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ADDRESSING CHALLENGES ACROSS THE VIRUS LIKE PARTICLE MANUFACTURING WORKFLOW

The ability to quickly build up vaccine manufacturing for new infectious disease threats has proved valuable during the COVID-19 pandemic, caused by the SARS-CoV-2 virus. Rapid, large-scale vaccine manufacturing matters for existing disease threats, too: pathogens' constant adaptations mean that production of existing vaccines must be agile enough to accommodate mutations.

"Vaccination is one of the most powerful techniques to protect humans and animals from infectious diseases," says Maya Yovcheva, a research scientist at Thermo Fisher Scientific. The World Health Organization estimates that vaccines saved 2-3 million lives in 2019 alone.[1]

Virus like particles are an increasingly popular platform for vaccine manufacturing because of their rapid production and the strength of the resulting immune response. Vaccines based on virus like particles for hepatitis B and human papillomavirus (HPV), which can cause cervical cancer, are available commercially.

Virus like particles are made from viral proteins that self-assemble into a structure resembling a virus's outer shell. These particles do not contain the pathogen's genetic material, so they cannot replicate. Proteins from different strains of the same virus can be included in a virus like particle to strengthen the resulting immune response.

Once a virus has been genetically sequenced, scientists can prepare vaccine candidates using virus like particles more quickly than when using traditional vaccine platforms. One of the most common approaches for making virus like particles is a protein expression system using baculoviruses.

The speed of the baculovirus expression system makes it a promising manufacturing platform to produce vaccines for viruses that mutate rapidly.

Scientists at Thermo Fisher Scientific are developing technologies for scalable and cost-effective production, screening, and purification of virus like particles.

BACULOVIRUS-BASED EXPRESSION SYSTEMS

The Gibco ExpiSf™ Baculovirus Expression System is the first baculovirus-based insect system for protein production with components whose ingredients are all known, or chemically defined. It can generate three times as much protein as other insect expression systems.[2]

This protein production kit contains a baculovirus generation kit, insect cells adapted for high-density suspension growth, chemically characterized growth media, and additives to enhance protein expression. All components of the system are engineered to work together for consistent, optimized performance (figure 1).

Figure 1: Components of the Gibco ExpiSf Expression System have been designed to complement one another for rapid, scalable, high yields of protein produced in insect cells via a baculovirus expression system.

Source: Thermo Fisher Scientific

Scaling protein expression is notoriously challenging, requiring extensive process development to identify conditions that deliver optimal protein yield as cultures get larger.[3] With cells derived to grow well in suspension, this system can be cultured on scales ranging from deep-well plates to medium-volume shake flasks and larger bioreactor vessels. When scaled up from a 25 mL to 400 mL culture volume, the kit produced protein levels largely within expected error, according to data presented in April 2021.[2] Working up to a 10 L culture volume in a 22 L wave bioreactor, cell growth reached approximately 70% of the growth achieved with shake flasks, which indicates the feasibility of scaling up to a large-batch manufacturing environment.[2]

The media component of modern protein expression systems contains many ingredients that provide nutrients for cell growth and function. This media traditionally contained animal-based serum, but natural variation in those components introduced variation in media performance during biopharmaceutical manufacturing. The biotechnology industry is increasingly developing chemically defined media to provide cell culture conditions that contribute to consistency in protein expression.[4]

The defined ingredients of the Gibco ExpiSf CD Medium provide lot-to-lot consistency, as well as consistent protein expression between cell culture

batches. The medium can be used for multiple steps during expression, including cell growth, baculovirus generation, and virus like particle production. It is free from animal components, serum, and protein, which removes concerns about possible negative immune reactions in clinical applications.[5] For large-scale production, the medium is available in a dry powder format created with Thermo Fisher Scientific's granulation technology. According to Yovcheva, the powdered format is an excellent option for a manufacturing environment, as it can be easily dissolved in various volumes of liquid.

Two additives in the Gibco ExpiSf Expression System are also key to consistency and high protein yields. A cationic lipid–based transfection reagent enables high-efficiency gene transfer and corresponding production of high titer baculovirus stocks. The transfection reagent reduces "the need for time-consuming amplification steps and is highly scalable," Yovcheva says. The other additive is a proprietary expression booster, included to maximize protein yields.

The Gibco ExpiSf Expression System offers a greater virus like particle yield from its cells than from conventional cells for both the Chikungunya virus and HPV, according to data in an April 2021 presentation.[2]

AFFINITY-BASED CAPTURE TO SPEED PURIFICATION

After retrieving virus like particles from a cell culture, the next step is purification. Conventional workflows often involve multiple purification steps including a number of polishing steps to remove remaining impurities. A major drawback of having several purification steps is loss of product for each of these separation steps, leading to a lower overall yield of the drug product.

A proven approach to minimize product loss is to start out with an affinity-based capture step, says Chantelle Gaskin, field application scientist at Thermo Fisher Scientific. In this step, the product passes through resin with target-specific affinity ligands attached to the resin beads.

Thermo Fisher has developed a diverse collection of affinity ligands, all tuned to bind their specific target molecule with high selectivity and specificity. However, purification of virus like particles for vaccines isn't amenable to off-the-shelf options. While it is possible to develop custom affinity resins to capture vaccine components, such an approach can be time-consuming. One way to make affinity capture generally practical for vaccine manufacturing is to append the target with a molecular tag.

For virus like particles, protein components can be expressed to carry a C-terminal tetrapeptide tag, also called a C-tag. The affinity tag is small enough to minimize potential impact on protein folding and function. In addition, regulatory agencies have approved leaving the tag on the final drug product, streamlining manufacturing.[6] C-tagged products can be easily separated from other components using a specialized chromatography resin that binds to the C-tag, such as Thermo Fisher's CaptureSelect™ C-tagXL resin.

It's worth noting that tag-based capture is shown to be useful in early stages of vaccine development, which typically involves screening of multiple candidates in parallel. A collection of vaccine targets such as various virus like particles, all carrying a C-tag, can be rapidly purified for further testing. Introducing C-tag during drug development streamlines purification strategies overall for successful candidates.

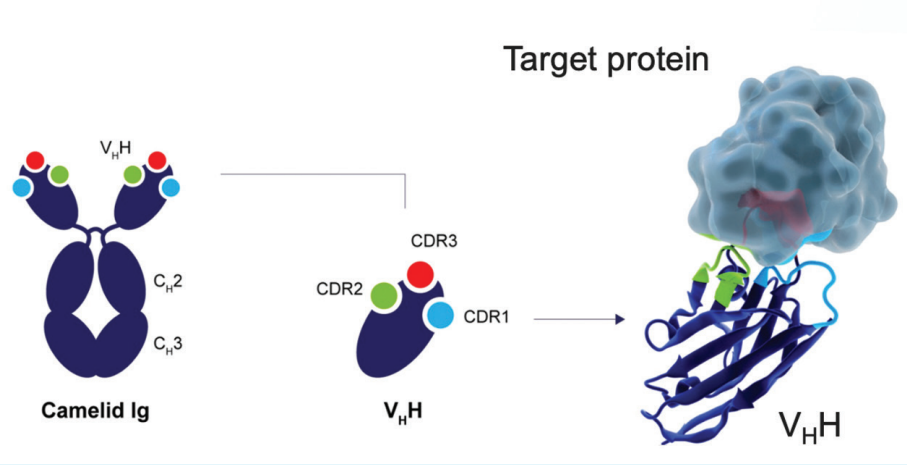
USING AFFINITY PURIFICATION FOR EFFECTIVE POLISHING

Using the baculovirus-expression system to produce virus like particles poses a unique purification challenge, as the baculovirus and virus like particles are similar in size and envelope structure. These shared traits make baculovirus and virus like particles difficult to separate using traditional purification schemes.

To rapidly and efficiently remove baculovirus impurities from virus like particles produced using baculovirus expression systems, Thermo Fisher has developed the POROS™ CaptureSelect™ BacuClear affinity resin. They started with the POROS™ bead, which has large through-pores that accommodate the purification of larger molecules such as viruses. They added an affinity ligand developed using their CaptureSelect™ technology (see sidebar) that specifically captures baculovirus particles and fragments, leaving virus like

A SMALL LIGAND WITH BIG BENEFITS

Many biotherapeutics lack established affinity purification options. Tunable affinity ligands, such as those used with Thermo Fisher Scientific's CaptureSelect™ affinity resin technology, open the door to new purification possibilities for complex biotherapeutics.



Affinity ligands derived from camelid immunoglobin (Ig) antibodies that have been pared down to the N-terminal domain (V_HH fragment), including just the variable antigen-binding regions, also called complementarity determining regions (CDR), (shown in green, blue, and red). The antigen-binding region of the affinity ligand can be tuned to specifically recognize and capture target molecules, such as proteins.

Source: Thermo Fisher Scientific

CaptureSelect™ ligands are structurally derived from heavy-chain-only antibodies found in camelids. The variable domain of these antibodies is called the V_HH ligand, and although being small (15 kD), these fragments harbor full antigen-binding capacity. In addition, these ligands are easily tunable through genetic engineering, enabling development of a specific ligand to target virtually any protein, antibody, or viral vector.

V_HH fragments' small size, rigid structure, and tunable recognition makes them useful as affinity ligands in applications that aren't possible with conventional antibodies.

particles free to pass through the resin. When researchers purified influenza virus like particles from baculovirus impurities using the BacuClear affinity resin, they found that more than 70 percent of baculovirus was removed from the product.[7]

QUALITY CONTROL TESTING

All biopharmaceuticals produced in host cells through biotechnology, such as the Sf9/baculovirus expression system, must meet quality control standards set by the World Health Organization (WHO), the US Food and Drug Administration (FDA), the European Union, and other governing regulatory agencies. One of those standards includes a limit on the amount of genetic material from host cells that may be present in a final therapeutic dose.

"Host cell DNA can impact product quality, efficiency, and safety," says Florian Durst, Thermo Scientific's senior field application scientist in the pharma analytics business unit.

The WHO and FDA both recommend that residual host cell DNA should be limited to under 10 ng per therapeutic dose.[8,9] The FDA recommends analytical methods for detecting residual DNA have a maximum sensitivity of 10 pg.

In biopharmaceutical manufacturing, residual host cell DNA may be quantified at any step after cell harvest. Testing at multiple stages within a workflow makes the development of a reliable quantification method difficult as it has to be compatible with various sample matrices.

Thermo Fisher has developed kits to quantify DNA from a range of host cells, plasmid vectors, and viruses, including the Sf9 insect cells and baculovirus used in virus like particle production, during biopharmaceutical manufacturing. The company's resDNASEQ™ system provides consistent assay performance via quantitative polymerase chain reaction (qPCR) analysis (figure 2).

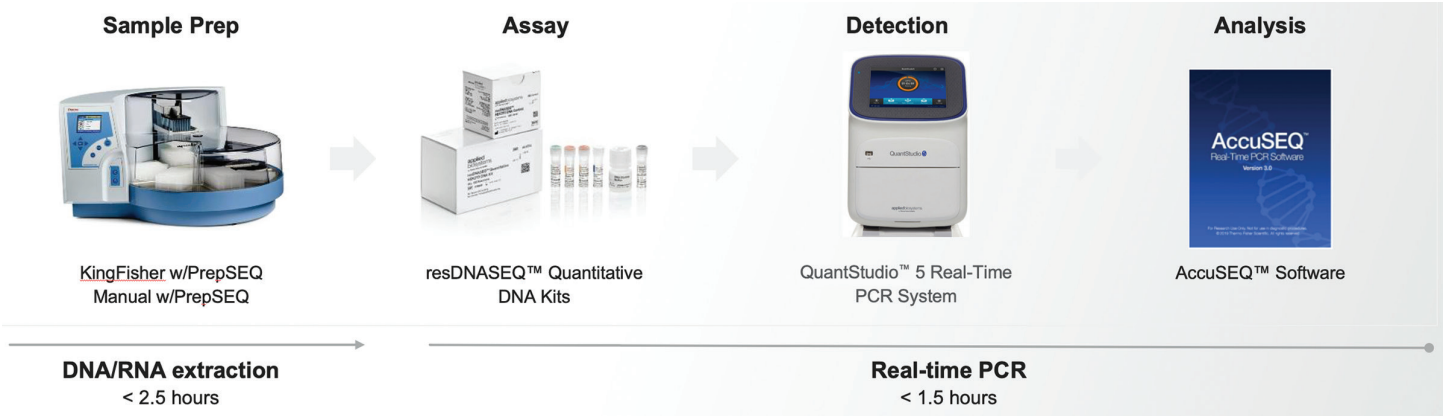


Figure 2: ResDNASEQ system integrates rapid sample preparation for nucleic acid extraction with a workflow for real-time quantitative polymerase chain reaction (PCR) analysis. Nucleic acid quantification can be used for rapid impurities and contaminant testing during regulatory quality control in biopharmaceutical production.

Source: Thermo Fisher Scientific

For the recently developed duplex Sf9 and baculovirus kit, the resDNASEQ™ workflow has a simultaneous limit of detection of 30 fg and limit of quantification of 300 fg for both baculovirus and SF9 residual DNA. Moreover, the method offers consistent results, as indicated by a coefficient of variation of less than 10%.[2]

Nucleic acid testing can also be used to detect regulated contaminants, such as mycoplasma, which are bacteria that can infect humans and are small enough to pass through most microbial filters.[10] Regulatory agencies worldwide now accept nucleic acid testing to show that processes used for biopharmaceutical production are free of mycoplasma. Previously, the only acceptable method for lot-release mycoplasma detection was a culture-based test that took 28 days.

Thermo Fisher Scientific's MycoSEQ™ Mycoplasma Detection System has been specifically developed and validated to meet regulatory requirements. The real-time, qPCR system can detect more than 90 mycoplasma, spiroplasma, and acholeplasma species without cross-reaction from closely related bacteria, with sensitivity to detect less than 10 copies of mycoplasma DNA per PCR reaction.[2] It has been implemented, and following appropriate validation, accepted by regulatory agencies for more than 40 commercial therapeutics for lot-release testing.

Virus like particles are a powerful platform to generate highly immunogenic vaccines, particularly for viruses that are mutating frequently. An integrated system of tools for protein expression, purification, and quality control testing can help support efficient and cost-effective virus like particle production on a commercial scale.

For more information, watch a webinar here.

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