# Rapid development of a serum-free, suspension-adapted CHO cell production process

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## Abstract

Chinese Hamster Ovary (CHO) cells are the industry standard for the production of recombinant therapeutic proteins. A suitable CHO production cell line should be able to grow to high cell density in fed-batch bioreactors, maintain high viability and stable expression throughout the duration of the production window, and grow in well-defined, serum-free media. The advantages of eliminating serum from the production process are well known and include lower costs and reduced risk of introducing adventitious agents. We have set out to generate a cell line with these favorable endpoints starting with CHO DG44 cells, an attached, serum-dependent cell line. Using a blend of proprietary media and an iterative serum-weaning process, we have created a stable, serum-free cell line that shows good growth and increased productivity in comparison to its progenitor cell line. This cell line fulfills other important criteria required for a production cell line including rapid recovery from transfection and ability to be cloned in serum free conditions.


## Cell Culture

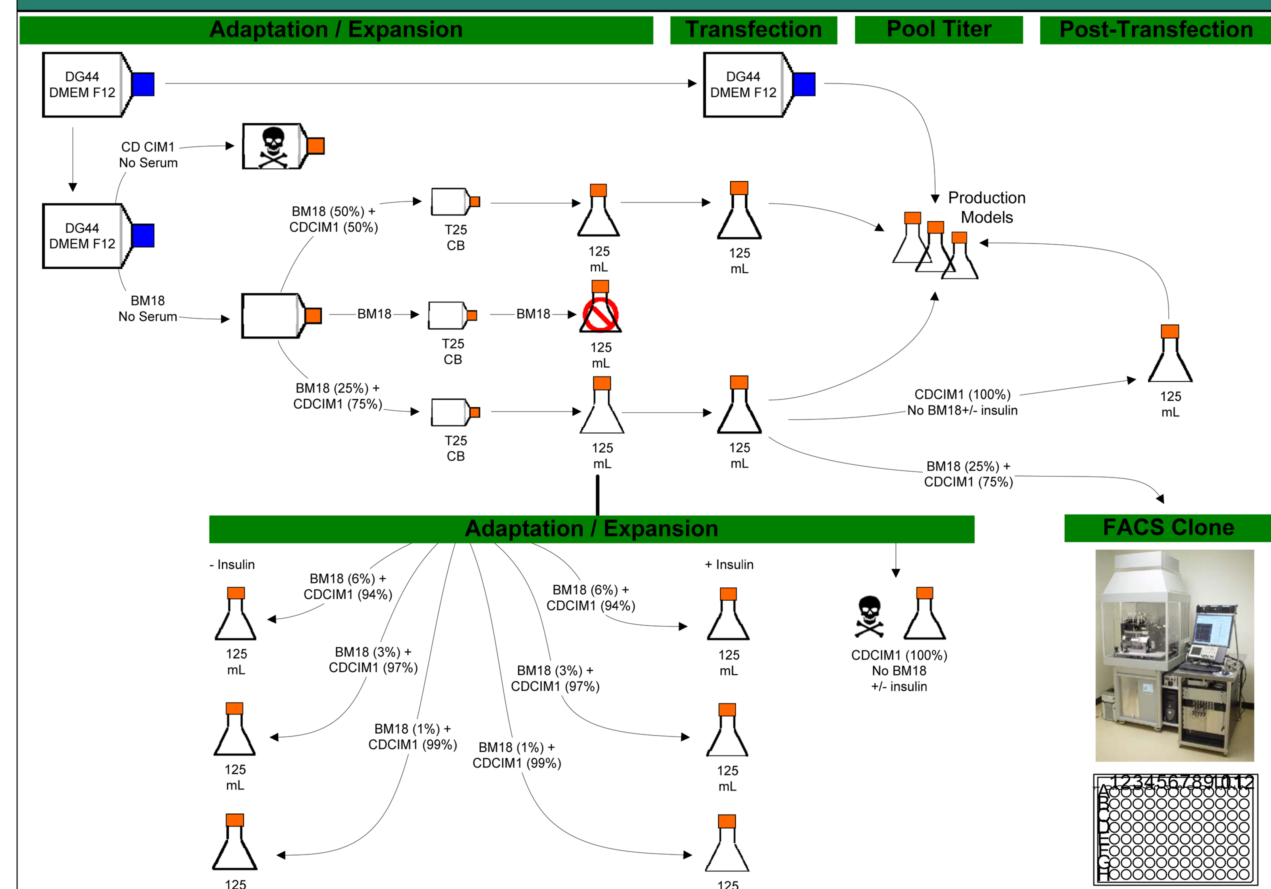
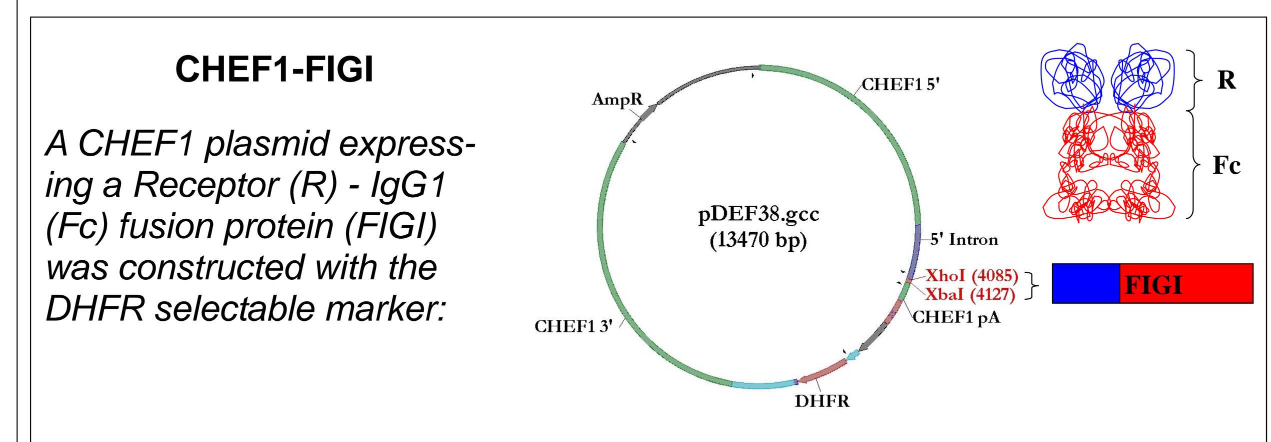



**Figure 1.** Cell culture process showing the DG44 adaptation pathway from DMEM F12 (lean media) to CD-CIM1, a proprietary rich production media.

## CHEF1® Expression Vector

The CHEF1 expression system was used to express the FIGI reporter protein (see below). CHEF1 takes advantage of the Chinese Hamster Elongation Factor (CHEF) promoter to drive high level, stable expression of heterologous genes in CHO cell lines. The CHEF1 promoter drives expression many fold better than the human EF promoter in CHO cells, presumably because hamster transcription factors easily recognize the hamster promoter (Running Deer and Allison, 2004*). CHEF1 vectors employ four regions of the hamster EF gene: 1) a 4 Kb upstream promoter and flanking region, 2) a single intron just upstream of the start codon, 3) the poly-adenylation (polyA) domain, and 4) 4Kb of 3' untranslated region (UTR) downstream of the gene-of-interest. The promoter, intron, and 3' UTR are all required for high level gene expression as truncations and deletions result in significantly reduced expression levels.

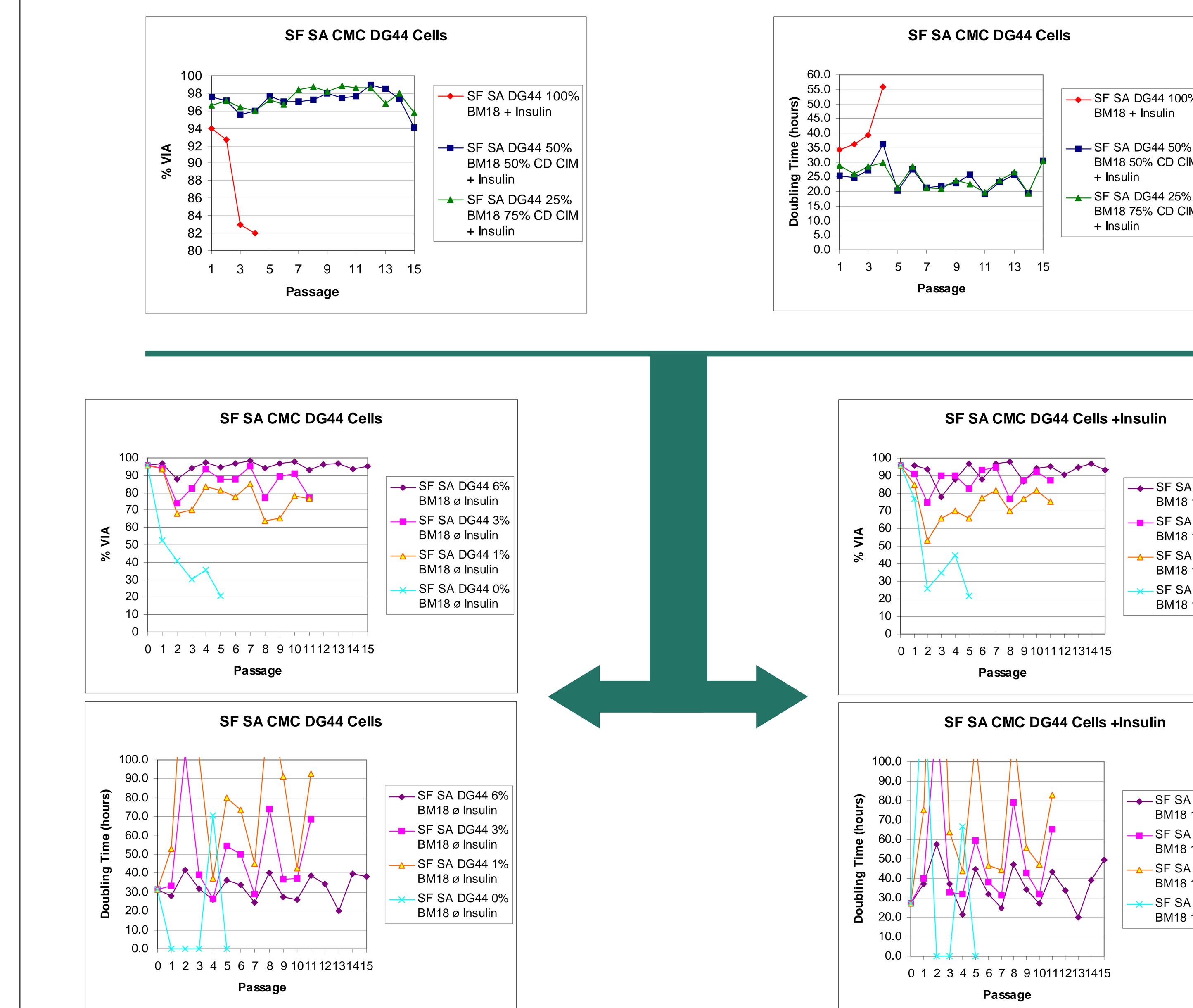



*Running Deer, J., Allison, D.S. High-level expression of proteins in mammalian cells using transcription regulatory sequences from the Chinese hamster EF-1a gene. *Biotechnology Progress* 20: 880-889; 2004.

## Cell Line Adaptation

CHO DG44* cells require serum for survival and grow as adherent cultures. One goal of this research was to adapt DG44 host cells to grow in suspension, in a proprietary serum-free media designated CD-CIM1™. This is a CMC ICOS proprietary chemically defined media developed to promote growth of a transfected DG44 cell line. To achieve serum independent, suspension growth, the attached DG44 host cells were first introduced into BM18, an animal component free (ACF), non-chemically defined proprietary media containing soy hydrolysates and insulin, then into blended BM18:CD-CIM1, and finally into BM18:CD-CIM1 in suspension. After three or more passages at greater than 90% viability cells were passed into new adaptation media.

*The Figure 1 adaptation pathway starts with attached DG44 cells growing in DMEM F12 transferring into BM18 with no serum, then into BM18:CD-CIM1 blended media without serum. The cells were first adapted to these serum-free media in tissue culture (TC) flasks then to suspension growth in their respective media. The graphs below show robust shake flask suspension growth in the blended media but poor growth in BM18 alone. The 25:75 (BM18:CD-CIM1) blend grew slightly better than the 50:50 blend, and was closer in composition to the desired 100% CD-CIM1, so it was further adapted to reduced BM18 blended media (i.e. 6%, 3%, 1% and 0% BM18 +/- insulin). Only the 6% BM18 (94% CD-CIM1) blend supported greater than 90% viable growth but the doubling times were longer than in the 25:75 blend. Cells did not grow in CD-CIM1 alone.*

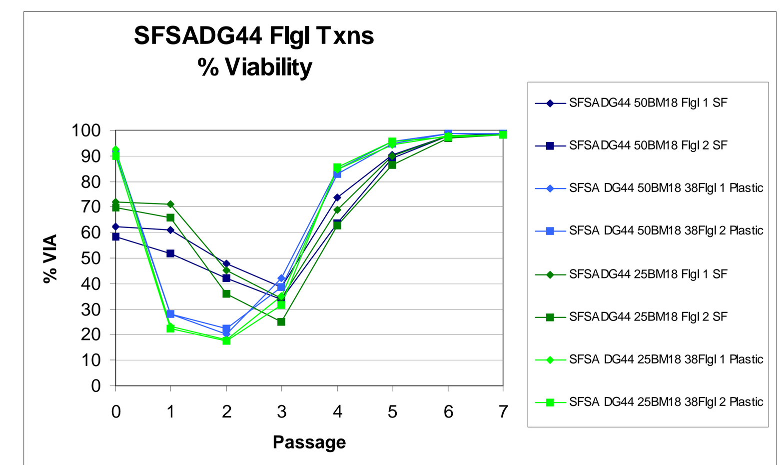



*CHO DG44 is a dihydrofolate reductase (DHFR) deletion mutant CHO cell line obtained from Lawrence Chasin (Columbia University). The origin of DG44 cells has been described by Urlaub et al., 1986 (Effect of Gamma Rays at the Dihydrofolate Reductase Locus: Deletions and Inversion. Somatic Cell and Molecular Genetics. 12:555-566).

## Serum Free Transfection

The 25:75 and 50:50 (BM18:CD-CIM1) adapted cells showed robust and stable growth performance and were used to test transfection capabilities. A CHEF1-FIGI reporter plasmid was electroporated into these cell lines and selected for DHFR by growth in their respective media lacking HT (hypoxanthine and thymidine). Transfected cells recovered in media plus HT for 3 days in TC flasks (plastic) or 6 days in Shake Flasks (SF) and then were selected for 6 or 7 passages (graph below).

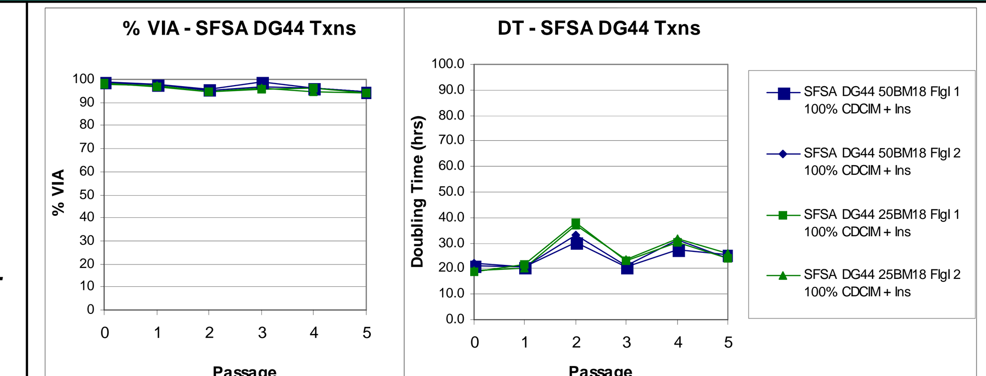
*The percent viable cells dropped rapidly after transfection when plated in TC flasks and stayed low for two passages. In shake flasks, the viability dropped through passage number three. Despite the different viability loss kinetics, the recovery profile looked similar starting at passage three for both attached (plastic) and suspension growth (SF). All cultures reached greater than 90% viability on passage 6 or 7, or approximately 14-16 days post-transfection.*




## CDCIM1 Adaptation of Transfected Cell Line

The serum-free, suspension adapted cells grown in 25:75 and 50:50 (BM18:CD-CIM1) blended media adapted well to growth in 100% CD-CIM1 only after transfection with CHEF1-FIGI.

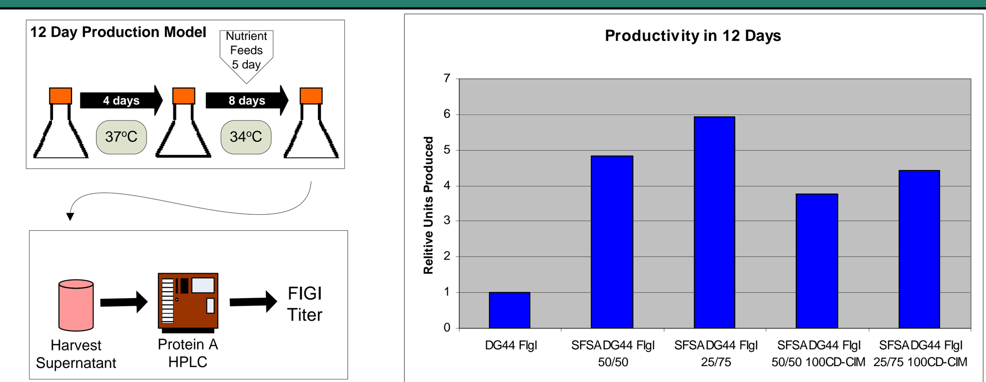
*Transfected (CHEF1-FIGI) cells were recovered to greater than 90% viability in 25:75 or 50:50 blended media then passaged into 100% CD-CIM1 starting at Passage "0" on the graph.*




## Productivity—Titer

Transfected pools expressing FIGI were grown in 12 day production models and assayed by Protein A HPLC (see diagram). Serum-free, suspension adapted (SFSA) cells grown in 50:50, 25:75, or 100% CD-CIM1 (adapted from both 50:50 and 25:75 blends) all produced more reporter protein than the attached, serum-dependent DG44 control cells.

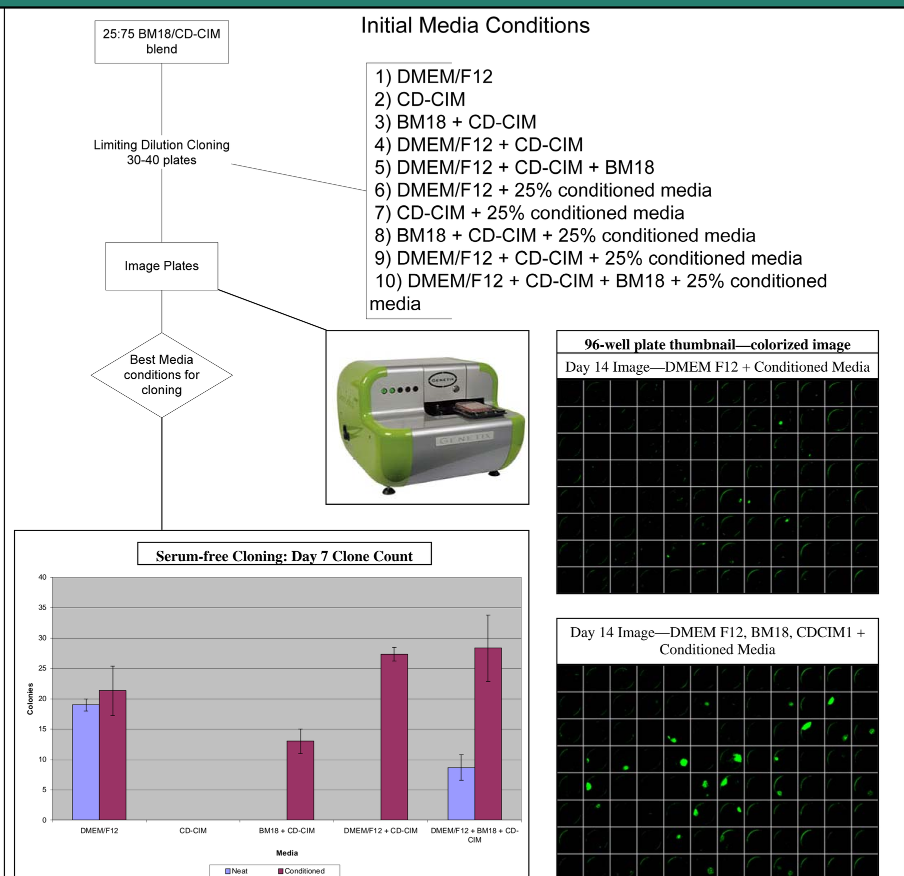
*Titers are relative values compared to DG44 (1 unit)*




## Serum Free Cloning

Preliminary attempts to clone serum-free cells in media without serum indicated that a combination of lean (DMEM F12) and rich (CD-CIM1 or BM18) media yielded the highest number of clones. The flow diagram outlines a strategy for developing a useful cloning media that yields clones in greater than 25% of wells in 96-well plates by limiting dilution. A blend of DMEM F12, BM18, and CD-CIM1 plus 25% conditioned medium gave the best growth and number of clones on Day 7 (see graph). By Day 14 the number and size of the clones in DMEM F12 alone was considerably reduced compared to DMEM F12, BM18, and CD-CIM1 plus conditioned medium, whereas the clonal growth in this blended serum-free media remained consistent (see 96-well plate images).

*Cells were diluted to 1 cell per well in their respective cloning media listed at right (3 to 4 96-well plates per condition). Plates were imaged on the Genetix CloneSelect imager starting on Day 1 after cloning and repeated regularly throughout a two week growth period. Clonal colonies were identified by the imager (see example at right) and confirmed monoclonal by visual inspection of Day 1 images.*




## Summary—Future Directions

- CHO DG44 were successfully adapted to suspension growth in a blend of serum-free BM18 and CD-CIM1.
- These serum-free, suspension-adapted (SFSA) cells can be transfected and selected with DHFR in about 14 days.
- Expression of the FIGI reporter protein was considerably better in the SFSA pools compared to DG44.
- Once transfected, the cells easily adapt to chemically defined CD-CIM1 media without significant loss of viability.
- A blend of DMEM F12, BM18 and CD-CIM1 plus conditioned media suitable for serum-free cloning was developed.

*The next stage of this project will be to develop a FACS based sorting and cloning program to enrich for high expressing clones. To this end we have developed an antibody staining enrichment protocol to sort the brightest (highest expressing) top 10 percent of cells. These isolated cells, grown out as a pool for 10 days, retain their high expression profile as seen in these FACS histograms. This enriched pool will serve as the starting population for FACS based single cell sorting and subsequent cloning.*