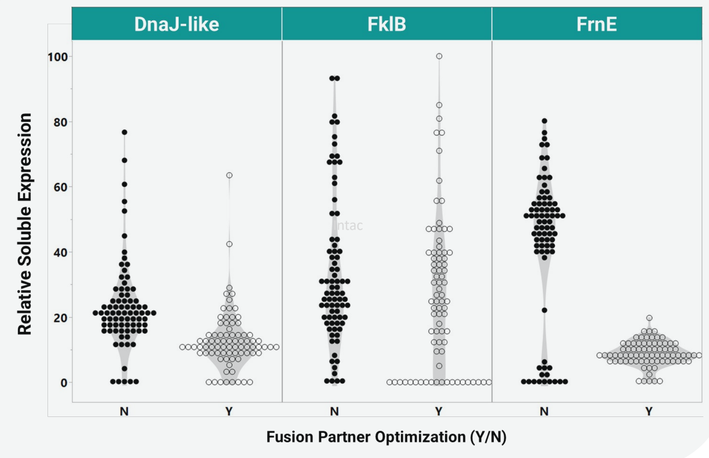
Fusion Protein Partner Screening



- Soluble titer measured by SDS-CGE and normalized to target peptide content
- Host strain selection significantly influenced fusion partner expression levels
- FklB fusion strains achieved highest soluble titers among candidates tested
- Selected folding modulators (FMO) enhance soluble expression

PRODUCT DEVELOPMENT

Teriparatide Fusion Partner Screening at 96 Well Scale



- Native fusion partner coding sequences produced comparable or superior expression relative to codon-optimized constructs
- Each fusion partner construct achieved high soluble expression at the 0.5 mL scale

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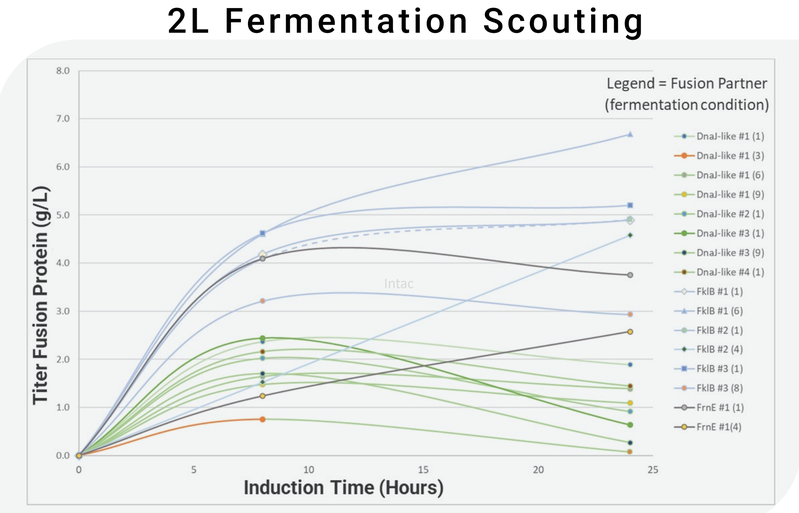
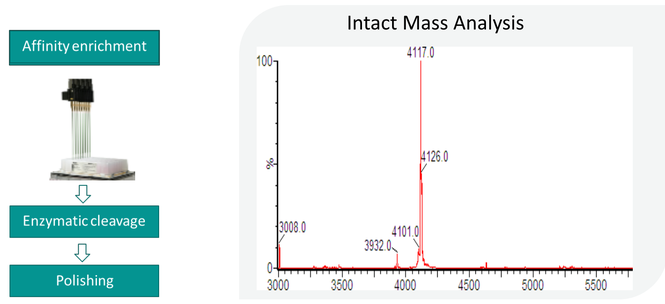
Pfenex Expression Technology®, based on *P. fluorescens*, leveraged native fusion partners to achieve soluble expression titers >6 g/L of recombinant peptide.

2L Fermentation Scouting



- Ten high-expressing strains were evaluated under variable fermentation conditions
- DNAJ-like protein fusion strains (green lines) appear to undergo degradation between 8-24 hrs post induction
- FklB fusion strains achieved highest soluble titers (>6 g/L) under non-optimized fermentation conditions

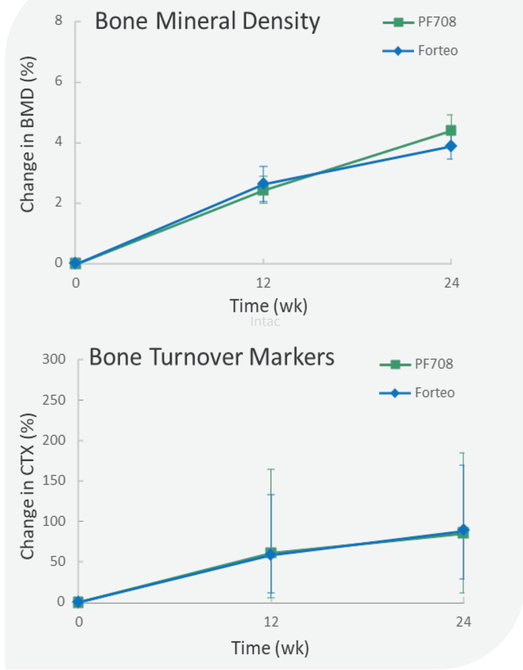
Recovery from Fusion Partner



- Target peptide was recovered from fusion protein with correct quality attributes via site-specific cleavage
- Fusion construct and strain selected for further process development

Clinical Bioequivalence Study

- Bioequivalent PK and PD profiles across multiple clinical endpoints
- No clinically or statistically significant differences in immunogenicity, bone mineral density and bone turnover markers



SUMMARY

Recombinant peptide production using *P. fluorescens* fusion partners achieves soluble expression of >6 g/L with target peptide recovery via site-specific cleavage. Comparative evaluation of three fusion partner constructs identified FklB-based strains as superior performers across variable fermentation conditions. A clinical bioequivalence study of teriparatide with reference product validates this approach as a manufacturing platform. Exclusively licensed to Alvogen, teriparatide was FDA approved in 2019.