pAVEwayTM – microbial expression system for the efficient large scale production of therapeutic proteins

pAVEwayTM is an innovative expression system in *E. coli* that enables rapid creation of novel recombinant strains and the development of downstream processes that ensure industry-leading product titres and efficient scale-up of the production of proteins of therapeutic utility. In addition to the potential for reduced cost of goods, pAVEway also enables antibiotic-free cGMP production in a tightening regulatory environment, without sacrificing yields and versatility. A wide range of proteins of therapeutic utility has been produced using pAVEway and a generic fermentation platform, with a variety of accumulation routes, including intracellular soluble, intracellular insoluble and periplasmic secretion.

THE pAVEway platform is based on a set of unique protein expression plasmids, which have been developed by Fujifilm Diosynth Biotechnologies (Figure 1). These plasmids employ a number of powerful promoters, such as λpL, tac and T7A3, to enable the use of a large range of *E. coli* hosts. The inclusion of a stability enhancement system in the plasmids, based on the cer recombination site, reduces the need for antibiotics to assure plasmid retention, thus helping to avoid any future regulatory sanctions on the use of antibiotics in the fermentation process.

Tight control of expression of the cloned protein is made possible using two perfectly palindromic lac operator sequences that are optimally spaced to provide a DNA loop in which the repressor tetramer can bind very tightly to the operators and completely shut off expression (Figure 2). This ability to totally turn off protein expression until the cells are induced with IPTG allows high biomass accumulation prior to induction and assures that all cells are capable of protein production upon induction (Figure 3). Such tight control also assures that the protein is not expressed prior to induction, avoiding plasmid instability due to metabolic load. Tight control of expression also helps to maximise titres, particularly of proteins that are potentially toxic to *E. coli*, and enables a generic high cell density fermentation protocol to be used for any protein, significantly shortening process development.

The expression level can be modulated by varying the IPTG inducer concentration (Figure 4), enabling the use of pAVEway in situations where the maximum rate of protein expression may not be needed for optimal accumulation of the appropriate form of the protein. For example, it may be beneficial to express a recombinant protein targeted for secretion or soluble intracellular expression slowly, so that the host secretion machinery or folding capacity is not overloaded. An overload can greatly reduce the growth and productivity of recombinant cells. Different combinations of the promoter region components can enable creation of a range of pAVEway vectors with expression kinetics that can be tailored to the requirements of a specific protein and its production route. High titres of a diverse range of biopharmaceuticals can thus be generated using pAVEway and the appropriate combination of host strain and fermentation conditions.

A panel of *E. coli* hosts has been developed that enables high expression levels and robust fermentation performance at high cell densities, as well as a set of platform fermentation processes suitable for laboratory and manufacturing applications. pAVEway thus enables rapid selection of an optimal expression system and early definition of the upstream production process.

**A proven system**

The unique features of pAVEway have enabled the production of more than 100 different therapeutic proteins at very high titres since its inception 10 years ago, from cytokines, fusion proteins, enzymes and vaccines to antibodies and antibody fragments, ranging in size from five to 400 kilodaltons (Figure 5). This confirms the ability of pAVEway to generate highly productive recombinant *E. coli* strains with excellent scalability for biopharmaceutical production.

**Fast track process definition**

pAVEway has a track record of enabling rapid entry into first clinical manufacture from recombinant plasmid construction (Figure 6). Starting from the protein of interest, we will discuss the preferred route of expression with you. Alternatively, a pAVEway study can be designed to rapidly assess the best expression route.

We will then clone the gene in a pre-defined set of pAVEway plasmids, transform these into a variety of host strains and grow them in a range of conditions. This allows rapid selection of an optimal expression system and early definition of the upstream production process. The entire development process, from cloning of the gene of interest to fermenter evaluation, can be completed in just four weeks.

One key to the speed of development using the pAVEway system is the ambrTM250
fully automated bioreactor system from Sartorius Stedim Biotech. It consists of 24 parallel and single use bioreactors, each independently controlled. The system is fully automated, with continuous monitoring and control. Identical bioreactors provide the confidence and reproducibility required for parameter screening and statistical analysis. Most importantly, the ambr250 system has fermentation process characteristics that are far more similar to industrial scale fermentation systems than traditional shaker flasks. As a result, fermentation parameters defined on the ambr250 system can be used to run processes scalable up to 3000 litres.

Using the ambr250 system, high throughput strain screening and fermentation process optimisation can be accomplished in parallel, thus speeding up process definition. Multiple design of experiment (DoE) fermentations can be carried out simultaneously in a scalable, reproducible system. This ensures that the maximum amount of statistically relevant data is collected using the minimum number of runs. High quality data and process understanding can thus be obtained much earlier in the development process, and rapid progression is achieved from gene to high titre, strain specific, and optimised fermentation processes that are robust, well characterised and suitable for cGMP manufacture.

The downstream data analysis from the ambr250 screening is also automated, further shortening the time to results. The Caliper Life Sciences LabChip® GX II automated electrophoresis system provides rapid and high throughput analysis of expressed protein titres, processing 96 samples in one run. The software allows users to visualise results via an electropherogram or virtual gel view. The data are also presented in tabular form, which can then be analysed or easily exported into a spreadsheet format.

Utilising the power of software packages such as JMP™ and Design-Expert™ provides statistical analysis and visual representation of the large quantities of information obtained. Design-Expert software enables breakthrough improvements to a product or process by not only screening for vital factors, but also determining ideal process settings for top performance and discovering optimal product formulations. We have developed custom scripts for effective mining of process data using JMP and can apply multivariate analysis to further define the design space at an earlier stage in the development timeline to get the most from our data.

Conclusion

The efficient expression of therapeutic proteins in microbial or mammalian systems is one of the major bottlenecks in the production of biopharmaceuticals. The combination of the unique and proven pAVEway™ expression system. The lac repressor tetramer binds to a pair of perfectly palindromic operators, one positioned upstream of the promoter and one downstream. This causes a DNA loop to form which, in combination with the increased affinity of the lac repressor for perfectly palindromic operators compared to native lac operator sequences, provides extremely tight repression. Addition of the inducer (IPTG, in yellow) displaces the lac repressor tetramer, allowing transcription of gene of interest mRNA to begin.