# Establishment of Recombinant CHO Cell Lines Under Serum-free Conditions

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**Abstract:** Due to regulatory requirements today's state-of-the-art cell culture production processes are performed in well defined serum- or protein-free media. Nevertheless, development of recombinant production cell lines is often performed under serum-containing conditions. Accordingly, after clonal selection the cells have to be adapted to serum-free medium, which bears the risk of losing productivity or product quality and significantly extends development time. Through extensive medium screening and testing of multiple transfection methods a CHO dhfr$^-$ host cell based procedure was established, allowing the serum-free performance of all steps being essential for the development of a recombinant cell line

**Key words:** recombinant; CHO dhfr-; serum-free; transfection; selection; amplification; development time; medium screening; single cell cloning; cell-specific productivity; medium optimization; cell line development

## 1. TRANSFECTION

Testing different methods, transfection under serum-free (SF) conditions was enabled using Nucleofection™ technology.

Nucleofection™ was successfully applied to various CHO cell lines, although transfection efficiency differed between the tested cells (Fig. 1A).



*R. Smith (ed.), Cell Technology for Cell Products*, 57–61.

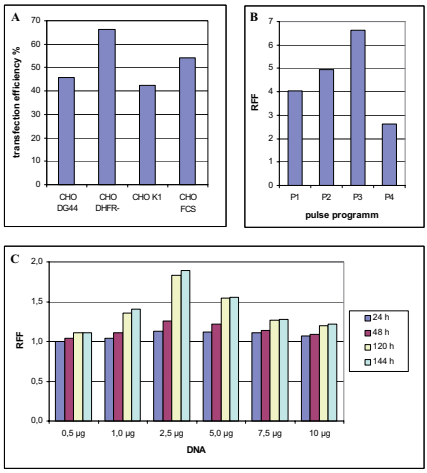


*Figure 1.* Optimization of cell transfection.

CHO cells were transiently transfected with an expression vector comprising a GFP reporter gene. Protein expression was quantified by fluorescence measurement; RFF = relative fluorescence factor; **A** Transfection efficiency for various CHO cell lines; **B** Optimization of the transfection efficiency of a CHO DHFR$^-$ cell line applying different pulse programs and **C** various DNA concentrations.

By optimizing the pulse program (Fig. 1B) as well as cell number / DNA ratio (Fig. 1C) a high efficient method for SF transfection was developed.

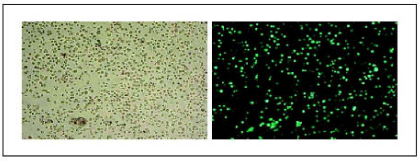

*Figure 2.* CHO DHFR$^-$ cell line transfected under optimized conditions.

Cells transfected with an expression vector comprising a GFP reporter gene are shown with bright field (left) and fluorescence microscopy (right).

## 2. SINGLE CELL CLONING

Under SF conditions the successful generation of clones derived from a single cell strongly depends on the medium used. Substantial medium screening and optimization yielded in a few SF formulations that are suitable to support single-cell cloning by limiting dilution.

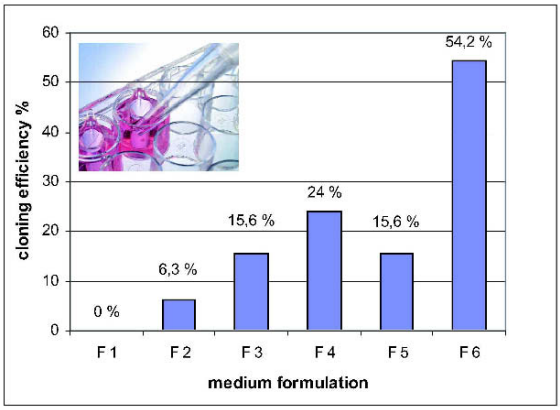


*Figure 3.* Cloning efficiency in different media.

To assess the suitability of different serum-free medium formulations (F1–6) for single cell cloning, in an initial screening 5 cells per well were seeded into 96-well plates and the number of wells showing cell growth were evaluated.

Choosing an appropriate medium single cell cloning, seeding one cell per well into 96-well plates, was enabled.

During the development of a growth factor producing cell line, single cell cloning gave rise to a five-fold increase of cell-specific productivity compared to the cell pool obtained after stable transfection of a CHO DHFR$^-$ cell line.

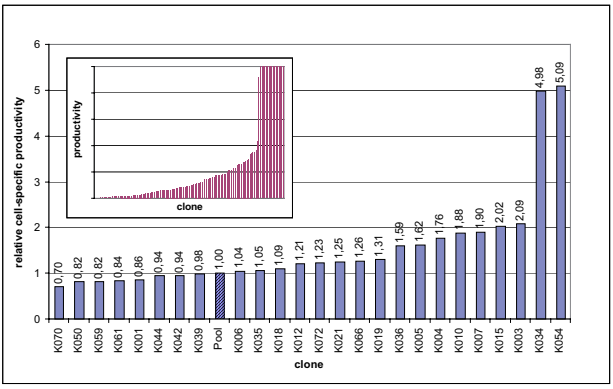


*Figure 4.* Productivity of single cell clones compared to the starting cell pool.

The cell pool was obtained by transfection of a CHO DHFR$^-$ cell line with a growth factor encoding gene and subsequent selection by hypoxanthine / thymidine deprivation. Several hundred clones were screened for productivity (insert). Cell-specific productivities for selected clones are shown in relation to the starting cell pool (set to 1).

## 3. MEDIUM OPTIMIZATION

Further increase of the cell-specific productivity of single cell clones was obtained by optimizing the SF medium. Various medium modifications were tested and an increase of cell specific productivity of up to 400% was reached.

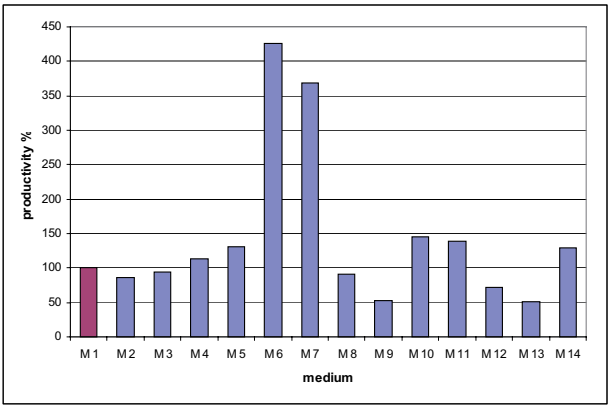


*Figure 5.* Medium optimisation.

Cell-specific productivity of a growth factor producing single cell clone grown in different medium modifications (M1-14). M 1, the medium formulation used to generate the recombinant cell line and the single cell clones, was set to 100%.

## 4. CONCLUSION

A method based on CHO dhfr$^-$ host cells was established, which allows the SF development of recombinant cell lines for the production of biopharmaceutical proteins.

All development steps, including transfection, selection, amplification and single cell cloning were performed under SF conditions.

In addition to regulatory compliance the established procedure significantly shortens the time for cell line development compared to a serum-containing process.