Benchmarks

Adaptation of BTI-TN5B1-4 (High Five) Insect Cells for Large-Scale Production in a Stirred Bioreactor

BioTechniques 27:448-450 (September 1999)

Recombinant baculoviruses are used for the production of eukaryotic proteins in insect cell cultures. Because the system is capable of producing very high levels of recombinant proteins compared to mammalian cells, baculovirus technology has found its way to a number of industrial applications (i.e., vaccine production) and x-ray crystallographic structure/function studies. Commonly, the Spodoptera frugiperda SF-9 cell line is used because of its ability to grow in suspension and to express a high level of recombinant proteins. Recently, an attachment-dependent cell line, the Trichoplusia ni BTI-TN5B1-4 (High Five), has been shown to be superior to the SF9 cell for expression of both cytoplasmic and secreted glycosylated proteins (3,6,10). This cell line can optimally produce 26-fold more human-secreted alkaline phosphatase and 28-fold more soluble tissue factor per cell than the SF9 in monolayer cultures (10,11). Previously believed to be attachment-dependent, High Five cells were not easy to grow in a bioreactor despite of the use of a microcarrier system (11,12). Several approaches have permitted these cells to adapt to single-cell suspension culture, either with a long adaptation period (6) or with the use of polyanions (1,2) or heparin (8). However, these methods can inhibit the baculovirus infection of High Five cells. Here, we propose an alternative method to adapt, in one step, High Five cells to single-cell suspension, based on the use of the serum-free medium (SFM) Xpress® (BioWhittaker, Walkersville, MD, USA).

High Five and SF9 cells were obtained from Invitrogen (Carlsbad, CA, USA) and ATCC (CRL-1711; Rockville, MD, USA), respectively. Xpress SFM was utilized for both cell lines. This medium is protein free and contains glucose as the only source of carbon. Its osmolarity and pH were estimated at 362 mOsm/kg and 6.2, respectively. No agents were added to the medium. A recombinant Autographa californica nuclear polyhedrosis virus (AcNPV) was used to express the human class II histocompatibility glycoprotein molecules, HLA-DR1, under control of the polyhedrin promoter. Recombinant baculoviruses carrying truncated genes for the α and the β subunits of human HLA-DR1 were generated in the insect cell line SF9 (7). Viral stock was produced by infection of SF9 at low multiplicity. Viral titer was approximately $3 \times 10^8$ plaque-forming units (pfu)/mL. Cells were infected with the baculovirus at a multiplicity of infection (MOI) of 10, according to the procedure of O’Reilly et al. (4), which evaluated cells at a lesser density. In our study, High Five cells were progressively adapted, in 175-cm² tissue culture flasks, to Xpress medium after 3 passages and were maintained at 27°C. This medium induced a morphological change: cells left their adherence and were able to grow in suspension with a doubling time of approximately 16 h and a maximal density of $4 \times 10^6$ cells/mL.

Once High Five cells were adapted to suspension culture, we determined fundamental parameters, such as the inoculum density and the time of infection (TOI) for a large-scale culture in a 15-L ADI 1030, Stirred-Bioreactor (Biocontroller, Applikon, The Netherlands). Minimal starting cell density of $2 \times 10^5$ cells/mL seemed to be optimal to rapidly reach the exponential phase of High Five cells. The TOI was evaluated at $1 \times 10^6$ cells/mL with an optimal production of recombinant HLA-DR1 from 72–96 h post-infection. HLA-DR1 was measured with an enzyme-linked immunosorbent (ELISA) assay. The supernatent containing HLA-DR1 molecules was directly coated with 0.1 M sodium carbonate (pH 9.6). L243 biotinylated monoclonal antibodies were used to detect the bound HLA-DR1. The revelation system was the streptavidin-peroxydase. Finally, under identical conditions (medium and the density of cells), HLA-DR1 expression in the High Five cells was higher than in the SF9 cells (Figure 1).

Because of the ability of High Five cells to grow easily in suspension and to

![Figure 1. Comparison of the expression rate between the High Five and SF9 cell lines. Cells were adapted in Xpress medium and were infected at $1 \times 10^6$ cells/mL. As detailed in the text, protein production was followed by ELISA.](image)

![Figure 2. Morphology of High Five cells from the batch just before infection with baculovirus, magnified 200x.](image)
have a higher expression rate than the SF9 cells, it was possible to use the *Trichoplusia ni* cell line for large-scale production in a fermentor. Consequently, we tested High Five cells in the stirred-bioreactor with a working volume of 15 L. The temperature was controlled at 27°C by a warmed belt. Oxygenation of the cell culture was performed with a dissolved oxygen level of 80% air saturation by adjusting with pure oxygen by means of a porous sparger. Agitation was fixed at 100 rpm. The pH was not adjusted because of the low variability of the pH medium. In this study, we characterized the performance of High Five cells in terms of growth, mortality, viability, morphology and recombinant major histocompatibility complex (MHC) product yield. The fermentor was inoculated at a density of $3 \times 10^5$ cells/mL. Medium was progressively added to maintain a density between 0.5 and $1 \times 10^6$ cells/mL to reach 15 L. The culture was followed during one week, and viability was determined by the trypan blue exclusion method.

As illustrated in Figure 2, results showed a good adaptation of the High Five cells for large-scale culture. They appeared to grow in single-cell suspension. The culture was relatively short and presented a low mortality (Figure 3). Indeed, 7 days are enough to reach a density of $1 \times 10^6$ cells/mL with a mortality <5% of the total cell number. After infection, we noted that the cells did not grow anymore, but were metabolically active, as evidenced by a high oxygen uptake and a low mortality. Moreover, the use of pure oxygen was an important factor for the viability of the cells. In addition, data showed that oxygenation with air could be a limiting factor (1,9). According to our experiments, the use of air led to an excessive bubbling responsible for an elevated cell mortality. For this reason, we recommend the utilization of pure oxygen. Finally, we obtained approximately 2 mg/L of recombinant proteins quantified by the Bicinchoninic Acid (BCA) assay (Pierce Chemical, Rockford, IL, USA). We concluded that the short culture time and the high expression rate of High Five cells permitted the acquisition of approximately 4-fold more HLA-DR1 compared to SF9 cells.

In summary, the major goals of this study were to evaluate the ability of High Five insect cells to grow in Xpress medium with the idea to use them in a stirred-bioreactor. Finally, we conclude that the use of High Five cells offers many advantages in relation to their expression rate, time of culture, viability and their short adaptation for large-scale culture.

To achieve our study, we characterized the performance of High Five cells in terms of nutrient consumption and by-product accumulation, which could be limiting factors for the culture (5,6,12). In that way, we could improve cell culture productivity by applying a fed-batch process.

![Figure 3. High Five culture in a 15-L stirred-bioreactor. BTI-TNSB1-4 cells were inoculated in a bioreactor at $3 \times 10^5$ cells/mL in serum-free Xpress medium. Once $1 \times 10^6$ cells/mL were reached, the cells were infected by the recombinant baculovirus AcNPV. Supernatent and cells were collected at 96 h post-infection.](image-url)
Single-Nucleotide Resolution of RNA Strands in the Presence of their RNA Complements

Benchmarks Vol. 27, No. 3 (1999)

Double-stranded (ds)RNA is important for a variety of biological systems. The discovery of the dsRNA-binding motif (dsRBM), coupled with the occurrence of this motif in a wide variety of functionally diverse proteins, has led to increased interest and study of dsRNA (6,14). For example, the dsRNA-activated protein kinase (PKR), an enzyme involved in the cellular antiviral response, contains two tandem copies of the dsRBM. In addition, the dsRNA adenine deaminases (dsRADS) contain three tandem copies of this motif (7). Likewise, the study of the RNA-dependent RNA polymerase (RdRP) activity associated with RNA virus transcriptases and replicases also requires the use of dsRNA. In each of these systems,