Rapid, high titer expression of antibody derivatives using *Pseudomonas fluorescens*

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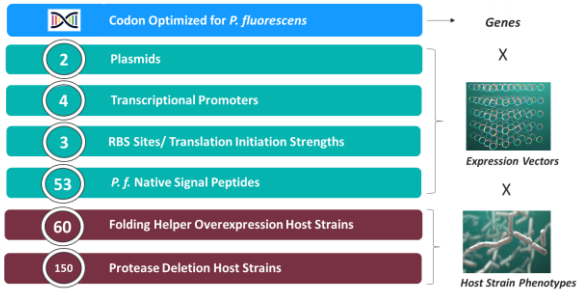
Abstract

While antibodies and their derivatives remain critical scaffolds for novel therapeutic development, scaling production in common hosts like E. coli is often difficult due to their structure and complex disulfide bonding. A Pseudomonas fluorescens expression platform has been developed that overcomes these challenges and enables rapid, high quality expression of therapeutic antibody derivatives. The Pfenex platform leverages a varied toolbox of expression plasmids and a wide array of different production hosts to quickly identify high producing strains. Using automation-enabled high throughput screening workflows, a production strain can be identified and scaled to a fermentation process in as few as 12 weeks. This platform screening process has successfully been used to produce high levels of a range of antibody derivatives, including a trimeric multifunctional antibody derivative, peptibodies, Fabs, and scFvs.

Methods

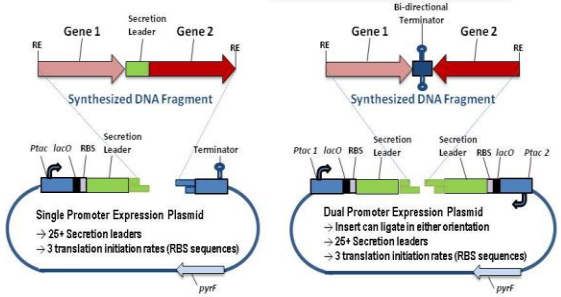
Pfenex *Toolbox*



Toolbox components are combined to produce thousands of unique expression strains to be screened in parallel

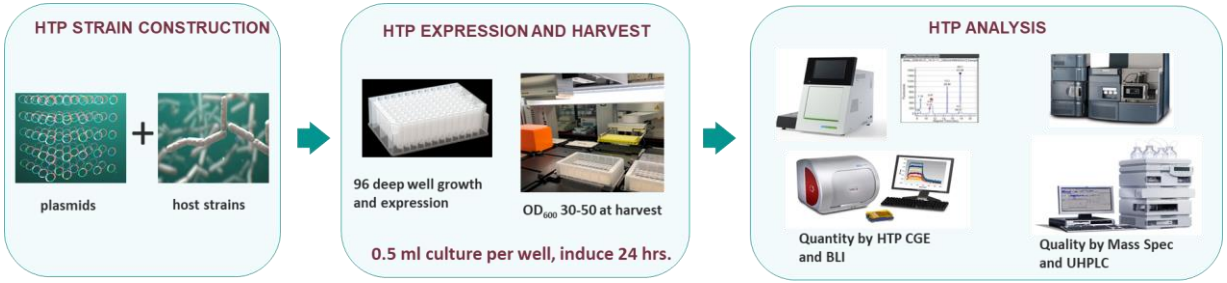
Rapid Cloning Process

Digest and ligate synthesized DNA fragment in single reaction with expression plasmid, and transform directly



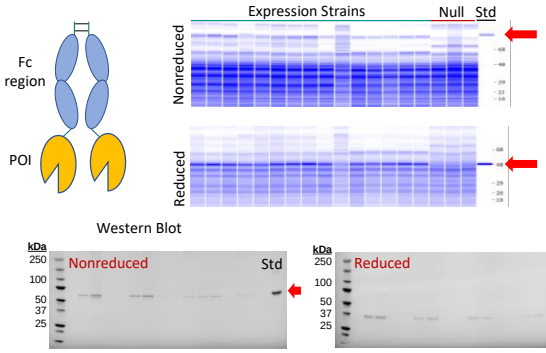
- Gene fragments synthesized; no PCR amplification or sequencing of target required
- Rapid cloning requires use of a single restriction enzyme
- Clone in-frame with different combinations of RBS strengths and periplasmic secretion leaders incorporated into single and dual operon expression plasmids
- Plasmids maintained by *pyrF* complementation

Methods *Automated, High-throughput Strain Screening and Analysis*



Case Studies

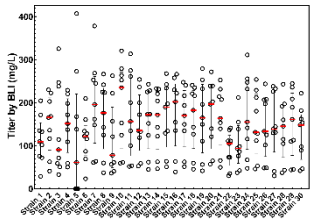
Protein-Fc fusion - Soluble Expression



Optimal strain produced more than 2 g/L of soluble assembled protein-Fc fusion

Trimeric Multifunctional Antibody Derivative Expression

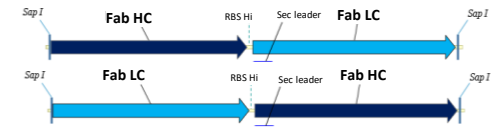
Screening of 270 expression strategies in high-throughput and functional binding assay to determine high producing strains

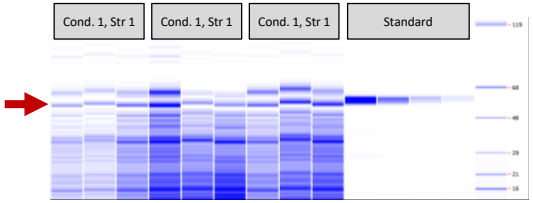


- Soluble active expression of more than 1 g/L achieved without fermentation optimization

FAB Expression and Optimization

Screen variable heavy and light chain expression ratios





Strain screening and fermentation scale-up resulted in over 4 g/L assembled (CGE and MS) and active (BLI) FAB produced

Summary

- Extensive expression toolbox: expression strategies and host strains
- Robust high throughput methods developed to identify best strains quickly to produce active proteins at high titers
- A wide variety of antibody derivatives can be produced using the platform

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