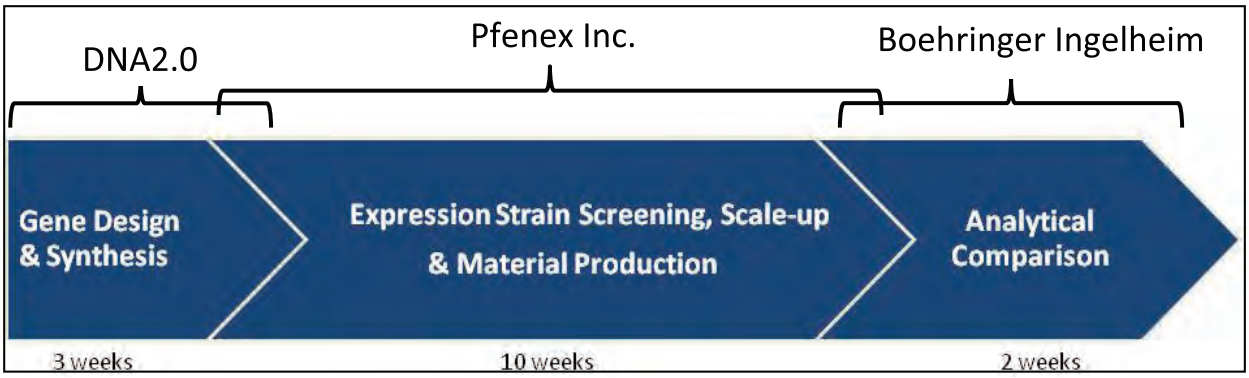
Strategic Partnerships can offer great advantages for time-to-clinic development of difficult to express proteins

The following case study illustrates the successful and rapid path from gene design and synthesis to protein production, as a result of the strategic partnership between Pfenex Inc and its strategic partners DNA2.0 and Boehringer Ingelheim. In this case study the experience and technologies from the three organizations were integrated to do a side-by-side comparison of different expression systems for a difficult to express Fab. The case study, led by Dr. Georg Klima, Head of Process Science – Microbial, Boehringer Ingelheim, outlines the expression of a Fab fragment through four different approaches including *E. coli* (periplasmic), *E.coli* (inclusion body), *Pichia pastoris* and *Pseudomonas fluorescens* (*Pf*ēnex Expression Technology™). The case study describes how a potential lead Fab fragment progressed from an amino acid sequence on paper to 500mg of 99% pure protein in 13 weeks, see Figure 1 below.

Figure 1



DNA2.0 is a California-based gene design and synthesis company. The company has developed a unique gene design technology – GeneGPS™. This patented technology employs gene synthesis and machine learning to identify and quantify gene design variables controlling protein expression yield in a number of expression hosts including *E. coli, Pichia pastoris* and mammalian cell lines. Together with strategic partner Pfenex Inc., the company is also developing these tools for *Pseudomonas fluorescens* (Figure 2). The sequence variables are combined in the redesigned synthetic genes for improved protein expression. Optimization of the DNA coding sequence of a recombinant protein adds yet another dimension to the multidimensional *Pf*ēnex Expression Technology™ toolbox of engineered expression vectors and hosts.

Close integration between DNA2.0 and Pfenex allows for rapid design of appropriate DNA sequences to accommodate the functional requirements of each new protein sequence. Efficient automated gene synthesis allows for consistent 2-3 week turnaround time from virtual sequence to validated physical construct delivered to Pfenex scientists. In this project, human Fab genes were sequence-optimized and synthesized by DNA2.0.

Figure 2

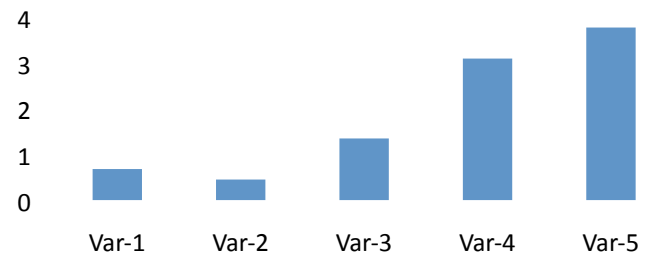


Figure 2. Gene constructs with varying synonymous codon usage for a specific protein target and expression level in *P.fluorescens*, as measured in specific expression level. Five variants showed dramatic difference in expression compared to the wild type sequence.

Pfenex Inc.'s *Pf*ēnex Expression Technology™ platform is a powerful protein expression technology, based on the microorganism *Pseudomonas fluorescens*. This platform allows for the very rapid, <5 weeks, construction and analysis of more than 1000 unique expression strains for optimal recombinant protein expression. Small scale fermentation range finding of top candidates enables selection of a single optimal expression strain as well as suitable fermentation conditions for initial test material production. Legacy recombinant protein expression hosts such as *E. coli* and yeasts tend to have high failure rates with regard to expression of soluble, active, full length protein. In addition, the time required to identify the optimal expression strain in these systems is protracted, resulting in significant opportunity cost for the drug developer. Leveraging the *Pf*ēnex Expression Technology™ shortens development timelines via higher success rates and lowers overall long term cost of goods associated with rapid process cycle times, high cell densities and low cost media. With the advent of biosimilars, it is imperative that innovators develop highly efficient and low cost processes to create an additional barrier to entry for competitors developing biosimilars that will ultimately compete with the innovator products in the marketplace.

The results of the study reveal that Pfenex was successful not only in delivering 500 mg of soluble, active, high quality, highly purified protein for pre-clinical evaluation, but was able to do so within **ten weeks** of receipt of the optimized gene; all three other expression approaches failed to express the active Fab molecule.

Host	Titer (g/L)	Product Location	Comments	Active
E. coli	Light Chain: 11 Heavy Chain : 3	Cytoplasmic Inclusion Body	No Assembly of HC & LC	No
E.coli	0	Periplasm	No Expression	No
P. fluorescens	*0.10 – 0.23** **not optimized*	*Periplasm (supernatant)*	*Soluble Expression*	*Yes*
Pichia pastoris	0	Medium	No Expression	No

Analytical Results of the *P. fluorescens* (*Pf*ēnex Expression Technology™) Expressed Fab			
Quantity of Fab Delivered	500 mg	Purity (WCX-HPLC P1+P2)	≥98%
Identity (ESI-MS)	Confirmed	Solubility (SEC-HPLC)	≥10 mg/mL
Purity (SDS-PAGE)	100%	Temperature Induced Denaturation (T_m) (DSC)	≥80°C
Purity (SEC-HPLC)	≥98%	Affinity K_D (Biacore)	0.26 pM

The *Pf*ēnex Expression Technology™ platform offers a robust solution to the real and opportunity costs incurred through the maintenance of several recombinant expression platforms. The value to the drug developer is clear; there are significant real and opportunity costs associated with using the traditional linear, and iterative approach to production strain development. Data derived from other expression hosts suggest development program delays in the range of five to 12 months on programs which have utilized such a serial approach. The real costs of such delays, including materials, FTE's and facility costs are in the millions of dollars and certainly millions of dollars more due to the delays of a product reaching the commercialization stage. As a result, utilizing a platform such as *Pf*ēnex Expression Technology™ earlier and throughout the development program provides tangible benefits not only in expediting programs by efficient protein production, but also by allowing programs to avoid attrition due to inability to produce product.

Within the strategic partnership between Pfenex Inc. and Boehringer Ingelheim, Pfenex´s cutting edge expression technology is combined with Boehringer Ingelheim's leading development and manufacturing services for biopharmaceuticals. This integrated service facilitates the speed and reliability with which new drugs can proceed through clinical trials. Within the collaboration, all types of services required to move from amino acid sequence to final drug product at various scales up to 6.000 L can be offered.