White Paper:
pAVEway™ expression system for the efficient expression of therapeutic proteins

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INTRODUCTION

One of the major bottlenecks in the production of biopharmaceuticals is the efficient expression of therapeutic proteins in microbial or mammalian cells. The *Escherichia coli* pAVEway™ expression system described here has been developed to ensure high product titres and efficient scale up to GMP manufacture, whilst minimising many common issues seen in other expression systems, such as 'leaky' expression (expression of recombinant protein in the absence of inducer).

HOW IT WORKS

The use of a number of powerful *E. coli* RNA polymerase promoters, such as T7A3, λpL and tac, opens up a large host range in comparison with the popular T7 system that is limited to hosts carrying the λDE3 prophage. Control over basal expression is provided by the use of perfect palindromic lac operator sequences (Figure 1).

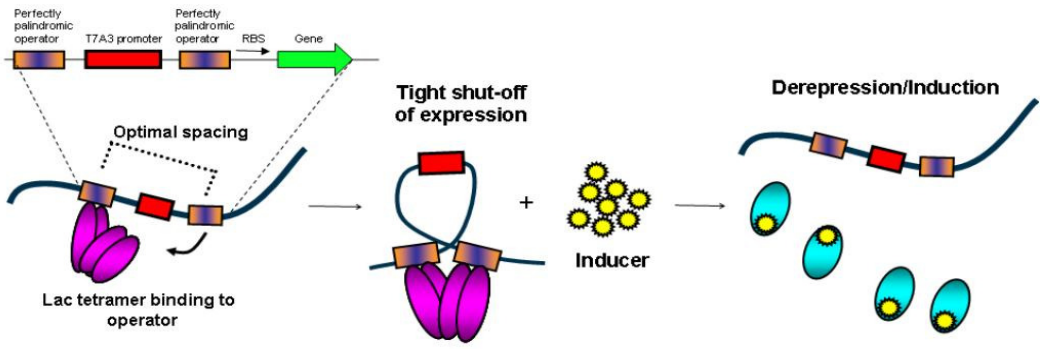

Figure 1: *Schematic representation of repression and subsequent induction in the pAVEway™ expression system. The lac repressor tetramer binds to each perfectly palindromic operator, one positioned upstream of the promoter and one downstream. This causes a DNA loop to form and in combination with the increased affinity of the lac repressor for perfectly palindromic operators compared to native lac operator sequences, extremely tight repression is observed. Addition of the inducer (IPTG, in yellow) displaces the lac repressor tetramer allowing transcription of gene of interest mRNA to begin.*

This high level of control is extremely important in large scale (≥5 L) fermentations as it allows high biomass accumulation prior to induction which can be critical when expressing proteins that are potentially toxic to *E. coli*. The ability to effectively 'switch off' protein expression until induction ensures that all cells are capable of protein production. For an expression system used in large scale manufacture this is a highly desirable characteristic and demonstrates control over the process. This also enables a generic high cell density fermentation protocol to be used for any protein, with no specific optimisation required, which gives the ability to go from gene to fermentation in as little as five weeks.

The rate of target gene transcription in the pAVEway™ system can be controlled by varying the concentration of inducer (IPTG). This can be utilised in situations where the maximum rate of expression may not be needed for optimal accumulation of the appropriate form of the protein (Figure 2).

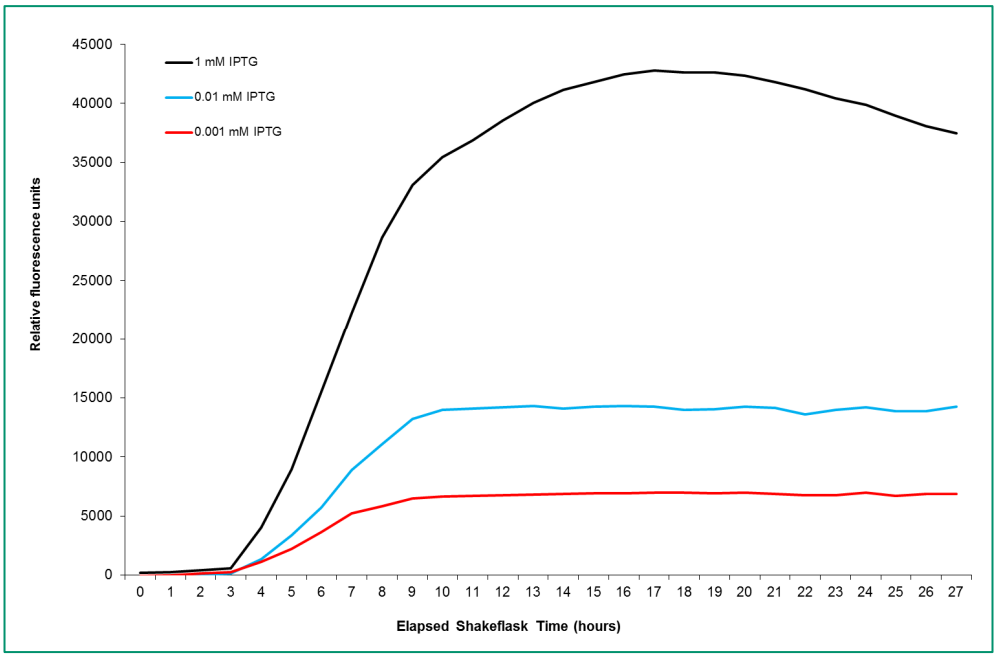

***Figure 2**: The effect of IPTG Concentration on GFP expression in pAVEway. IPTG Concentration is proportional to expression rate. This allows the production rate to be matched to protein folding and secretion rates. Induction takes place at 3 hours post inoculation. GFP Production monitored for further 24 hours.*

The pAVEway™ system allows for true 'tuneability', giving homogenous expression populations at a given IPTG concentration (Figure 3). This means that every cell is producing at the same rate, rather than expression being reliant on a population subset with some cells not induced and some cells fully induced.

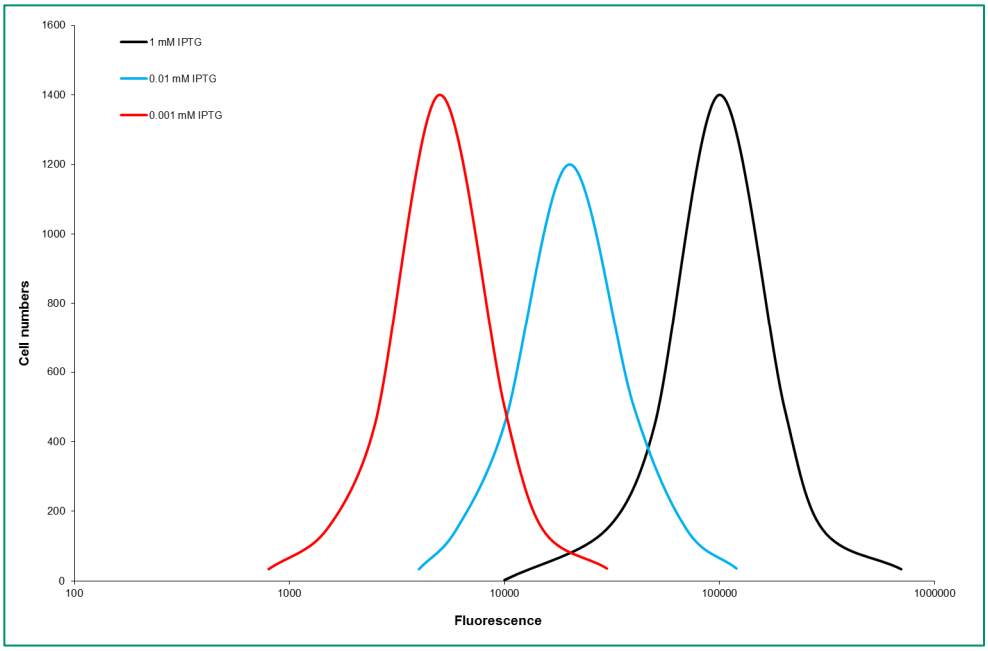

***Figure 3**: Flow Cytometry analysis of GFP expression in pAVEway™. The pAVEway™ system allows for tunable expression to match the cellular protein folding and secretion capability. Flow Cytometry data shows a single discreet population peak at each IPTG concentration. This shows every cell is contributing to expression rather than a small subset population.*

The utility of this can be shown when expressing a recombinant protein targeted for secretion or soluble intracellular expression. Fine control over the expression is required, so that the host secretion machinery or folding capacity is not overloaded as this can greatly reduce the growth and productivity of recombinant cells.

Different combinations of the two promoter region components lead to a range of pAVEway™ vectors with expression kinetics that can be tailored to the requirements of a specific protein and its production route. When combined with generic high cell density fermentation protocols, high titres of a diverse range of biopharmaceuticals, from viral and bacterial proteins through to complex mammalian proteins such as growth factors, cytokines and antibody fragments have been produced.

Since the pAVEway™ system was launched in 2007, a wide experience base has been generated by covering such a diverse range of molecular types. This confirms the ability of the system to generate high productivity *E. coli* strains with excellent scalability for biopharmaceutical production.

Continuing development of the pAVEway™ system will provide additional benefits for users. To further facilitate increased secretion, FUJIFILM Diosynth Biotechnologies has developed a family of secretion leaders. It is useful to be able to direct secreted proteins to either the co-translational translocation pathway, also known as Signal Recognition Particle (SRP), or the post-translation translocation pathway, known as the SEC pathway. The family includes secretion leaders which can direct proteins to either translocation route. When the secretion leaders are coupled to the pAVEway™ system, expression vectors high level secretion of recombinant protein can be achieved.

The presence of antibiotic in fermentation media is becoming an increasing regulatory concern, so the recent development of an antibiotic free pAVEway™ system without compromising product yield highlights its versatility.

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