Application Note

An Accelerated Method for Production of Recombinant Proteins using UCOE™ Expression Technology

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INTRODUCTION

UCOE (Ubiquitous Chromatin Opening Elements) technology[1] is a breakthrough solution for **rapid protein production and cell line development** using mammalian systems. Using this technology, researchers can obtain gram level quantities of protein material from highly productive stable cell pools in less than four weeks. Application of the UCOE technology to the production of stable pools results in cultures containing a larger percentage of highly expressing clones as compared with pools generated with conventional non-UCOE vectors. High yielding, stable cell lines can be derived in less than 60 days, screening less than 100 clones, and without the need for amplification. UCOE technology is compatible with standard industry platforms, such as CHO cells, CMV IE promoters, commonly used plasmids, transfection/selection agents, media etc.

DESCRIPTION AND FORMAT

A broader use of recombinant therapeutics requires the development of improved, cost effective manufacturing processes to result in increased product yield and affordability. Advances in the understanding of the chromatin structure/activity relationship[2, 3, 4, 5] permitted the development of the UCOE expression technology. UCOE technology provides a significant increase in transcription efficiency and in the stability of protein expression in mammalian cells. UCOE elements are isolated from methylation-free CpG islands associated with housekeeping genes[6, 7]. The technology utilizes a number of different UCOE elements, namely the 3.2 kb RPS3 and the 8kb, 4kb and 1.5 kb hRNP, which can be used alone or in combination with each other. These elements create and maintain an "open" chromatin environment around the chromatin-integrated transgene, ensuring it is transcriptionally active. UCOE elements prevent transgene silencing and promote consistent, stable and high level gene expression, irrespective of the chromosomal integration site. UCOE technology provides a substantial improvement to current protein manufacturing methods[8]. To exemplify its broad application, data obtained for a recombinant antibody and the mammalian Target of Rapamycin (mTOR) are shown below.

PERFORMANCE

UCOE technology dramatically increases the proportion of high expressing cells within stably transfected pools

Figure 1 shows that UCOE technology dramatically increases the proportion of high expressing cells in stably transfected pools. CHO-S stable pools established with UCOE-containing EGFP vectors showed significantly higher percentage (70% and 80%) of highly expressing clones than those obtained with the non-UCOE vector controls.

UCOE technology enables easier and faster selection of stable, high yielding cell lines

Since the utilization of UCOE elements result in increased frequencies of highly expressing clones (Fig. 1), it is much

easier and faster to find high yielding clones with the productivity and stability required for large scale manufacturing (Fig. 2). Figure 2 shows expression levels of antibody protein by clones isolated from pools generated with UCOE and non-UCOE vectors.

Timeframe for generation of a high yielding cell line, screening only 80 clones

Day	
0	Transfected CHO-S cells (humanized IgG1 monoclonal antibody)
1	Selection and simultaneous cloning by limiting dilution
19	Grow up clones in 24-well plate + initial screen for productivity
26	69/80 clones positive
28	24-well to 6-well – quantitative screen for yield
33	Top 12 clones selected for further analysis in shake flasks
36	1st exhaustion assay set up
43	2nd exhaustion assay set up
46	1st exhaustion assay harvest
55	2nd exhaustion assay harvest
55	Selection of clone for further scale-up work

The experiment testing the timeframe was performed in serum-free media. At Day 55, following screening of just 80 clones, 8 high expressing clones were selected and evaluated with respect to titers. The resulting antibody titer of one of these clones from unoptimized CD CHO shake culture was determined to be 300 mg/L. This clone when cultivated in a 2 L non-optimized fed-batch bioreactor generated a titer of 1.4 g/L. The stability study using the top clones was initially performed under selective pressure and after 65 days the selection was removed and the evaluation continued for a total time of 135 days. This analysis showed that the expression of these clones was stable even after removal of the selective antibiotic (data not shown).

Application of UCOE technology to the expression of difficult proteins

The Target of Rapamycin (mTOR) protein, a 289 kDa kinase, proved to be a difficult protein to express due to its size and susceptibility to protease degradation. Our previous attempts to express mTOR using conventional, non-UCOE expression vectors were unsuccessful. Data presented in Figure 3 demonstrates that UCOE technology can aid in

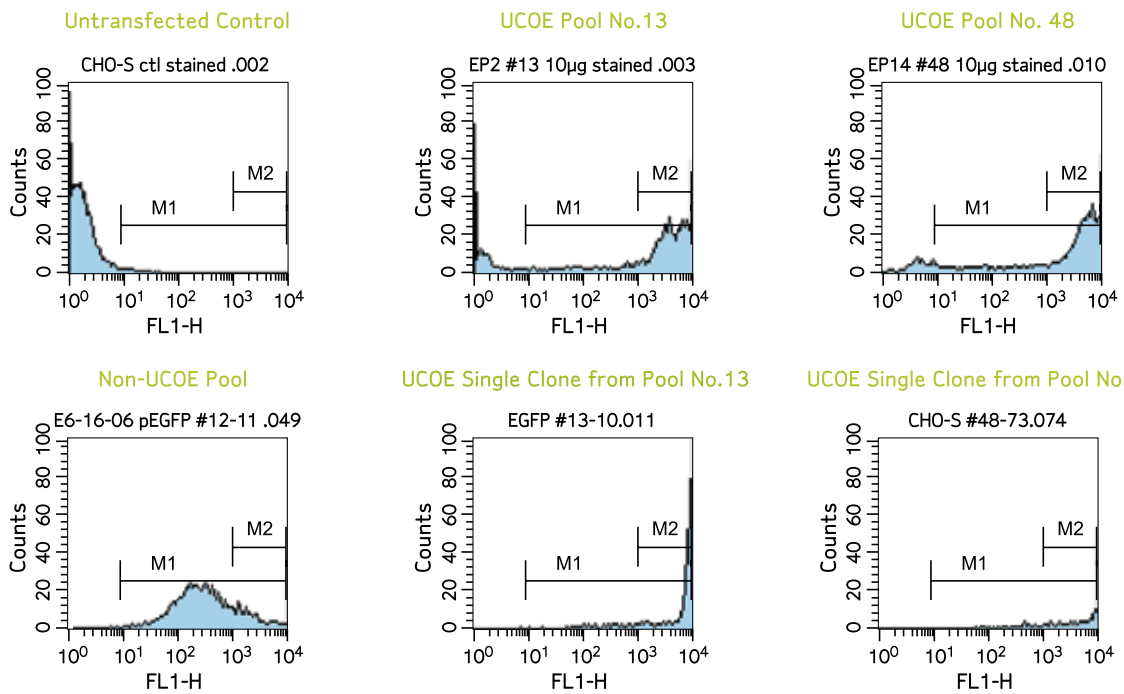


Figure 1.

FACS analysis of CHO-S cells expressing EGFP protein. Following stable transfection with UCOE-containing and non-UCOE control vectors the stable pools were analyzed by FACS. In addition, the pools were subcloned and the single clones subjected to the FACS analysis as well. The histograms show the increasing green fluorescence on a logarithmic scale (FL1-H) and the number of events on a linear scale (counts). Data demonstrates that more than 70% of cells in analyzed pools express the EGFP protein at a very high level compared with only 12% in a control pool. Furthermore, the clonal cultures isolated from the respective pools contained more than 98% cells expressing at a very high level.

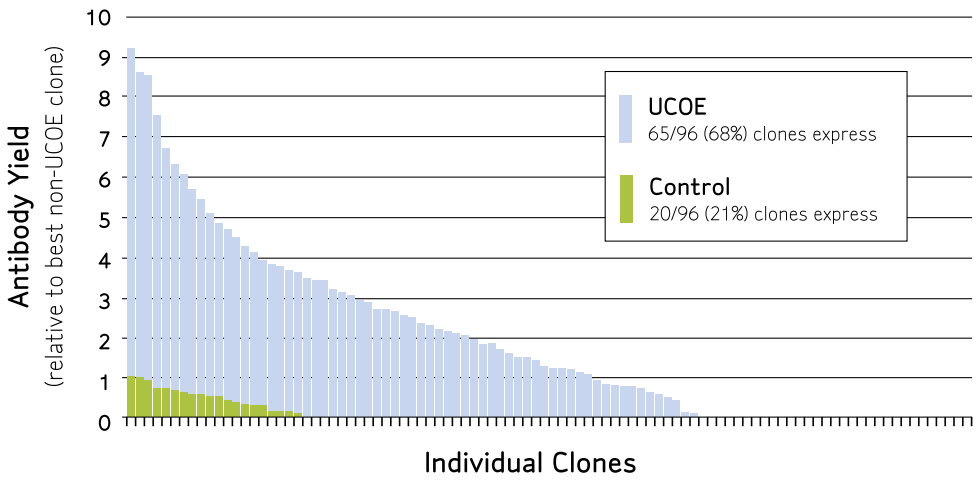
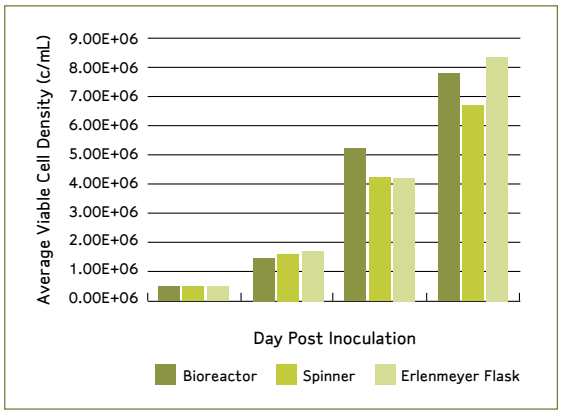


Figure 2.

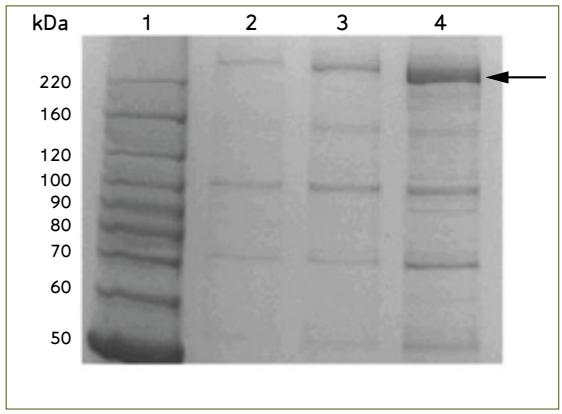
Expression levels of antibody protein produced by clones generated with UCOE and non-UCOE vectors. Separate plasmids containing heavy and light chains of antibody were co-transfected into CHO-S cells followed by drug selection and screening of 96 randomly selected clones. The expression level (titers of secreted protein) for the individual clones was evaluated using ELISA assay. Protein yields from UCOE-containing clones were several folds higher than those obtained from non-UCOE technology counterparts.

projects where expression of a protein presents a challenge. The mTOR production studies were carried out using a clonal line and three different culture formats (bioreactor, spinner and shake cultures). In this particular experiment, the protein expression levels for the 3 day cultures were as follows: 81 µg/L (shake culture), 236 µg/L (spinner flask), and 500 µg/L (bioreactor). It is worth noting that cell harvest was performed on day 3 where the cell density and the productivity of the cultures were not at their maximal levels. The average viable cell densities of all three cultures on day 3 were approximately 8 x 1 0E6 cells/mL as shown in Figure 3a. The purified from each culture were subject to Coomassie stained SDS-PAGE gel analysis. Figure 3b shows expression levels and quality of the purified mTOR. The study demonstrates the applicability of UCOE expression technology to projects where the protein expression may present a challenge.

Non-optimized Batch Production



a. Average Viable Cell Density



b. Coomassie Stained SDS-PAGE Gel

Figure 3.

Three different production formats used for expression of full length mTOR utilizing the UCOE system and the CHO-S cells. A Bioreactor, Spinner and an Erlenmeyer flask were each inoculated from the same cell stock and at the same cell density. a) All three culture formats are showing robust cellular growth and high viability until the day of harvest. The average viable cellular density was evaluated based on trypan blue exclusion. b) Coomassie stained SDS-PAGE gel showing purified mTOR. Lane 1: Molecular weight markers; Lane 2: mTOR expressed in an Erlenmeyer flask format; Lane 3: mTOR expressed in a Spinner format; Lane 4: mTOR expressed in a Bioreactor format. mTOR is approximately 289 kDa and its gel localization is indicated by the arrow. These results were further verified by western blot analysis (data not shown).

CONCLUSIONS

Addition of a UCOE element into a vector results in increased frequency of highly expressing pools that are stable for at least 100 days. Expression levels from the UCOE-containing vectors are 10 to 50 fold greater than those obtained from the non-UCOE technology counterparts. The technology allows for substantially-increased yields of recombinant proteins from both pools and clones, and enables production of gram level quantities of protein from stable pools in less than 2 months. Highly productive clonal lines can be isolated by screening less than 100 clones in less than 2 months. UCOE technology maximizes speed, stability, and yield of recombinant protein expression.

UCOE Expression Technology Applications

Production of therapeutic proteins: Preclinical studies and large scale manufacture

Cell-based screens: Consistent levels of gene expression, improved speed and reliability

Gene therapy: Fewer, more consistent integration events

Transgenic animals: More consistent expression across all cell types

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LICENSING OPPORTUNITIES

Non-exclusive licenses are available for use of the UCOE expression technology for research or manufacturing purposes.

For customers in North America, please contact Jack Zhai, Manager of Business Development – North America, **jack_zhai@millipore.com**, 508-572-2050.

For customers outside of North America, please contact John Wynne, Director of Business Development, **john_wynne@millipore.com**, +44 (791) 248-7375.

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