



UCOE™

Expression Technology

**Maximizes protein expression in
mammalian cells**

- ▶ Over 50% of UCOE-derived clones have higher expression than the best non-UCOE-derived clones
- ▶ UCOE-derived vectors produce substantially more expressing clones than non-UCOE-derived vectors
- ▶ Cell lines stable over 130 generations
- ▶ High-yielding cell lines can be derived in less than 60 days, without amplification
- ▶ Small (1.5-8kb) DNA elements, capable of making two protein chains per plasmid, e.g., antibodies
- ▶ Express Antibodies, Receptors, Enzymes, Cytokines, etc.
- ▶ Integrates with industry standard platforms, e.g, CHO cells, CMV promoters, plasmids, transfection agents, media, etc.

SPEED. STABILITY. SUCCESS.

UCOE (Ubiquitous Chromatin Opening Element) expression technology gives major improvements in gene expression in stably-transfected mammalian cells through effects on the structure of chromatin. UCOE expression technology prevents transgene silencing and gives consistent, stable and high-level gene expression irrespective of the chromosomal integration site. UCOE expression elements are small DNA elements (isolated from the area around house-keeping genes, which need to be active most of the time) that create a transcriptionally active, open chromatin environment around an integrated transgene, maximizing its potential to be transcribed into protein, irrespective of the position of the transgene in the chromosome.



The leader in bioprocessing solutions

Millipore is a valued partner to leading pharmaceutical and diagnostic companies worldwide. Our products and services are used in the production of many approved therapeutics and diagnostic kits currently on the market. Decades of experience utilizing the highest quality materials and proactive safety standards enable Millipore to deliver value and performance.

In addition to UCOE expression technology, Millipore offers a wide range of products and services for cell culture and diagnostic applications, including:

- EX-CYTE® growth enhancement media supplement
- Incelligent™ Insulin (recombinant human) and other cell culture supplements
- Application specific grades of Probumin™ BSA
- Over 60 BIOSCOT® monoclonal antibodies
- LucraTone™ supplements

For more information on these products and services, please contact Millipore customer service at 800.431.4505 (outside US 678.728.2246).



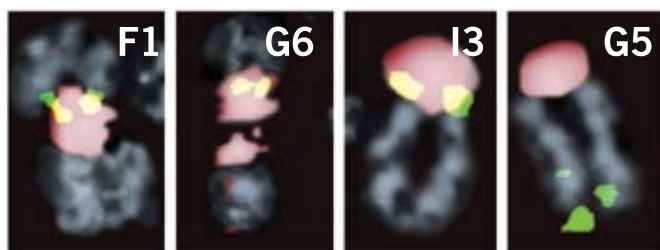
Successful Integration

The successful integration of a transgene into mammalian cells is largely dependent upon the chromatin structure around the integration site. Most mammalian chromatin exists in the closed (heterochromatin) form, which is transcriptionally silent. With conventional vectors, most integrations suffer from position effects and chromatin shutdown, which leads to gene-silencing and unproductive integration events. As a result, pools of stably-transfected cells generally show low productivity and stability, as only a few cells might be good producers, and gene silencing and overgrowth of non-producers rapidly diminish the overall productivity of the pool. This also makes it very difficult and time-consuming to find high-expressing stable clones needed for large-scale manufacturing.

Efficient transgene expression from a centromeric location

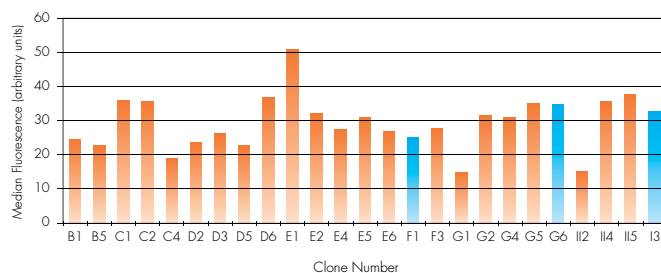
UCOE's have been shown to allow active expression of a transgene, even in the dense, transcriptionally silent centromeric region of the chromosome.

Figure 1. Fluorescent *In-Situ* Hybridization



Fluorescent in-situ hybridization (FISH) analysis of metaphase spreads of stably-transfected mouse L cells, showing metaphase chromosomes hybridized with a multimerized murine gamma-satellite repeat fragment to highlight centromeres (red staining) and the transfected gene construct to probe for the integrated transgenes (green staining). Co-localization of the two signals gives yellow foci within the red centromeric regions. Three 16A2-EGFP-transfected clones show three integration events (F1, G6, I3). Typical integrations within the chromosomal long arm seen with most of the clones are also shown (G5).

Figure 2. Clonal Gene Expression



Clones possessing a single copy of 16A2-EGFP; reporter (EGFP) gene expression was assessed by FACS analysis. The three light blue histogram bars highlight clones harboring a centromerically integrated transgene

Dramatic Increase of high-expressing cells

UCOEs dramatically increase the proportion of high-expressing cells within a stably-transfected pool of cells for over 130 generations. Inclusion of UCOE expression technology in the expression construct ensures that the integrated transgene is in an 'open' conformation, maximizing the potential of each integration event, and ensuring more consistent high level expression.

Figure 3 shows CHO-K1 cells transfected with hCMV-EGFP expression construct or an identical construct with UCOE expression technology inserted. Transfected pools were drug selected and analyzed for fluorescence level by FACs after 33 generations. (Similar results seen after 200 generations).

Benefits of UCOE Expression Technology

Boosts Expression

UCOE expression technology gives a 9- to 56-fold boost in the production level of a secreted protein (Erythropoietin). A single UCOE element placed upstream of the 5' end of the promoter and transgene is sufficient to boost expression twenty-fold or more.

Figure 4 shows CHO-K1 cells transfected with erythropoietin expression constructs with or without UCOE expression technology. Drug selected pools were assessed for erythropoietin production over an extended time period. Two experiments are shown, using two different vector backbones.

Rapid small-scale production of a monoclonal antibody

The productivity and stability of the individual cells allows pools to be a source for rapidly generating gram quantities of proteins in 3 to 4 weeks. This has potential advantages over transient transfection, minimizing the quantities of DNA and costly transfection agent, as well as allowing aliquots of the pool to be stored for future use if the protein is required at a later stage.

Figure 5 shows 3×10^7 CHO-S cells transfected with 90 μ g of an antibody expression plasmid either with or without a UCOE™, drug selection was applied and once sufficient cell number was generated, cells were seeded at a cell density that would allow growth to 10L. On day 16 a small non-feed addition was made to a proportion of the cultures (+opti). A fraction of the cells were grown and the data shown are an extrapolation of the yield if 10L of culture had been generated.

Isolation of antibody clones

Because the majority of cells are stable high-expressors, it is much easier and faster to find high-yielding clones with the productivity and stability required for manufacturing, as opposed to the "needle-in-a-haystack" approach using conventional vectors.

Figure 6 shows vectors encoding heavy and light chains (separate plasmids) co-transfected into CHO-S cells followed by drug selection and screening of 96 randomly selected clones.

Figure 3.

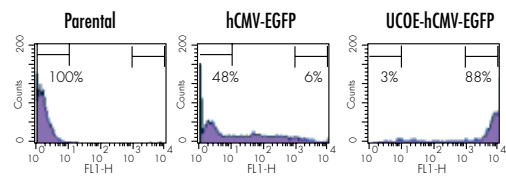


Figure 4.

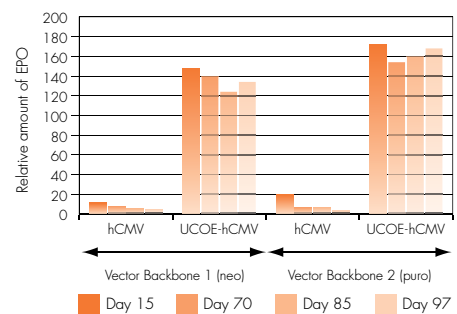


Figure 5.

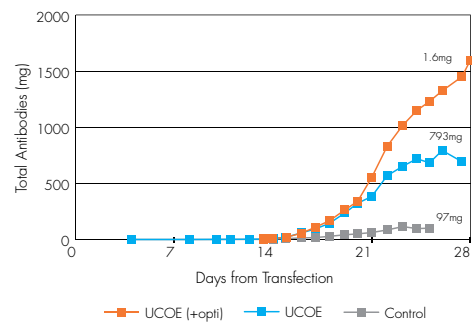
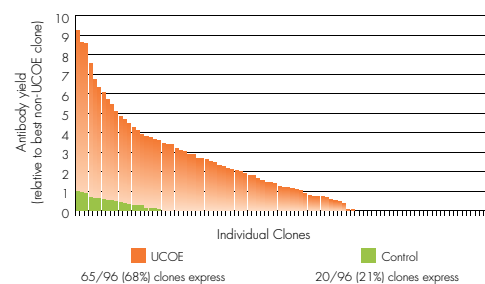


Figure 6.



Advantages of UCOE Expression Technology

Research	Bioprocessing
<p>Rapid: Grams of protein in <30 days from stable pools of cells</p> <p>Boosts: Expression levels up to 20-fold higher than conventional vectors without UCOEs</p> <p>Stable: Pools of cells stable over 130 generations</p> <p>Reproducible: Stably-transfected pools can be frozen, providing working stocks for further production without the need for re-transfection</p> <p>Easy to use: Small DNA elements, capable of making two protein chains per plasmid, e.g., antibodies. Flanking elements are not required.</p> <p>Versatile: Antibodies, EPO, GPCRs, Enzymes, Cytokines, etc., expressed</p> <p>Compatible: Enhances industry standard platforms, e.g., CHO cells, CMV promoters, etc.</p> <p>Serum-free: Protein-free media used throughout</p> <p>Scalable: Up to 100L in shake flasks and fermentors</p> <p>Transferable: UCOE-containing vectors are also suitable for large-scale manufacture</p> <p>Advantages over transient transfections: gram quantities of protein without the high costs of transfection agent for large-scale transients</p>	<p>Rapid: High-yielding cell lines can be derived in <60 days, without amplification</p> <p>More productive integration events: UCOE vectors give substantially more expressing clones than non-UCOE vectors</p> <p>Higher expression: >50% of UCOE clones have higher expression than the best non-UCOE clones</p> <p>Stable: Cell lines stable over 130 generations</p> <p>Amplification not required: UCOEs boost production without amplification, with 1-5 copies per cell, reducing the chance of instability caused by the high copy numbers seen with amplification</p> <p>Easy to use: Small (1.5-8kb) DNA elements, capable of making two protein chains per plasmid, e.g., antibodies</p> <p>Versatile: Antibodies, EPO, Receptors, Enzymes, Cytokines, etc., expressed</p> <p>Compatible: Enhances industry standard platforms, e.g. CHO cells, CMV promoters, etc.</p> <p>Serum-free: Chemically-defined, protein-free media used throughout</p>

Uses of UCOE Expression Technology

Research	Bioprocessing
<ul style="list-style-type: none"> • Rapid production of up to gram quantities of proteins for research, animal models, toxicity studies, etc. • Stable pools transfected using UCOE vectors yield substantially more antibody and more EPO than pools transfected using non-UCOE control vectors. • 1.6g of monoclonal antibody and 2.5g of EPO produced using UCOE-containing vectors from CHO-S stable pools grown to 10L scale within 28 days of transfection using shaker flasks. • Using pools transfected with UCOE vectors is a cost-effective alternative to scaled-up transient production, as grams of protein can be prepared within a month of transfection, providing significant savings in the costs of transfection agent and vector DNA • Speeding up production of gram quantities of proteins offers substantial time-savings in the race to get products into the clinic 	<ul style="list-style-type: none"> • Generation of manufacturing cell lines • Over two-thirds of UCOE clones are positive, with over half giving higher yields than the best non-UCOE clones • UCOE clones are stable >130 generations, without drug selection • UCOEs reduce the number of cell lines that need to be screened in order to obtain high productivity clones • Yields of >800mg/L of antibody have been achieved in shake flasks from clones generated using UCOE vectors, w/up to 1.4g/l in 2L bioreactors
	<h3>Other potential applications</h3> <p>Generation of highly stable transgene expressing cell lines for drug discovery, cell-based screening, gene therapy and transgenic animal generation, where more consistent expression throughout the a variety of cell types or tissues is required.</p>

To learn more about UCOE supplements:

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Licensing Opportunities: UCOE expression technology has been licensed to a number of pharmaceutical and biotechnology companies across North America, Europe and Japan and is covered by 55 issued and pending patents worldwide. Non-exclusive licenses are available for use of the UCOE expression technology for research or manufacturing purposes.

References:

Antoniou, M. et al "Transgenes encompassing dual-promoter CpG islands from the human TBP and HNRPA2B1 loci are resistant to heterochromatin-mediated silencing" *Genomics* 82 (2003) 269-279

Benton, T. et al "The use of UCOE vectors in combination with a preadapted serum free, suspension cell line allows for rapid production of large quantities of protein" *Cytotechnology* 38 (2002) 43-46.

Williams, S. et al "CpG-island fragments from the HNRPA2B1/CBX3 genomic locus reduce silencing and enhance transgene expression from the hCMV promoter/enhancer in mammalian cells" *BMC Biotechnology* (2005), 5:17

