

WHITE PAPER

# Paveway™ PLUS Microbial Expression System for the Efficient Production of Therapeutic Proteins

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FUJIFILM Biotechnologies

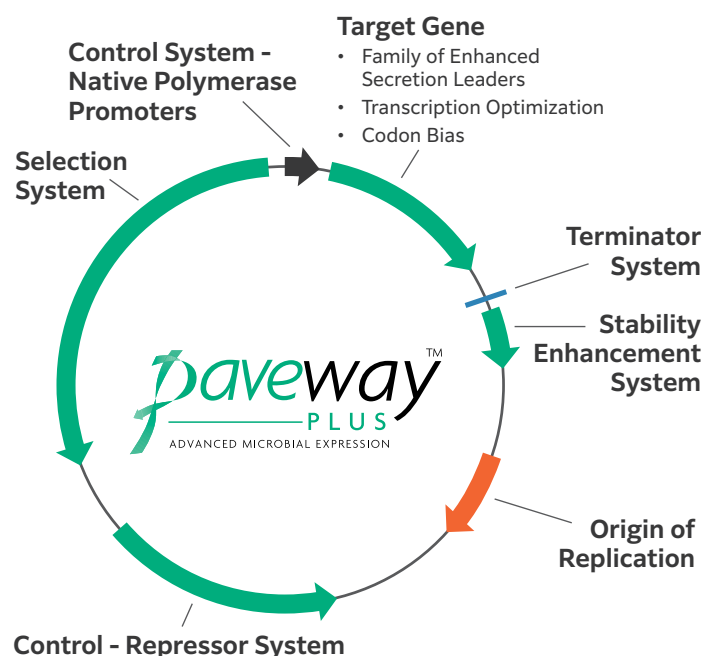
**Paveway™ by FUJIFILM Biotechnologies is an innovative expression system in *Escherichia coli* (*E. coli*) that enables the creation of novel recombinant strains, with lead strain selection based on titer possible within 6 weeks of program initiation.** Since its launch in 2007, Paveway™ has become a well proven *E. coli* expression system, with >140 different therapeutic proteins successfully expressed with a variety of accumulation routes, including intracellular soluble, intracellular insoluble and periplasmic secretion. In addition to the potential for reduced cost of goods, Paveway™ also makes possible antibiotic-free cGMP production, without compromising yields and versatility.

Our Paveway™ PLUS advanced workflow launched in 2022 enables strain selection based on both product titer and quality, with lead strain identification within 11 weeks of program initiation.

## Better by Design

The platform is based on a set of unique protein expression plasmids which have been developed by FUJIFILM Biotechnologies (Figure 1). These plasmids use a number of powerful promoters, such as  $\lambda$ pL, tac and T7A3, to enable the use of a broad range of *E. coli* hosts. The inclusion of a stability enhancement system in the plasmids, based on the cer recombination site eliminates the need for antibiotics to assure plasmid retention.

**Figure 1. The family of Paveway™ PLUS plasmids for the high-titer expression and cGMP production of therapeutic proteins.**



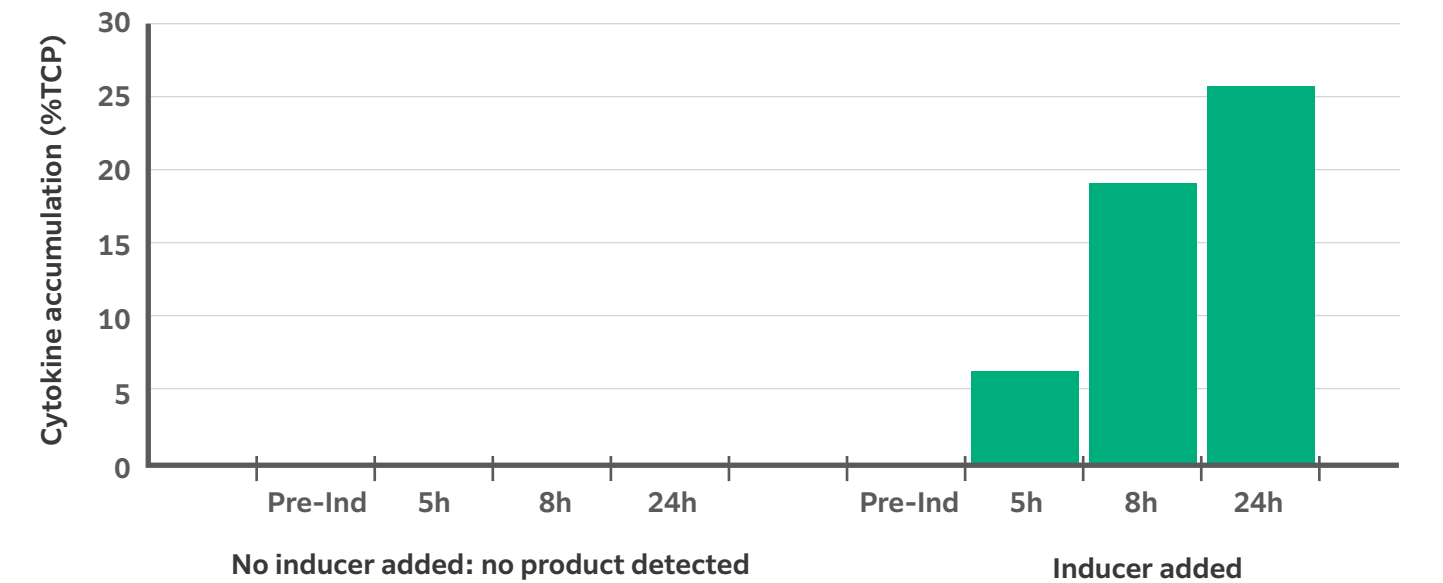
Tight control of expression of the cloned protein is possible using two perfectly palindromic lac operator sequences that are optimally spaced to provide a DNA loop in which the repressor tetramer can bind very tightly to the operators and completely shut off expression (Figure 2).

Figure 2. How the control system works



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).

Figure 3. Tight control of protein expression in the Paveway™ PLUS system, showing cloned protein expression before and after addition of the IPTG inducer. No expression is detected before the addition of IPTG, and strong expression is observed after IPTG induction.



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 maximize titers, particularly of proteins that are potentially toxic to *E. coli*, and it enables a generic high cell density fermentation protocol to be used for any protein, shortening process development.

The expression level can be modulated by varying the IPTG inducer concentration (Figure 4), enabling the use of Paveway™ PLUS in situations in which 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™ PLUS vectors with expression kinetics that can be tailored to the requirements of a specific protein and its production route. High titers of a diverse range of biopharmaceuticals can thus

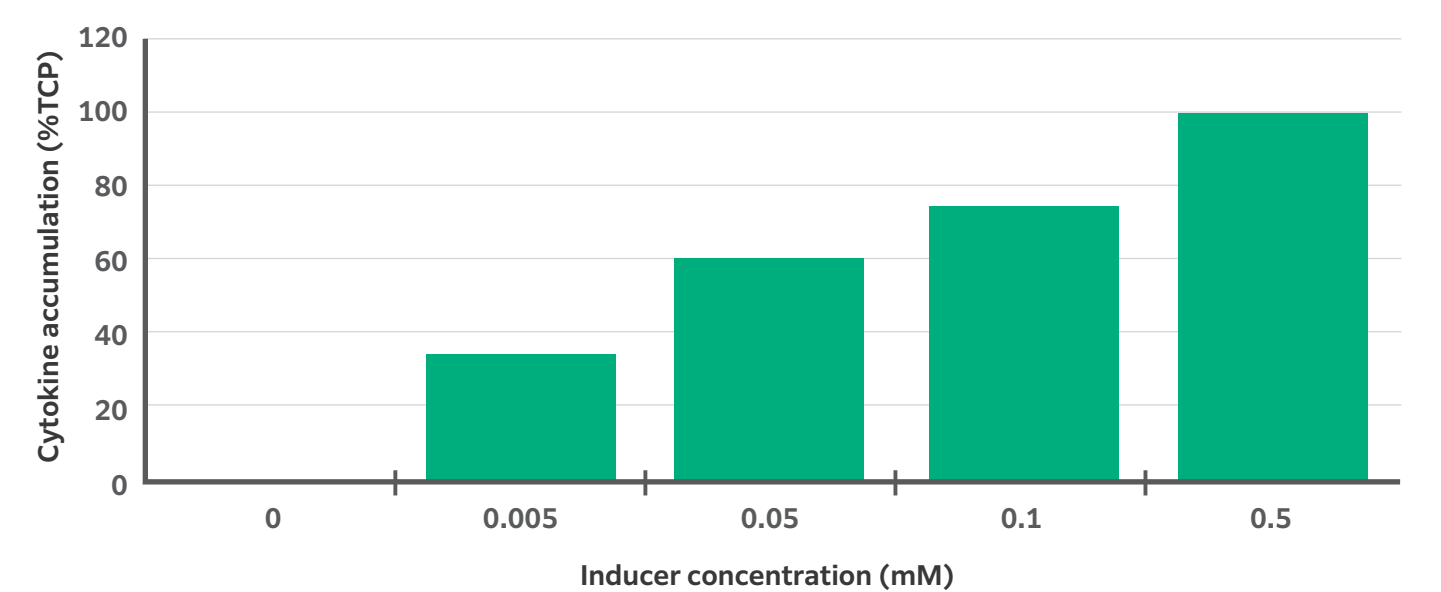
be generated using Paveway™ PLUS and the appropriate combination of host strain and fermentation conditions.

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™ PLUS thus enables rapid selection of an optimal expression system and early definition of the upstream production process.

## A Proven System

The unique features of this expression system have enabled the production of more than 140 different therapeutic proteins at remarkably high titers since its inception over decade ago, from cytokines, fusion proteins, enzymes, and vaccines to antibodies, ranging in size from 5 to 400 kilodaltons (Figure 5). This confirms the ability of the system to generate highly productive recombinant *E. coli* strains with excellent scalability for biopharmaceutical production.

**Figure 4. Modulation of protein expression levels by varying the concentration of inducer, using the Paveway™ PLUS system.**



**Figure 5. Illustration of the wide variety of proteins that have been expressed by multiple routes (soluble, insoluble inclusion bodies, and secreted).**

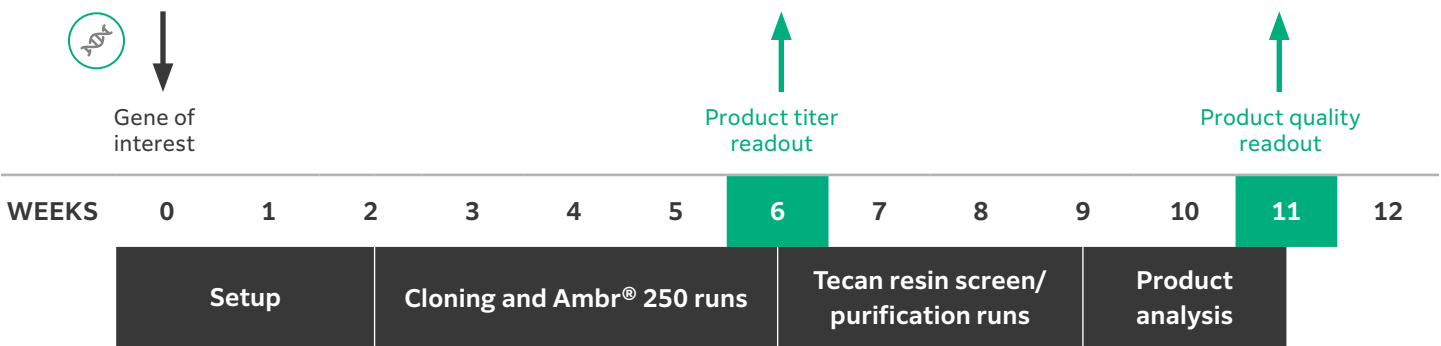
Protein	Cellular location	Titre g/L
GST-3C protease	Intracellular soluble	8
Recombinant vaccine 1	Intracellular soluble	6
Erythropoietin	Intracellular soluble	13
Tumour Necrosis Factor-cx	Intracellular soluble	14
Recombinant vaccine 2	Intracellular IBs	13
Interferon-cx2	Intracellular IBs	14
Antibody fragment	Intracellular IBs	10
Recombinant vaccine 3	Secretion soluble	8
Fab fragments (various)	Secretion soluble	0.5–1.5
Nanobodies and Single Chain Fragment Variable (ScFv)	Secretion soluble	7–12

## Fast Track Process Definition

We have a record for enabling rapid entry into first clinical manufacture from recombinant plasmid construction. Starting from the protein of interest, we will discuss the preferred route of expression with you and design a process to rapidly assess the best expression route. We will then clone the gene in a pre-defined set of 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 6 weeks including assessment of titer. For clients selecting the Paveway™ PLUS advanced workflow, informed risk-based decisions on strain selection using product quality data can be made at week 11 (Figure 6).

**Figure 6. Fast track development of the cloned strain, the fermentation process and titer evaluation are completed in 6 weeks, whilst with the advanced Paveway™ PLUS workflow product quality data is available at week 11.**



Using the Ambr® 250 system, both high-throughput strain screening and fermentation process optimization can be accomplished, thus speeding up process definition. Multiple design of experiment (DoE) fermentations can be conducted simultaneously in a scalable, reproducible system. This ensures that the maximum amount of statistically relevant data is collected using the minimum number of weeks. 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-titer, strain-specific, and optimized fermentation processes which are robust, well characterized, and suitable for cGMP manufacture.

The downstream data analysis from the Ambr® 250 screening is also automated, further shortening the time to results. The Perkin Elmer LabChip® GX II automated electrophoresis system provides rapid and high-throughput analysis of expressed protein titers. 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 out of our data.

## Success Stories

Our original Paveway™ system was used to successfully manufacture SYN-004, Synthetic Biologics' proprietary oral beta-lactamase enzyme for the prevention of *Clostridium difficile* (*C. difficile*) infections, in quantities sufficient for use in Phase I and II clinical trials. SYN-004 is believed to be the first and only therapy designed to neutralize intravenous beta lactam antibiotics excreted into the gut. It is intended to protect and maintain the balance of healthy bacterial flora in the gastrointestinal tract and to potentially prevent the devastating effects of *C. difficile* infection. *C. difficile* is a multidrug-resistant bacterium that infects ~450,000 – 500,000 U.S. patients each year, and almost 30,000 patients die from this infection annually in the US alone. The U.S. Centers for Disease Control and Prevention (CDC) have classified *C. difficile* as an "urgent public health threat," surpassing methicillin-resistant *Staphylococcus aureus* (MRSA) as the number one hospital acquired infection in the United States. Expression titers were increased by more than 25-fold, versus the *Bacillus* expression platform used previously by Synthetic Biologics. This was confirmed during the initial cGMP production run on the FUJIFILM Biotechnology Paveway™ platform.

In a press release, Jeffrey Riley, Chief Executive Officer of Synthetic Biologics, stated "We are extremely impressed by the quality, quantity, and efficiency of the initial SYN-004 API manufacturing work by FUJIFILM Biotechnology. We not only exceeded our yield expectations, but also provided exemplary customer service through its diligent approach and skillful execution in developing the cGMP process for SYN-004."

## 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 our unique and proven plasmids, fast process development and automated downstream analysis ensures rapid progression from gene to optimized, high-titer fermentation process. Our system also provides the in-depth process understanding and robustness required for cGMP manufacture and ability to efficiently express have been proven in more than 140 proteins of therapeutic interest. Furthermore, our Paveway™ PLUS advanced workflow launched in 2022 enables strain selection based on both product titer and quality, with lead strain identification within 11 weeks of program initiation.

Learn more about [Paveway™ PLUS](#).

## About the Author



### CHRIS LENNON

Director, Microbial Upstream Process Development, FUJIFILM Biotechnologies

Chris is the Director of Microbial Upstream Process Development, leading the Molecular Biology, Microbiology &

Microbial Fermentation teams at FUJIFILM Biotechnologies in the UK. The Microbial Upstream Process Development group is responsible for the generation of mammalian expression vectors and microbial expression vectors and strains, non-GMP microbiological testing plus fermentation and primary separations process development. Chris has over 20 years' experience at Fujifilm and was part of the team that developed the company's proprietary Paveway™ PLUS microbial expression technology.



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