Optimizing Virus-Based Expression Systems

Rapid Approach for Real-Time and Direct Virus Quantification

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Since its first use in the 1980s, the utility of baculovirus to express proteins has grown in both scope and diversity. This system is ideal for work that requires superior fidelity in protein function as it delivers high levels of recombinant protein with post-translational processing. Baculovirus-mediated expression of recombinant proteins is a complex, multistage, time-consuming process with many steps. These include inoculation of cell-based growth systems, scale-up to production volume, harvest, purification, concentration, and packaging.

As a result, there are significant opportunities for optimization at different points in the system. Virus quantification is an especially notable source of delays, since many methods require days or weeks to complete. The focus of this tutorial is to describe an analytical platform designed to improve quantitation of baculovirus, as well as quantitation of the resulting expression products, such as virus-like particle-based vaccines (*Figure 1*). Particular emphasis is placed on real-time, in-process quantification and how using this information can significantly improve outcomes.

Virus Counter® Technology

The Virus Counter 3100 (*Figure 2*) was specifically created to address the need for faster virus quantification. In a preparatory step, viral genomes are stained with a fluorochrome that emits in the yellow region of the visible spectrum and proteins in viral capsids are stained with a fluorochrome that emits in the red region of the spectrum.

In the instrument, stained viral particles are hydrodynamically focused through a laser probe region and subsequently detected via fluorescence on two separate optical channels, with simultaneous events counted as intact virus particles. The number of simultaneous events counted during the analysis time is used in combination with sample flow rate to calculate the concentration of virus particles per milliliter of sample. This universal approach allows for the detection of a wide variety of viruses using a simple assay.

Baculovirus Production

The Virus Counter was initially investigated as an alternative to traditional viral plaque assay for the quantification of baculovirus stocks, establishing significant and consistent correlation between total particle count and infectivity.[1,2] While plaque assays require approximately 5 days to measure baculovirus titer, a Virus Counter measurement takes less than 10 minutes per sample, allowing the next step in the process to be initiated a full week sooner. Consequently, the conversion from plaque assays is accelerating through the adoption of this new technology by researchers in this application area.

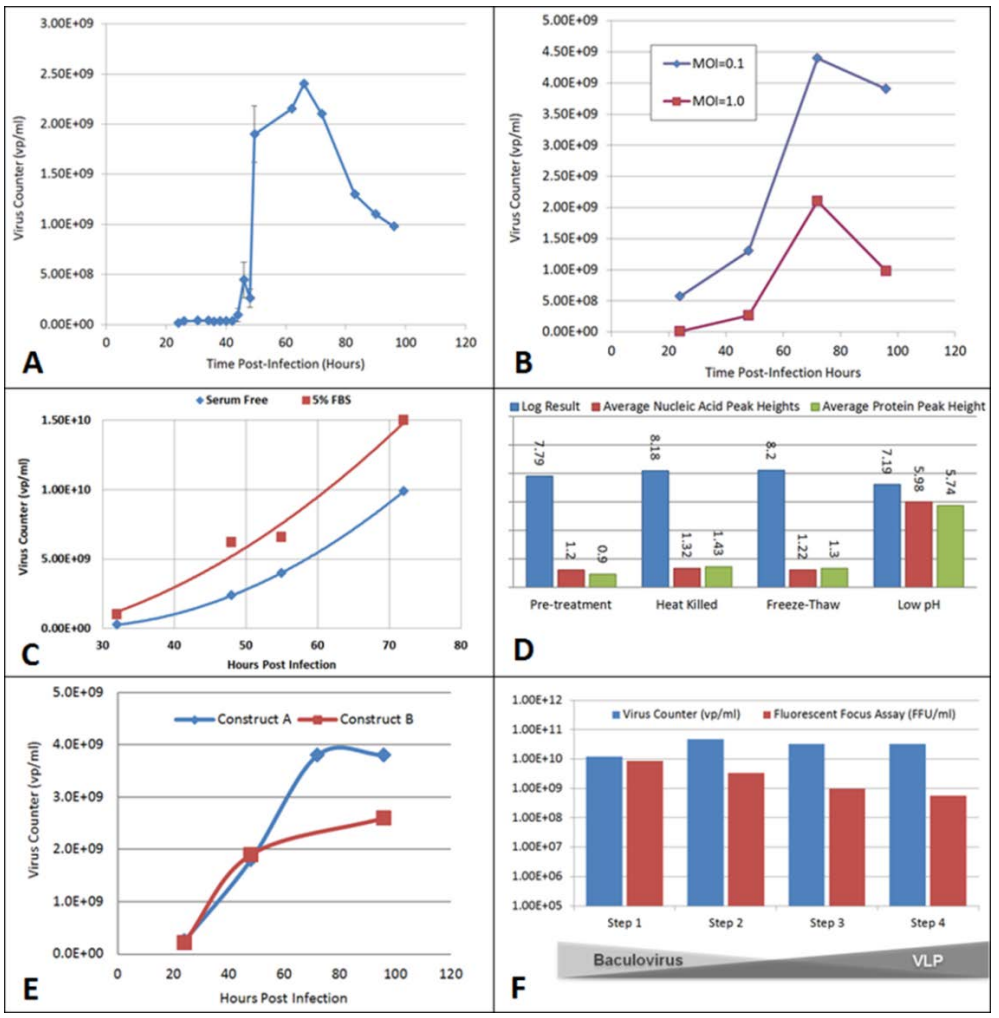

Figure 3. Real-time, in-process monitoring of baculovirus growth using the Virus Counter. (A) Baculovirus particle counts per mL of culture supernatant for a 10-L baculovirus expression using Sf9 cells in serum-free medium. Samples were withdrawn at timed intervals between 24 and 96 hours post-infection. (B) Baculovirus particle counts per mL of culture supernatant for two 10-L stirred-tank bioreactor runs utilizing the same baculovirus construct at two MOI values. (C) Comparison of baculovirus growth in two different media formulations sampled between 32 and 72 hours post-infection. (D) Effect of different conditions on baculovirus aggregation compared to untreated sample. (E) Gene inserts encoding different expressed proteins have a significant impact on baculovirus titer. (F) Decreased infectivity relative to total particle indicates removal of baculovirus and enrichment of VLP product during purification.

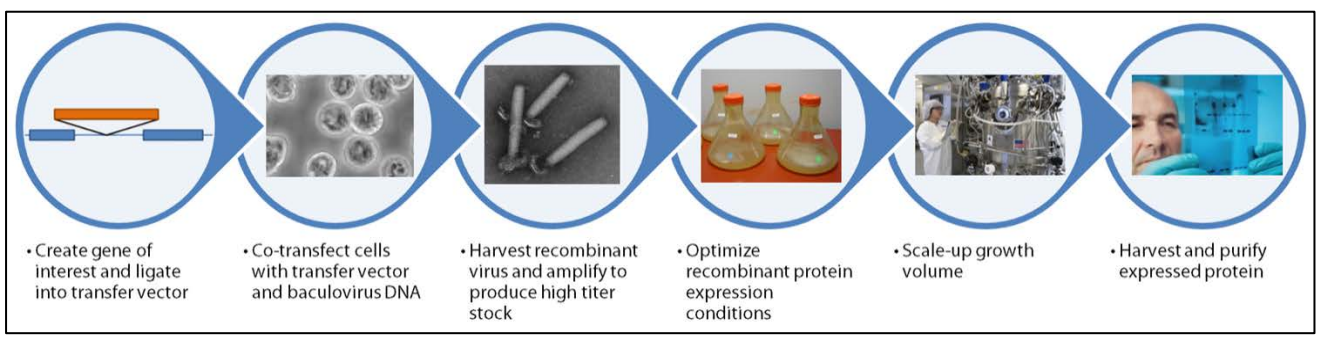

Figure 1. Schematic illustrating the primary steps of using baculovirus systems to express proteins.

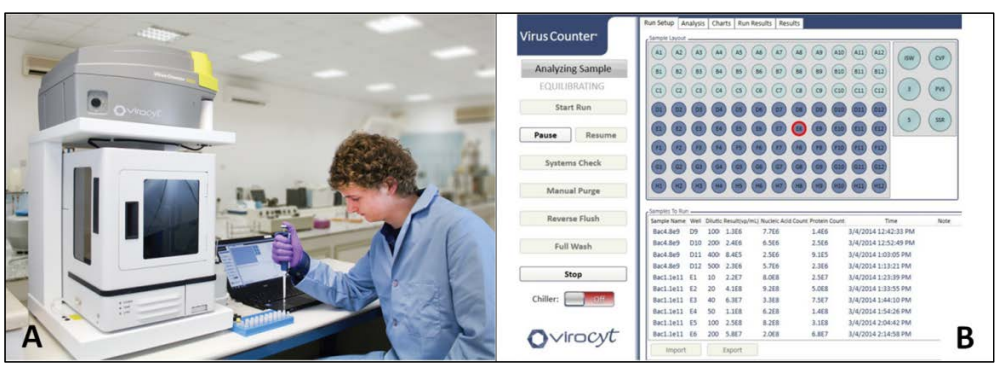

Figure 2. (A) The Virus Counter 3100 (top) with attached 96-Well Autosampler (bottom). (B) Screenshot of Virus Counter software in Autosampler mode illustrating the graphical interface and well mapping capability.

It is also possible to employ the Virus Counter to optimize viral yield and quality by monitoring the impact of a variety of factors, such as temperature, pH, media composition, and infection conditions. This technique can also be used with different types and sizes of growth systems (shaker flasks, spinner bottles, bioreactors) for a number of variations of the expression technology (traditional baculovirus, BacMam, Baculovirus Infected Insect Cells).[2,3]

Figure 3A provides an example of real time, in-process precision for monitoring baculovirus amplification. The data reveal a rapid, almost 10-fold increase in baculovirus titer from $2.6x10^8$ to $2.2x10^9$ virus particles per mL (vp/mL) in the culture supernatant between 48 and 62 hours post-infection. Interestingly, the titer peaked at 66 hours post-infection and decreased over the final 30 hours of culture, highlighting the importance of frequent sampling of growth cultures.

The relationship between multiplicity of infection (MOI) and the kinetics of viral growth in 10-liter bioreactors is illustrated in *Figure 3B*. For both MOI values tested (0.1 and 1.0), there was a significant increase in virus titer between 48 and 72 hours post-infection, followed by a decline in titer in the final hours. A twofold increase in virus particles in the bioreactor run infected at an MOI of 0.1 was observed relative to the higher MOI. Baculovirus expression in Sf9 cells infected with an MOI of 0.1 rely on a secondary round of infection approximately 24 hours post-infection, which may explain the higher level of virus in the process fluid throughout the sampling timeframe. Not surprisingly,

the addition of serum to the cell media also increases virus titer by half a log (*Figure 3C*).

The aggregation behavior of baculovirus particles has also been studied in different conditions, including higher temperatures, repeated freeze thaw cycles, and reduced pH (*Figure 3D*), with acidity the only factor capable of inducing significant aggregation. These findings are particularly relevant for determining the quality of viral stocks following long-term storage prior to their use.

Another important consideration is the effect the expressed protein has on the culture. Depending on its biological role, a protein may either positively or negatively influence cells and/or viruses. This has been demonstrated by tracking the behavior of baculovirus preparations containing different genes inserts (*Figure 3E)*. Based on this information, one might opt to harvest the Construct A Culture at 80 hours post-infection while continuing to incubate Construct B for a longer period of time until maximum titer is reached.

Protein Expression

Virus-Like Particles (VLPs) are an emerging method of creating vaccines in which proteins are assembled into structures lacking viral genetic material, creating an immunogenic but non-infective means of eliciting a protective response against the virus of interest. A number of baculovirus-based systems have been used to produce VLP vaccines, and—as a result—there is a growing requirement to quickly and accurately quantify both baculoviruses and VLPs throughout production, harvest, and purification.

Since most VLPs are non-infective (especially those intended for use as vaccines), traditional infectivity assays like plaque titer are not an option; and, there is a general lack of methods available for quantifying VLPs.

Figure 3F tracks the decrease in infectivity at each step of the purification process as baculovirus is removed while the total particle count increases or remains the same, suggesting an enrichment of the non-infective VLP component.

Summary

Although this tutorial has focused on a specific growth system, these considerations are translatable to monitoring other processes for which operational control is crucial. Tracking factors such as multiplicity of infection, media conditions, and incubation periods prior to starting GMP production is essential to avoid significant problems during the actual run. Equally important is the increased process control derived from close oversight of both the baculovirus amplification rate and the resulting protein expression during the large-scale culture.

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References

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