The use of UCOE vectors in combination with a preadapted serum free, suspension cell line allows for rapid production of large quantities of protein

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Abstract

UCOE vectors contain non-tissue specific chromatin-opening-elements that permit rapid expression of a protein in an integration independent manner. Efficient expression can be derived from a single copy of an integrated gene site resulting in a higher percentage of cells expressing the marker gene in the selected pool in comparison to standard non-UCOE containing vectors. This, in combination with the utilization of a serum-free, suspension adapted parent cell line allows for rapid production of large quantities of protein in a short period of time. Utilizing this system more than 300 mg of a recombinant antibody has been produced in less than 1 month from transfection pools in shake flask. Selected subclones have been scaled into small bioreactors in less than 2 months, producing significant quantities of monoclonal antibody using a protocol generic for the parent cell line. The increased efficiency obtained with the UCOE vector reduces the number of transfectants which need to be screened in order to obtain high productivity subclones. Transfection of a standard host cell line, preadapted to grow in a large-scale setting, allows for rapid cell line development decreasing the transition time from research into development and manufacturing. Alternatively, the traditional approach of using a parent cell line which requires serum-free and suspension adaptation after transfection further increases the need for screening a large number of subclones, because many of the subclones will not be able to grow under conditions that allow large-scale protein production. The use of a preadapted cell line can reduce the time required to develop a cell line from months to weeks.

Abbreviations: UCOE, universal chromatin-opening-elements

Introduction

A method for rapidly producing large quantities of protein in a short period of time is a requirement in today's biotechnology industry. Utilizing a careful list of requirements, we have designed a system that couples an efficient vector system with a cell line that has 'scale-up' friendly characteristics allowing for rapid production of recombinant proteins in a mammalian system.

The vector system requirements included standard ones, such as ease of manipulation. In addition the expression vector needed elements that would make high levels of expression site-of-integration independent. This is achieved using elements that would make expression resistant to silencing, and thereby allow for sustained, stable expression over long periods of time. The final requirement was that the vector would need to contain elements that express at high levels in different cell types in different species.

Figure 1. Antibody Expression Cassettes. The 8kb UCOE was inserted upstream of the hCMV promoter in vectors that contain the light or heavy chain of a humanized monoclonal antibody. The vectors were co-transfected into CHO-S cells and subcloned. Other vectors used in this work replace the transgene with Green Fluorescent Protein.

The cell line chosen should be one that rapidly grows to high cell numbers in a suspension setting in chemically-defined, protein-free media. The cell line would need to be easy to transfect and be susceptible to standard selection protocols. It would need to be easy to subclone. It would need to be able to express the recombinant protein at high levels with the desired secondary modifications.

This paper describes the assembly of a system which pairs a vector that contains components that provide site independent, high levels of expression with a preadapted serum free cell line that can be rapidly scaled up to produce large amounts of protein rapidly.

Vector component selection

Universal Chromatin Opening Elements (UCOE's) are polynucleotide sequences that maintain chromatin in an 'open' configuration (Crombie and Antoniou, 2000). The inclusion of a UCOE in an expression vector upstream of the promoter provides high levels of expression that are independent of integration site and are resistant to silencing.

Vectors with and without the UCOE, a CMV promoter and different transgenes were constructed for evaluation. For the evaluation of the vector and the cell line, vectors which expressed green fluorescent protein (GFP) and the heavy and light chains of recombinant antibodies (Figure 1) were constructed. Evaluations of the vector were conducted in CHO-K1 (ATCC CCL-61) cells grown in F12 with 10% FCS and in a suspension adapted CHO cell line described below, grown in protein-free media.

Experiments were performed comparing expression of GFP from vectors with and without UCOE's. In these experiments CHO-K1 cells were stably transfected and single cells clones were derived from the selected pool. Each of the clones was analyzed for expression by flow cytometry (Figure 2). The addition of

Figure 2. UCOE vs. non-UCOE expression levels. CHO-K1 cells were stably transfected with vectors that express GFP, driven from the hCMV promoter, with and without the UCOE. The population of clones that contain the UCOE vectors demonstrate a higher percentage of cells that produce detectable levels of GFP. The individual clones demonstrate higher production levels when the UCOE is included in the vector.

the UCOE resulted in more clones with higher levels of expression.

In experiments where CHO cells were co-transfected with heavy and light-chain vectors, with and without UCOEs, each vector contained a selectable marker. The pools were selected and the antibody production levels tracked during the selection process (Figure 3). The inclusion of the UCOE resulted in a dramatic increase in expression levels.

Cell line selection

A Chinese Hamster Ovary subclone (D'Anna et al., 1997; D'Anna et al., 1996; Deaven and Petersen, 1973; Puck et al., 1958.) that has been adapted to a commercially available chemically-defined, protein free-media (Gorfien et al., 1998) was evaluated to determine if it met the requirements described above.

Figure 3. Antibody expression levels in CHO cells transfected with and without UCOEs. CHO cells were co-transfected with vectors containing the cDNA encoding the heavy and light chain of a humanized monoclonal antibody. Expression levels were monitored during selection. The population that was transfected with a UCOE containing vector produced increasing amounts of antibody during the selection period while little increase was detected in the population that was transfected with the non-UCOE containing vector.

Figure 4. Growth curve of CHO-S cells. CHO-S cells grown as recommended in shake flasks in CD CHO media demonstrate 12-16 hour doubling times and reach high cell densities.

The cell line, CHO-S (Invitrogen/Gibco), has a 12 to 16 hour doubling time in shake flask cultures reaching a peak cell density of $9-11 \times 10^6$ viable cells/ml (Figure 4). They are susceptible to hygromycin at 400 ug ml^{-1} and geneticin (G418) at 600 ug ml^{-1} . The cells grow as attachment independent single cells even in a stationary culture (data not shown).

Master and working cell banks were developed utilizing a chemically-defined, protein-free freezing media. The culture viability upon recovery was better than 95%. The cell banks were tested for mycoplasma by DNA fluorochrome and direct culture and found to be negative.

The presence of the $\text{Gal}\alpha \rightarrow 3\text{Gal}\beta \rightarrow 4\text{GlcNAc-R}$ (Gal-Gal) carbohydrate residue on antibodies used as human therapeutics has been associated with rapid

Figure 5.

protein clearance from the serum. As a result, the ability to produce recombinant protein without this residue is advantageous (Borrebaeck and Ohlin, 1993.; Kagawa et al., 1988). Utilizing the FITC labeled IB₄ lectin and flow cytometry (Cho and Cummings, 1997; Gorelik, et al., 1995) we were able to demonstrate the Gal-Gal residue is not present on the cell surface (Figure 5). Mass spectroscopy of a purified recombinant protein produced in the cell line has not demonstrated the presence of the Gal-Gal residue (data not shown).

UCOE vectors in suspension adapted, serum-free CHO cells

Suspension, serum-free adapted CHO cells were co-transfected with vectors containing UCOE antibody expression cassettes. Transfected cells were selected using hygromycin and G418 and subclones were selected. This process took approximately 5 weeks. The subclone was scaled into a 2L bioreactor to perform final parameter optimization before being scaled into a 100L bioreactor where 20g of antibody was produced.

A rapid timeline for protein production is made possible by having a cell line that is well characterized prior to transfection. Having the subclones in a serum free media and in suspension resulted in an almost immediate transfer into stirred tank bioreactors. The development of general protocols for the bioreactors allowed for a smooth transition and rapid scale-up.

Summary

The utilization of vectors containing UCOEs in a suspension adapted cell line allowed for rapid development and scale-up for production of antibody. The

UCOE allowed for screening of a small number of subclones to obtain a clone that was capable of producing 200 mg L⁻¹ of antibody in a 5 week period in serum free conditions. The use of a pre-adapted suspension line allowed a rapid scale-up into stirred tank bioreactors with predefined protocols.

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