Improving Protein Yields in Mammalian Cells

UCOE Tool Facilitates Cell-Line Production

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The development and manufacture of recombinant therapeutics requires the production of stable, high-yielding cell lines. The success of this process depends to a large degree on both the method chosen for transfection and clone selection, and also the cell line selected for culture.

The identification of high-yielding cells has traditionally been a lengthy process that involves sorting through thousands of clones in search of the few that, to some extent by chance, express the recombinant protein at suitably high levels. While optimizing media, feed strategy, and bioreactor parameters play an important role in ensuring maximum protein production, the optimization process starts at the very beginning, at the level of DNA.

The site of gene integration during transformation can determine the levels of protein produced—if a gene integrates into an area of the host genome that is transcriptionally silenced (heterochromatin), expression levels will be low. Genes that are constantly or regularly active are found within euchromatin and have high levels of histone acetylation. In contrast, heterochromatin is generally characterized by extensive histone deacetylation and high levels of histone methylation.

A number of strategies have been developed to change the structure of chromatin surrounding the integrated gene in order to circumvent the issue of integration site-specific repression.

In collaboration with King's College London, **Cobra Biomanufacturing** (www.cobrabio.com) has identified short polynucleotide sequences encompassing genetic regions surrounding housekeeping genes that maintain an open chromatin structure, thus allowing high levels of transcription of the subsequent genetic sequence. The DNA elements have been termed ubiquitous chromatin opening elements (UCOEs) and now are proving to be of great value in bioprocessing.

UCOE Technology

UCOEs consist of methylation-free CpG islands that encompass the promoters of housekeeping genes, for example the dual divergently transcribed heterogeneous nuclear ribonucleoprotein A2/B1 (HNRPA2B1)- heterochromatin protein 1Hs-ɣ (chromobox homolog 3, CBX3) promoters. CpG island-containing fragments of 4–8 kb from the HNRPA2B1-CBX3 (RNP) loci have been incorporated into eukaryotic expression vectors and have resulted in a 10- to 25-fold increase in productivity compared to vectors without a UCOE insertion.

The UCOE is always positioned upstream of the typical expression cassette, consisting of a cytomegalovirus (CMV) promoter, the gene of interest, and a polyadenylation sequence. Transfecting cells with such a vector results in a vastly increased proportion of high expressing, stable clones in the cell population, making the process of clone selection and culture much faster and easier. For antibody production, a dual UCOE vector is used with separate cassettes for heavy and light chain expression. Following transfection, the linearized UCOE expression cassette typically integrates into the chromosome at 5–10 copies per cell.

The value of this technology to the bio-

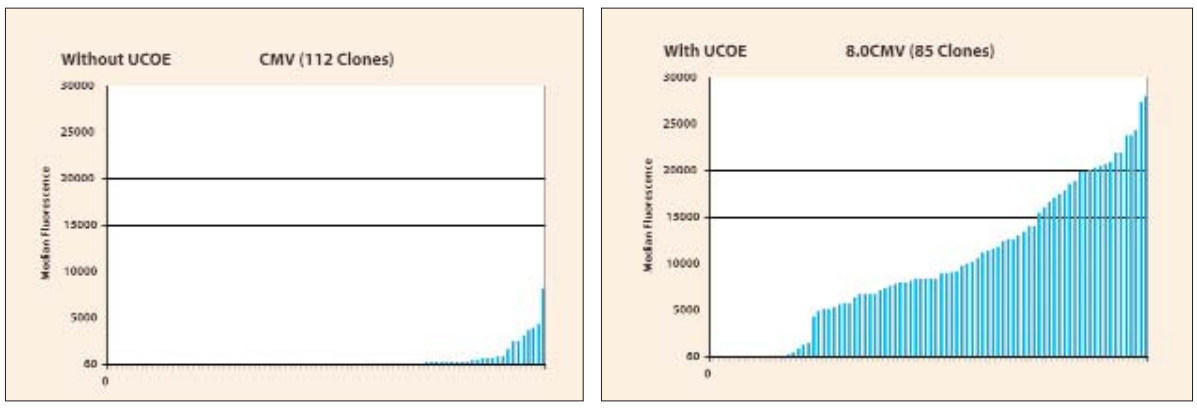

Figure 2. CHO cells were stably transfected with a human CMV-EGFP reporter construct, with or without an upstream 8 kb UCOE fragment. Median fluorescence indicates the level of reporter gene expression achieved in individual clonally derived fragments.

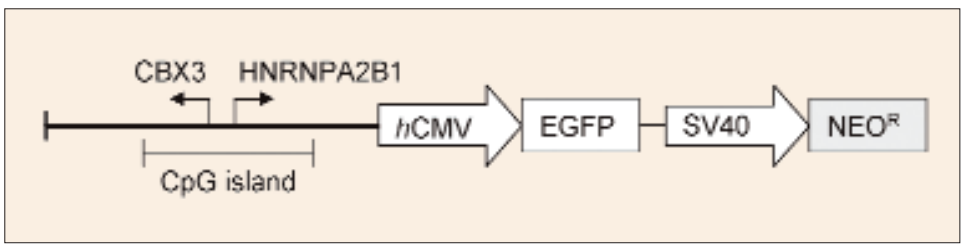

Figure 1. EGFP expression construct: All constructs carried the NEO^R gene preceded by the SV40 promoter for selection of stable transfectants of CHO-KI cells.

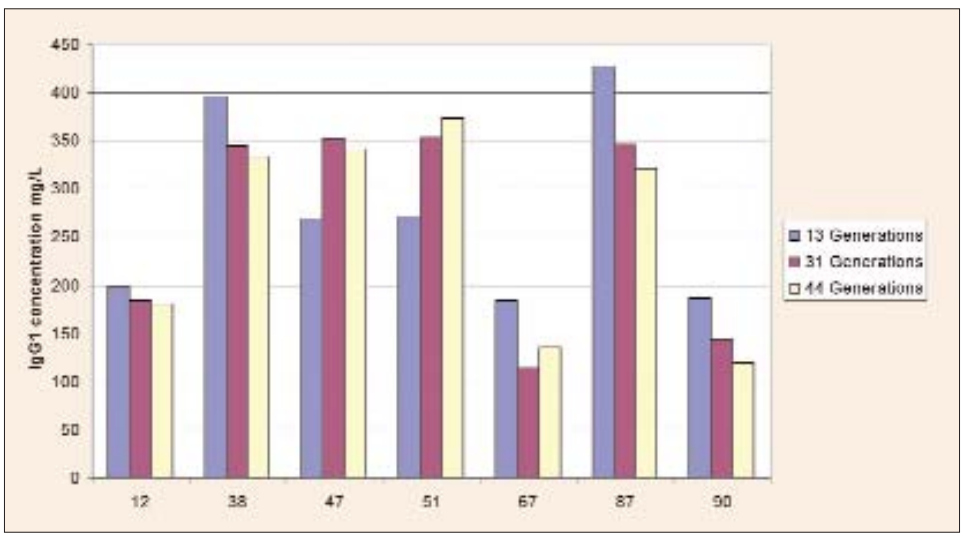

Figure 3. Stability of IgG1 expression in seven CHO-S clones over 50 generations: Overgrowth yields were determined at the beginning, middle, and end of the stability study.

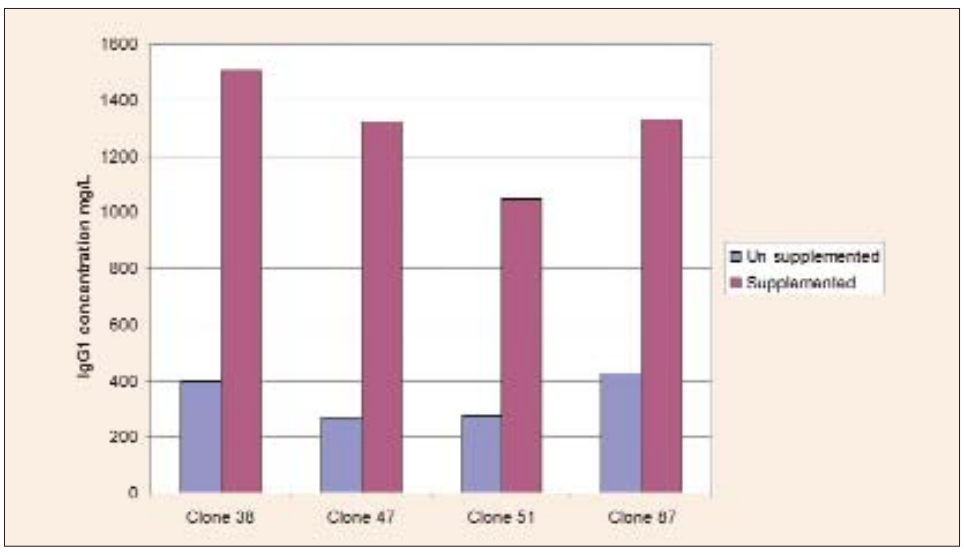

Figure 4. IgG1 expression in CHO-S clones in protein-free media with and without an unoptimized supplemental feeding regime.

process industry is, however, dependent on more than just the successful transformation and isolation of clones—cell line selection and development are equally important.

Cobra Biomanufacturing has developed the maxXpress system, which combines UCOE-containing clone production with custom cell-line development. The cell line used should be suited to the specific production task proposed. Requirements include a cell line that rapidly grows to high cell numbers in a suspension setting, in chemically defined or protein-free media. The cell line would also need to be easy to transfect and be susceptible to standard selection protocols. It would need to be easy to subclone and also express the recombinant protein at high levels with the desired secondary modifications.

Optimizing Protein Yields

UCOEs were initially isolated and evaluated by incorporation into vectors for the expression of enhanced green fluorescent protein (EGFP) from the hCMV promoter (*Figure 1*). Evaluations of the vector were initially conducted in CHO-K1 and after selection and isolation, each clone was analyzed for expression by flow cytometry.

Figure 2 illustrates the high percentage of cells that produce detectable levels of EGFP when compared to clones containing the same vector without the UCOE. It is clear that on an individual basis, cells containing the UCOE vector demonstrate higher production levels than cells containing the vector without a UCOE. This dramatically reduces the number of clones that therefore need to be screened.

The stability of expression from a UCOE vector has been demonstrated by studying the expression of an IgG1 during continuous cell culture of a suspension CHO-S cell line over 50 generations, with expression yields being determined at the start, middle, and end (*Figure 3*). It can be clearly seen that expression is stable for these higher expressing clones.

UCOE technology enabled the selection of high expressing clones capable of producing 1.5–2 g/L of protein within ten weeks. This compares favorably to traditional methods that can take up to 10–12 months. *Figure 4* shows that productivities can be increased from around 0.4 g/L to 1.5–1.9 g/L with minimal optimization in protein-free media with supplemental feeds.

The technology therefore enables not only the isolation of high expressing clones but can also allow the selection of high expressing pools, produced by transfecting the culture with the UCOE expression plasmid, followed by antibiotic selection. Such pools can be isolated within 2–3 weeks of transfection with a minimal quantity of plasmid DNA.

These pools can be used to generate research cell banks without the need for repeat transfection, and demonstrate high levels of productivity up to orders of magnitude above that achievable with transient transfection. This has allowed Cobra to supply large quantities of material for early-stage downstream purification (DSP) development for a number of clients.

In one example a human IgG1 was expressed in an initial shake flask media screen to 645 mg/L; this was followed by a 5 L fermentation, which allowed the development of a purification scheme prior to the isolation and generation of material from clones.

An IgG1 fusion has also been expressed at 500 mg/L in shake flask cultures using a generic medium developed for our CHO-S cell line; this has again allowed the development of a early-stage DSP process with the supply of sufficient quantities of material for activity studies. The production of material in pools is now routinely used at Cobra to reduce project timelines significantly.

UCOE technology is of huge value to the bioprocess industry if combined with informed cell-line development.

The engineering of expression vectors containing UCOEs enables the rapid and easy production of stable, high-expressing cell lines that are suitable for large-scale production and the rapid isolation of high expressing pools. Protein yields of 2 g/L can now be obtained with a reduced drain on resource, providing a cost-effective production solution to the biopharmaceutical sector. GEN