*Pf*ēnex Expression Technology™
Frequently Asked Questions

Why was *Pf*ēnex Expression Technology™ developed?

Answer: We developed *Pf*ēnex Expression Technology to greatly accelerate the biopharmaceutical development process by more rapidly providing higher quality protein, and by increasing the number of protein therapeutics that qualify as being commercially viable through greatly decreasing the costs of production.

What are the advantages of *Pf*ēnex Expression Technology?

Answer: *Pf*ēnex Expression Technology is a robust *Pseudomonas fluorescens*-based platform with high throughput methodology for optimized strain identification for protein production. It is an extraordinarily flexible platform, being used to produce innovative therapeutics and vaccines, research/tool proteins, prospective lead therapeutics and vaccine antigens as well as biosimilars in a variety of therapeutic areas. *Pf*ēnex Expression Technology has also been used successfully as a high throughput screen for discovery based applications, particularly in early stages of hit-to-lead applications and novel antigen and antibody identification. The technology provides a system by which proteins can be produced with the highest speed, quality, and yield, facilitating product development and maximizing the pipeline with proteins in development, and ultimately, into the marketplace. *Pf*ēnex Expression Technology's unprecedented success rate in the expression of high titers of soluble, active protein clearly differentiate this platform from any other available in the industry today and makes a compelling case for selecting ***Pf*ēnex first** for the expression of all protein types.

What is the success rate with *Pf*ēnex Expression Technology and how does this compare with other expression systems?

Answer: When opting for ***Pf*ēnex first**, scientists can be assured that in >95% of cases the protein will be successfully expressed at first screening in an active form thus allowing very rapid progression into pre-clinical or clinical studies speeding a decision on advancing or early termination of lead candidates. This *Pf*ēnex advantage significantly reduces the potential for rework or adding expensive process steps. Expression challenges with *E. coli* have been well documented; hence, using *E.coli* as a first expression option is burdened with a high failure rate. The data suggest when expressing a protein in *E.coli* one can anticipate a 75% failure rate with success being defined as first-time production of high titers of soluble, active protein which can progress a program to the next step in the development value chain. Therefore, in fully 75% of programs one will be required to evaluate a refold step or alternative expression host, consuming additional time and resources, incurring delays in development and resultant

higher COGS. In contrast, the success rate in *Pf*ēnex with complex proteins which previously failed to express in a soluble form in *E. coli* (and in some cases additional expression systems) is 83% in reaching higher titers of soluble and active protein.

What types of biotherapeutics can be expressed using this system?

Answer: A wide variety of aglycosylated proteins can be expressed with *Pf*ēnex Expression Technology, including antibody fragments and derivatives, vaccines, vaccine antigens research/tool proteins, biosimilars, and other complex disulfide-bonded proteins.

How is the *Pf*ēnex Expression Technology bacterium (*Pseudomonas fluorescens*) superior to *E. coli* and yeast for the production of biopharmaceutical proteins?

Answer: In comparison to *E. coli* and other prokaryotic expression platforms, *Pseudomonas fluorescens* uses unique pathways in the metabolism of critical carbon sources. These differences result in reduced production of metabolic byproducts that have been shown to negatively impact cell growth and heterologous protein expression. *Pseudomonas fluorescens* also has a 40% larger genome as compared to *E.coli*, which has allowed us to identify and develop many new protein expression elements, expanding the capability of the platform. As compared to yeast, *P. fluorescens* will not glycosylate proteins, thus ensuring that the protein of interest is free of heterologous glycans as is often found with proteins expressed in yeast systems, and the availability of *Pf*ēnex strains engineered to over-express folding modulators or with deletion of proteolytic activities enhances the quality of expressed proteins. From this foundation, we have invested a significant amount of research and development in creating new expression elements and host strains through the application of sophisticated genomic tools. This expression "toolbox" makes *Pf*ēnex Expression Technology the most advanced and robust protein production platform available.

How quickly can a production strain be developed using *Pf*ēnex Expression Technology?

Answer: The *Pf*ēnex Expression Technology platform assures the first time, significant production of virtually any aglycosylated protein in a scalable system within five weeks from commencement of gene cloning, enabling the rapid advancement of protein leads into proof-of-concept or clinical studies. Once potential production strains are identified through this first stage, within an additional three weeks the fermentation process can be assessed through the use of small scale, high throughput (DOE) methods. These range-finding experiments allow the testing of eight sets of fermentation conditions per selected strain in one week. From these fermentations a large amount of protein can be derived for extensive characterization.

How is *Pf*ēnex Expression Technology high throughput approach unique?

Answer: The *Pf*ēnex Expression Technology platform enables the rapid identification of production strains that are capable of expressing aglycosylated proteins of interest at high yields in soluble and active form. The high throughput approach employed by the *Pf*ēnex Expression Technology platform enables parallel screening and differentiation of hundreds and potentially thousands of host strain/expression plasmid combinations within five weeks. Fermentation development for *Pseudomonas fluorescens* is very rapid (48 fermentation experiments in one week) using DOE techniques at very small (5 ml)

controlled fermentation volumes with verification at the 1 and 20 L scales. In addition, the fermentation is very robust and scaleable to large volumes at extremely low cost. Advantaged, scalable protein recovery technologies also enhance the overall efficiency and yield of proteins produced in the *Pf*ēnex Expression Technology manufacturing process. This entire process depends upon the application of the *Pf*ēnex toolbox. This completely unique, off-the-shelf set of tools to enhance and augment the expression of recombinant proteins in *P. fluorescens* contains a wide range of options for gene expression that have been shown to affect the amount and quality of expressed proteins, as well as a very large number of pre-engineered host strains (all derived from a single fully characterized parent) having phenotypes known to also affect the quality and amount of expressed protein. This combination is the most potent weapon available today to ensure the rapid construction and characterization of highly effective hosts for the expression of recombinant proteins.

Why is protein produced with *Pf*ēnex Expression Technology of higher quality?

Answer: Proteins expressed in *Pf*ēnex Expression Technology are of high quality due to specific components of the platform being designed to ensure soluble and active expression through the avoidance of proteolytic clipping and post translational modifications along with enhancements to solubility through protein folding improvements. Expressing soluble active protein *in vivo* as opposed to protein refolding, as is often the case with proteins expressed in *E. coli*, is a significant quality advantage. Secretion of proteins to the periplasm of the *P. fluorescens* cell, while simultaneously over-expressing folding modulators, enables the proper formation of disulfide bonds, and subsequently the use of non-disruptive, scalable periplasmic release methods for protein recovery which do not break the inner membrane of the cell. Periplasmic extracts obtained with *Pf*ēnex Expression Technology are of much higher purity than standard bacterial lysates thus simplifying downstream purification processes with increased overall yields. A large collection of protease knockout strains helps to ensure that the expressed protein is not subject to proteolytic clipping. Finally, *Pf*ēnex will not glycosylate proteins, thus avoiding the costly problem of extensive heterogeneity frequently encountered in yeast expression systems.

Are there any unique production or analytical issues associated with this system?

Answer: The fermentation, primary recovery, and downstream purification processes can all be performed using standard equipment used in the GMP production of pharmaceuticals. Fermentation methods coupled with effective, scalable periplasmic release techniques developed for the *Pf*ēnex Expression Technology platform actually reduce some of the complexities often experienced using other microbial systems such as *E. coli* or *Pichia*. Analytical methods used in the characterization and testing of recombinant proteins produced in other microbial expression systems are useful for the characterization of products produced with *Pf*ēnex Expression Technology. Further, a host cell protein assay kit specific to *Pseudomonas fluorescens* is available through Cygnus Technologies (Southport, NC).

Why are regulatory risks lower with the *Pf*ēnex Expression Technology platform?

Answer: *Pf*ēnex Expression Technology is a lower risk platform due to acceptance by domestic and international regulatory agencies in multiple human clinical trials. We believe there are no barriers to acceptance by FDA or other regulatory bodies. Very positive responses have been received from customers and regulatory consultants. Since the species and this strain of *Pseudomonas fluorescens* are not pathogenic, it has the same BSL-1 status has *E. coli*, *Pichia*, *Saccharomyces*, and other host cell strains used in the manufacture of biotherapeutics. Antibiotics or animal-derived components are not used at any point in development or manufacturing processes.

For more details:

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