Obtaining high yields of soluble protein with fast-growing Vmax™ Express Competent Cells

Introduction

E. coli is a well-established molecular biology host system that is often a first-choice for recombinant protein expression. Although it has been modified and adapted over decades for protein expression applications, challenges such as low expression levels and the expression of proteins as insoluble inclusion bodies still frequently occur. Attempts to overcome these issues, including adjustments to growth conditions, are often unsuccessful, forcing researchers to turn to more complex, expensive, and time-consuming expression platforms. To address these challenges and allow the use of vectors, equipment, and reagents commonly used for *E. coli*, a team of SGI scientists developed a non-*E. coli* expression strain: Vmax™ Express. This rationally designed recombinant protein expression system is an alternative to traditionally used *E. coli* systems and offers a significantly faster growth rate and the ability to generate larger amounts of protein per liter of cells. See the following section and Figure 1 for a brief overview of the history of these host systems.

How *E. coli* became the workhorse of the biotech industry: A Historical Perspective

The ubiquitous use of *E. coli* in academic and industrial laboratories is rooted in history. *E. coli* has been studied in the laboratory since the 19th century after it was first identified as a human gut bacterium in 1885 by Theodor Escherich[1]. This gram-negative, rod-shaped bacterium was used in early studies investigating bacterial physiology and bacteriophage. Additional isolates were later found, further characterized, and studied throughout the first part of the 20th century. Around 1940, the "Phage Group" was formed and came to include some of the most influential scientists of that time, including Max Delbrück, Salvador Luria, Alfred Hershey, James Watson, Renato Dulbecco, Matthew Meselson, and Franklin Stahl, among others. In 1944, to standardize, simplify, and unify phage research, the Phage Group led by Delbrück, Luria, and Hershey drafted the "Phage Treaty," urging all bacteriophage researchers to focus on seven specific "T"-type bacteriophages that infect *E. coli*. That declaration and the subsequent breakthrough studies that followed, cemented the role of *E. coli* in molecular biology labs. Subsequently, pioneering studies that laid the early foundation for understating the genetic code were performed in *E. coli*. Because this organism was already in widespread use, it was also the system used for elucidating the basic mechanisms of transcription, translation, and DNA replication. By the 1970's, standardized *E. coli* protocols (e.g., molecular cloning) and reagents further established the pervasive use of *E. coli* throughout molecular biology labs. It comes as no surprise then, that the first report of recombinant expression of a human protein (somatostatin) by a team of scientists at Genentech in 1977 (rapidly followed by the production of human insulin in 1978) was in *E. coli*. Since then, *E. coli* has continued to be the most common molecular biology workhorse for cloning and protein expression studies[2].

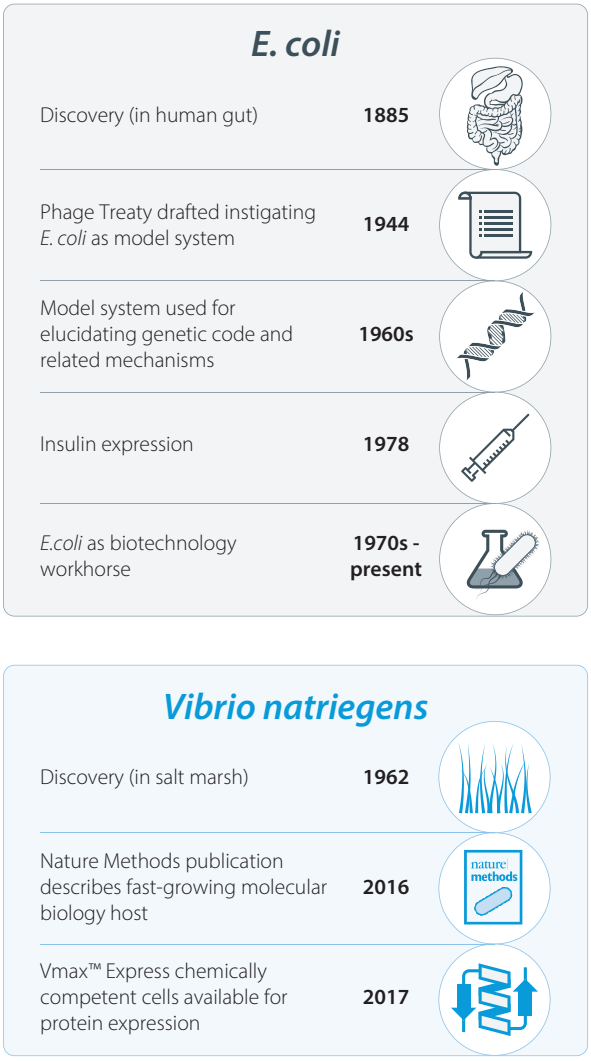

Figure 1. Historical overview of *E. coli* and Vmax™ host systems.

The Need for Alternatives to *E. coli* for Recombinant Protein Expression

E. coli offers many characteristics that have contributed to its widespread use. It is described in the literature as fast-growing and it is relatively inexpensive to culture and manipulate. Common laboratory strains are harmless and *E. coli* cloning and recombinant protein expression protocols are well-established. However, 70–80% of proteins expressed in *E. coli* form insoluble inclusion bodies[3]. While minor incremental improvements to *E. coli* strains and protocols have been made to address some of these drawbacks, when a protein will not express in *E. coli*, alternative systems that use different expression vectors, approaches, and equipment are often required.

Considering synthetic biology and genetic engineering tools now available, scientists at Synthetic Genomics Inc. (SGI) postulated that an entirely new, rationally-selected, and engineered prokaryotic system could avoid some of the difficulties inherent in *E. coli* protein expression and could improve upon the traditional *E. coli* workflow. Specifically, the team sought to identify a fast-growing host to accelerate and improve molecular biology workflows.

Thus, the search for a new host and model microorganism began, leading to a report from the early 1960s on the bacterium *Vibrio natriegens*[4], a non-pathogenic, gram-negative bacterium naturally found in marine environments. One of the most compelling features of *V. natriegens* is that under ideal conditions it has a doubling time of less than 10 minutes. In contrast, the doubling-time of *E. coli* under optimal conditions can be 20 minutes or more. Due to the exponential nature of bacterial growth, *V. natriegens* exhibits a significant reduction in the time required to hit a target biomass level. Leveraging the genetic tools and methods developed during the construction of the first minimal synthetic cell[5], SGI and SGI-DNA research teams used *V. natriegens* to create a new protein expression host, Vmax™ Express[6].

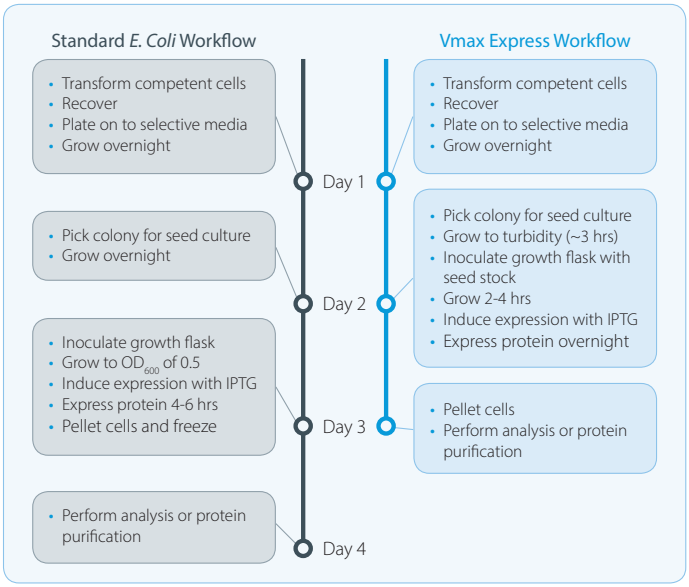

Figure 2. Vmax™ Express generates recombinant proteins 1 Day Faster than *E. coli*.

Vmax™ Express Competent Cells: A New Host System with Advantages Over *E. coli*

Vmax Express Competent Cells are engineered to retain the benefits of traditional bacterial protein expression systems—low cost, ease-of-growth, and compatibility with plasmids and antibiotics already in widespread use. With an accelerated doubling time, Vmax Express shortens protein expression workflows by one day (see Figure 2). Additionally, the data presented in this application note demonstrate that Vmax Express cells produce greater amounts of biomass and generate more soluble protein in less time than *E. coli*.

Methods

To compare the growth rate of Vmax Express to common *E. coli* strains, overnight cultures of Vmax and several *E. coli* expression strains were inoculated to an OD_{600} of 0.03 in Brain Heart Infusion Broth + v2 salts and cultured in a baffled flask at 37°C with agitation at 200 RPM. Expression studies were performed at temperatures optimal for each organism, as noted below. To evaluate recombinant protein expression levels in Vmax™ Express compared to *E. coli* BL21(DE3) cells, expression plasmids for green fluorescent protein (GFP), type I PQQ dehydrogenase, DHG dehydrogenase, alditol oxidase, glucose dehydrogenase, and uronate dehydrogenase were introduced into Vmax Express cells. Protein expression was induced using 1.0 mM IPTG at an OD_{600} of 0.5. After induction, cells were incubated for 4 and 24 hours. BL21 cells and Vmax Express cells were grown at their optimum temperatures of 37°C and 30°C, respectively. Samples of IPTG-induced (I) cells and uninduced (U) cells were analyzed. For GFP expression, UV light was used to visualize active GFP in equal volumes of each cell lysate. Lysates were examined by SDS-PAGE followed by Coomassie Blue staining to visualize the amounts of expressed 27 kDa GFP protein. Uronate dehydrogenase expression was also analyzed further. Following induction and incubation to allow for recombinant protein expression, cells were lysed and centrifuged to remove insoluble protein. Both the soluble and the insoluble pellet fractions were analyzed on an SDS-PAGE gel stained with Coomassie Blue.

Results

Vmax Express demonstrated faster growth and a greater accumulation of biomass relative to other commonly used expression systems (Figure 3). Significantly greater biomass in Vmax Express was evident after only 90 minutes of growth. After approximately 2.5 hours of growth, Vmax Express cells exhibited roughly 10 times the amount of biomass of all other strains tested. The greater amount of biomass evident in Vmax cultures continued for all subsequent times tested, including when cultures were harvested 8 hours post-inoculation. As shown in Figure 3, the Vmax strain also generated clearly visible colonies after 6 hours of growth on agar plates in contrast to *E. coli* strains that required an overnight incubation.

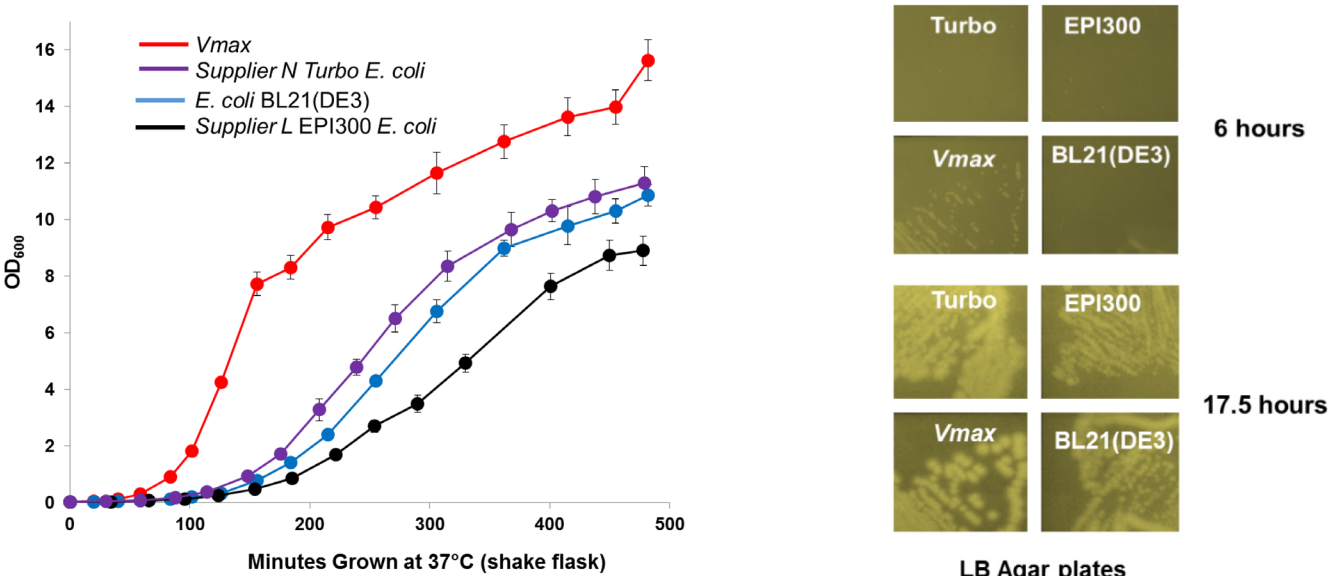

Figure 3. Vmax Express demonstrates faster growth and a greater accumulation of biomass in comparison to commercially available *E. coli* strains.

Next, we evaluated recombinant GFP expression in Vmax Express cells at multiple timepoints. Expression was monitored by fluorescence and by Coomassie Blue staining of SDS-PAGE gels. Vmax Express demonstrated a consistent increase in the amount of protein expressed in IPTG-induced cultures at all time points tested (4, 6, and 24 hours) and at both 30°C and 37°C (Figure 4).

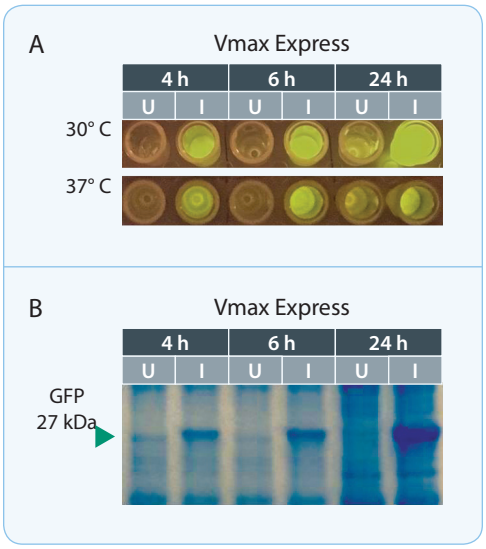

Figure 4. High levels of GFP expression in Vmax™ Express cells.

A common problem associated with *E. coli*-based expression systems is that proteins expressed *E. coli* are often found in insoluble inclusion bodies. In contrast to *E. coli* BL21 strains, Vmax Express has been shown to improve solubility for some proteins. One example, the expression of uronate dehydrogenase, is shown in Figure 5. In *E. coli*, recombinant uronate dehydrogenase is found in the insoluble fraction. In contrast, with Vmax Express, recombinant uronate dehydrogenase is visible in both the insoluble and soluble fractions, with most of the protein found in the soluble fraction.

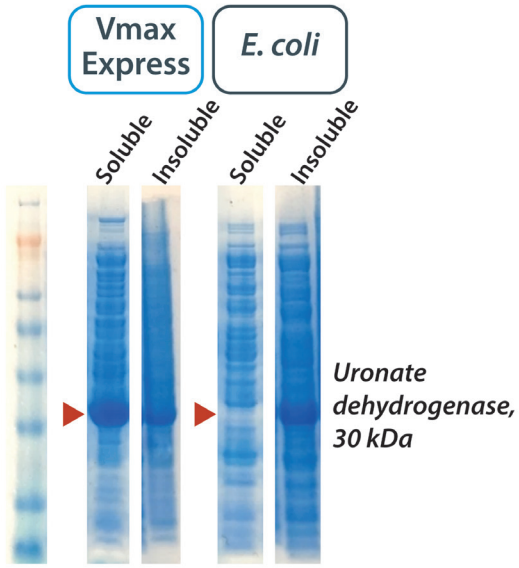

Figure 5. Most recombinant protein is visible in the soluble fraction in Vmax Express, in stark contrast to *E. coli*.

To quantify recombinant protein expression levels in Vmax Express and *E. coli*, we expressed a protein under comparable conditions in shake flasks in both systems (Figure 6). The total cellular biomass of Vmax Express, measured as wet cell weight, was three times greater than the total cellular biomass of *E. coli*. Following nickel-affinity chromatography of a His-tagged recombinant protein, we performed agarose gel electrophoresis and measured the amount of purified protein from the two different cell types. As shown in the gel image in Figure 6B, the amount of purified protein from Vmax Express cells was greater than the amount of purified recombinant protein from *E. coli*. When quantified by measuring the yield from the two cells, we observed a nearly 4-fold greater yield per unit of culture in Vmax Express cells.

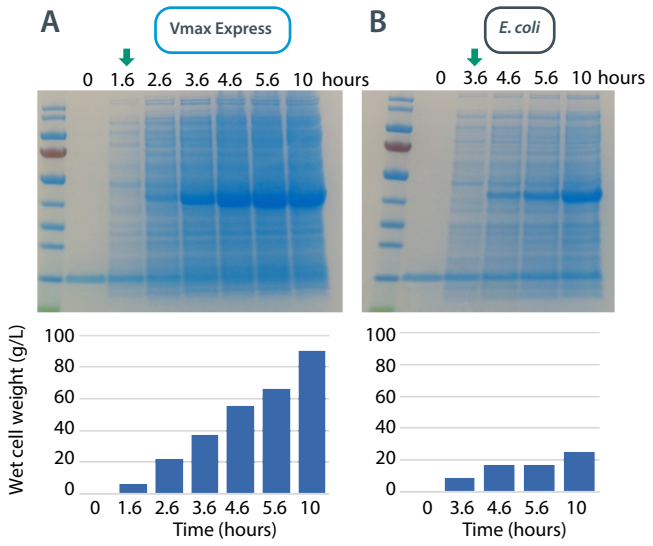

Figure 6. Using a fermenter, recombinant protein expression in Vmax Express is induced earlier, resulting in greater biomass and more expressed protein than in *E. coli*. Induction of expression is denoted by the green arrow in the gel images above. Vmax Express cells were induced after 1.6 hours of growth in a fermenter; *E. coli* was induced at 3.6 hours. Total protein was collected at the indicated time points and used for gel electrophoresis. Graphs below the gel images illustrate the measured biomass from each cell type at the times indicated.

Lastly, we compared the expression levels of many proteins in *E. coli* BL21 (DE3) cells and Vmax Express. As shown in the following table, superior expression was observed in Vmax Express cells for 5 out of 6 proteins analyzed.

Conclusions

For largely historical reasons, *E. coli* is the most commonly used bacterial strain for molecular biology. Despite incremental modifications to *E. coli* expression systems, scientists still face a multitude of challenges such as low levels of expression and poor solubility. These challenges may be overcome with the rationally engineered, novel expression system, Vmax Express, a bacterial host strain with a doubling time at least twice as fast as *E. coli*. With the ability to generate larger amounts of biomass per volume of cells, Vmax Express can produce greater amounts of protein. Compared to *E. coli*, Vmax Express yields substantially higher levels of soluble protein faster for most proteins tested. Because it is compatible with growth media, plasmids, and workflows commonly used for *E. coli*, Vmax Express is a convenient alternative for prokaryotic recombinant protein expression and may help avoid issues frequently encountered with other prokaryotic expression systems.

References

1. Blount ZD. The natural history of model organisms: The unexhausted potential of *E. coli*. Elife. 2015 Mar 25;4:e05826
2. Lee SY. High cell-density culture of *Escherichia coli*. Trends in biotechnology. 1996 Mar 1;14(3):98-105.
3. Yang Z, Zhang L, Zhang Y, Zhang T, Feng Y, Lu X, Lan W, Wang J, Wu H, Cao C, Wang X. Highly efficient production of soluble proteins from insoluble inclusion bodies by a two-step-denaturing and refolding method. PloS one. 2011 Jul 29;6(7):e22981.
4. Eagon RG. P*seudomonas natriegens*, a marine bacterium with a generation time of less than 10 minutes. Journal of bacteriology. 1962 Apr 1;83(4):736-7.
5. Hutchison CA, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF. Design and synthesis of a minimal bacterial genome. Science. 2016 Mar 25;351(6280):aad6253.
6. Weinstock MT, Hesek ED, Wilson CM, Gibson DG. *Vibrio natriegens* as a fast-growing host for molecular biology. Nature methods. 2016 Oct 1;13(10):849-51.

Table 1. Superior recombinant protein expression in Vmax Express cells

Protein	Size (kDa)	BL21 (DE3)	Vmax Express	Findings
Type I PQQ Dehydrogenase	69	✗	✓	Insoluble in BL21. Soluble in Vmax up to 24 hr post-induction.
DHG Dehydrogenase	25	✓	✗	Protein expressed in BL21. Minimal protein detected in Vmax
Alditol Oxidase	41	✓	✓+	Expressed in both. More protein and biomass using Vmax.
GFP	27	✓	✓+	Expressed in both. More protein and biomass using Vmax.
Glucose Dehydrogenase	40	✓	✓+	Expressed in both. More protein and biomass using Vmax.
Uronate Dehydrogenase	30	✗	✓	Insoluble in BL21. Soluble in Vmax up to 24 hr post-induction.

Complete product information and additional resources are available at **sgidna.com/pages/vmax-express-reagents**

SGI-DNA, Inc.
9535 Waples Street, Suite 100
San Diego, CA 92121 USA
North America: (855) 474-4362
Outside North America: (858) 228-4115