Protein Production in Insect Cells Using Flow Electroporation: A Superior Alternative to Baculovirus Expression

Insect cells are used for the industrial manufacturing of many products that are currently in clinical trials or already available on the market for veterinary and human applications. These cells offer an attractive means of protein production as they post-translationally modify proteins in a manner similar to that of mammalian cells. Furthermore, they are easy to culture with simplified cell growth that is readily adapted to high-density, suspension-based growth in serum- and protein-free media. Although a variety of transient transfection methods and recombinant baculovirus platforms are regularly used for insect cell protein expression (1, 2, 3), not all of these systems fulfill the scalability and reproducibility requirements for biomanufacturing.

Baculovirus-mediated protein production is an extended, multi-stage process. Often it takes 6 to 8 weeks for the creation of a baculovirus virus stock that is used for the final infection of insect cells for protein expression. MaxCyte electroporation offers a unique solution that provides a rapid, high efficiency, fully scalable means of insect cell-based protein production. In contrast to other transfection methods and baculovirus expression systems, MaxCyte electroporation requires no specialized constructs, viral stock production, engineered cells, or chemical reagents. The MaxCyte system allows for progression from plasmid to high-yield protein production within days. MaxCyte flow electroporation reproducibly (co)transfects cells, producing high transfection efficiencies and cell viabilities for a variety of commonly used insect cell lines, including Sf9, Sf21, and SL3 cells. The platform can transfec 5E5 cells in seconds up to 2E11 cells in less than 30 minutes, allowing for fast, high-level protein expression.

Insect cells are often used in the production of virus-like particles (VLPs). VLPs are a promising avenue of

3-Step Electroporation and Insect Cell Protein Expression Procedure

Step 1: Insect Cell Preparation
Harvest cells and suspend cells in 50% media & 50% electroporation buffer

Step 2: Electroporation
Mix DNA and cells, transfer to processing assembly & electroporate

Step 3: Recovery & Culture
Mix cells with culture media & DNAse I, incubate for 30-40 minutes at 27.4°C, followed by standard culturing and protein purification

Figure 1: Rapid, Streamlined Procedure for MaxCyte Electroporation. Insect cells are harvested, washed, mixed with expression construct(s), electroporated using the MaxCyte STX, incubated for a short recovery period, and cultured after transfection using standard conditions. This procedure allows progression from gene to protein in 3 days.
vaccination with an inherently higher level of safety compared with inactivated or live attenuated viral vaccines. These particles consist of one or more recombinantly expressed viral structural proteins that self-assemble into immunogenic complexes that closely mimic the three-dimensional structure of the native virus but lack the viral genome.

In this application note, data are presented showing high efficiency, high cell viability, and rapid production of VLPs following transfection of Sf9 cells using the MaxCyte STX® Scalable Transfection System. Results from comparisons to baculovirus expression demonstrate the superior quality and speed of MaxCyte electroporation for large-scale, insect cell protein production.

Materials
Sf9 cells (ATCC: CRL-1711)
HyClone™ SFX-Insect™ Media (Thermo Scientific SH3027802)
DNAse I (Sigma D4263)
Qiagen Plasmid Plus Giga Kit (Qiagen 12991)
Corning 125 mL Erlenmeyer Flasks (#430421)
EMD Millipore 71235-pIEX-4 DNA
Falcon™ Tissure Culture Plates (353046)
MaxCyte STX® Scalable Transfection System
MaxCyte OC-400 Processing Assemblies
MaxCyte Electroporation Buffer

Methods

Cell Culture and Electroporation
Sf9 insect cells were cultured in Thermo Scientific™ HyClone™ SFX-Insect™ Media in a 27.4°C, 0% CO2 incubator, with shaking at 150 RPM. Cells were passaged every 4 days, and split 1 day prior to electroporation to ensure they were in log phase growth at the time of transfection.

Cells were pelleted at 250 X g for 5 minutes and were resuspended in a 50:50 MaxCyte electroporation buffer:media mix to a final concentration of 1E8 cells/mL. Cells were mixed with plasmid DNA (0 μg or 1-2 μg/1E6 cells) and transferred to OC-400 processing assemblies. Cells were electroporated using the MaxCyte pre-programmed Sf9 protocol. Immediately after electroporation, 1/10th volume of DNase I (stock solution = 2,000 Kunitz units in 1 mL of dH2O) was added directly to the processing assembly. Samples were transferred to 125-mL shake flasks containing an equal volume of culture media (400 μL media for a 400-μL electroporation volume). After a 30-minute incubation at 27.4°C in a 0% CO2 incubator with no shaking, the cells were diluted in cell medium at a density of 2E6 cells/mL. The flasks were transferred to a shaking incubator at 27.4°C at 0% CO2. On days 1, 2, and 3 post electroporation, 1 mL of cultured Sf9 cells was added to a 6-well plate, diluted with 2 mL of culture medium, and imaged using bright field and fluorescence microscopy.

Analysis of GFP Expression and Viability
A green fluorescent protein (GFP) expression plasmid was generated by cloning GFP cDNA into the expression vector pIEX-4 (Novagen 71235-5). Cells were electroporated with the pIEX4-GFP plasmid and GFP expression analyzed on days 1, 2, and 3 post electroporation using a FACSCalibur™ flow cytometer (Becton Dickinson). Cell viability was assessed by trypan blue exclusion using a hemocytometer.

VLP Antigen Expression
Genes encoding three antigens that co-assemble into VLPs were cloned into a plasmid containing three independent copies of the Orgyia pseudotsugata multicapsid nucleopolyhedrosis virus immediate-early 2 (OpIE2) promoter. The expression plasmid was purified and used to transfect Sf9 cells via electroporation (2 μg of plasmid DNA per 1E6 cells). Control VLPs were generated by transduction of Sf9 cells with recombinant baculovirus. Cell culture supernatants were collected on days 1, 2, and 3 post electroporation or baculovirus infection. Supernatant sample were analyzed via SDS-PAGE.

Results & Discussion

Simple Protocol for High Efficiency Transfection
Figure 1 illustrates the straightforward, three-step procedure for MaxCyte electroporation: i) cell harvesting and preparation; ii) cell electroporation, and iii) post electroporation recovery and cell culturing. Specifically for Sf9 cells, the cells are harvested via centrifugation, resuspended in MaxCyte electroporation buffer, mixed with
plasmid DNA, and transferred to a processing assembly for electroporation. Electroporation on the MaxCyte STX® Scalable Transfection System is a push-button process using a pre-programmed, Sf9-optimized electroporation protocol. Following a brief recovery period, transfected Sf9 cells can be cultured using standard conditions.

Electroporation of Sf9 cells with a GFP expression plasmid led to >90% transfection efficiency by day 3 post transfection (Figure 2). Cell viability was >90% at all time points with no differences in cell viability levels between electroporated and control, non-electroporated cells.

Plasmid to Protein in as Few as 3 Days
Baculovirus-mediated protein production is an extended, multi-stage process (Figure 3). This 6- to 8-week process requires construction of expression plasmid(s), transfection of insect cells, viral stock preparation, and subsequent infection of insect cells, all prior to final production and purification of the recombinant protein of interest. In contrast, MaxCyte electroporation directly transfects Sf9 cells with the expression construct(s) of interest, allowing for strong protein expression within days of transfection. There are generally no differences in culturing of insect cells during the period of protein production following either MaxCyte electroporation or the final baculovirus infection. Baculovirus-expressed proteins, however, require additional steps during protein purification to remove contaminating baculovirus proteins, which increases the time and cost of the protein production process while reducing overall production efficiency. Thus, MaxCyte electroporation represents a more rapid and streamlined means of protein production in insect cells.

Rapid VLP Production With No Baculovirus Contamination
In these studies, Sf9 cells transfected via MaxCyte electroporation with an expression construct encoding three antigens that co-assemble into a VLP resulted in significant secretion of VLPs within 48 hours post transfection (Figure 4). In tandem, a baculovirus expression system was used to produce VLPs containing the identical three antigens. Cell supernatants were collected from baculovirus-infected Sf9 cells and from cells directly transfected with the expression construct. SDS-PAGE analysis following concentration via sedimentation through a sucrose cushion shows the presence of the three VLP antigens in cell supernatants from all electroporated and baculovirus-infected cells; however, baculovirus protein contaminants were also present in supernatants from baculovirus-infected cells. This is consistent with the literature (4), which documents the propensity for baculovirus protein contamination creating purification challenges and yield loss when using a baculovirus expression system. Overall, these results demonstrate the extremely rapid and high quality nature of direct insect cell transfection using MaxCyte electroporation, which can further streamline protein production by eliminating the need for baculovirus usage even for more complex molecules such as VLPs.

Conclusions
MaxCyte electroporation represents a rapid, streamlined, and scalable means of expressing proteins such as VLPs in
insect cells. High transfection efficiencies and cell viability lead to strong expression of proteins with post-translational modifications similar to those produced in mammalian cells. The streamlined nature of MaxCyte transient transfection allows the progression from plasmid to protein in as few as 3 days in contrast to baculovirus-mediated expression, which can take 6 to 8 weeks. MaxCyte electroporation also eliminates the potential for baculovirus particle contamination, which can lengthen the purification process, lower purification yields, and increase costs. Overall, the simplicity of MaxCyte electroporation, its capacity for high quality transfection of up to 2E11 cells in less than 30 minutes, and the ease of insect cell culture combine to create a truly scalable protein expression platform for use in biomanufacturing.

References


Further Reading

