Leibniz Universität Hannover
Institute of Technical Chemistry, Callinstr. 3, 30167 Hanover
Partner in Research
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Selection of High-Producing Cells via Cell Sorting using an Affinity Matrix

Larissa Behr[1], Pierre Moretti[1], Fabienne Anton[1], Cornelia Kasper[1], Thomas Scheper[1]
[1]*Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover, Germany*

Abstract

A limiting factor in protein production using animal cells is often the low productivity of the cells. Several ways exist to optimize the yield of a cell culture process, e.g. variation of culture conditions, composition of medium or feeding strategies. But the basis of a production process is a stable, robust, long-living and high-producing cell line. Especially the selection of those high-producing clones is a major challenge in biotechnology. The target protein production of such rare cells is energy consuming which could result in low growth rates. This could lead to overgrowth of non- or low-producing cells and therefore to a low product yield in a process. The selection of high-producing clones is usually performed by limited dilution methods in 96-well plates (single clone selection). After a first selection in producers and none-producers, the productivity of every chosen clone has to be determined via a batch cultivation and the determination of the productivity afterwarts. This method is very expensive and extremely time and labour intensive.
The aim of this study was the increase of productivity of a mouse hybridoma cell line via a surface affinity matrix staining and the selection of high-producing cells via FACS (Fluorescence activated cell sorting). Afterwards the productivity of the new cell population was determined with batch cultivations in spinner flasks. This method reduces the number of batch cultivations and the time distance to a following cell sort to further increases the productivity of the cell population.

Materials and Methods

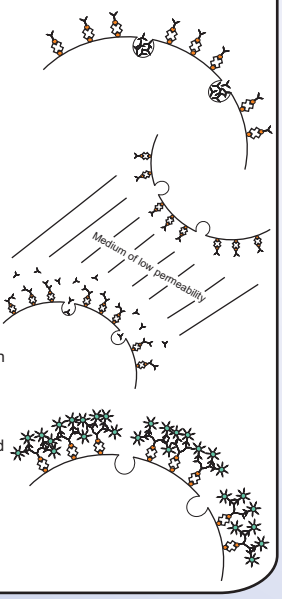
Cell lines: Mouse hybridoma cell line IV F 19.23 producing antibodies against penicillin G amidase.
Medium: SFM (Serum Free Medium) for hybridoma culture (Invitrogen) with the addition of 0,5 mg/ml Penicillin and Streptomycin (PAA Laboratories GmbH).
Cell density: Trypan blue exclusion method
Antibody concentration: Mouse-IgG ELISA (Roche Applied Science)
Cell sorting: The cell sorting was done with a FACS Vantage SE (BD Bioscience).
Cell cultivation: The batch cultivations were performed in 100 ml working volume spinner flasks (Techne) in an incubator at 37°C, 25 rpm and 5 % CO_2 atmosphere. Starting cell density: $0{,}15*10^6$ cells/ml.

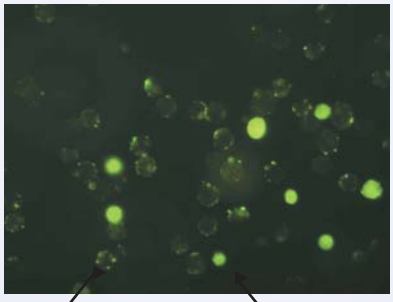
Affinity matrix staining

1. Step:
The affinity matrix staining of the hybridoma cells starts with the binding of first Biotin, then Avidin and finally Biotin labled antibodies.

2. Step:
Capturing of the secreted IgG1 on the cell surface. The low permeability of the medium inhibits the diffusion and binding of antibodies produced by different cells. Consequently none-producing cells will have no bound IgG1 on their cell surface.

3. Step:
Binding of FITC labled antibodies to the bound IgG1. Low-producing cells will be less stained than high producing cells. None-producing cells will not be stained at all.



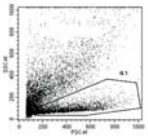


Cell with affinity matrix staining

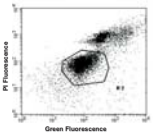
Dead cell

Gating during the sorts

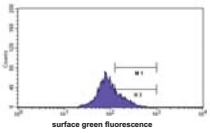
The cell sorting was performed using a flow in air sorter from BD Bioscience. Shortly before each sort the cells were stained with Propidium Iodide (DNA Dye) to divide the living from the dead cells. As the picture to the left shows the dead cells give a high fluorescence signal. Therfore, they have to be excluded to avoid the selection of dead cells falsely assuming to be high producing cells.



1) Selection of living cells: The graph shows the the size vs. granularity of the cells. The cells with the lowest granularity (mostly live cells) are gated.



2) Selection of living cells: A further selection of living cells is done in this diagram where only the cells with the lowest propidium iodide staining are gated.



3) Sorting of 20 % of living cells with the highest surface fluorescence asuming to be the high-producing cells.

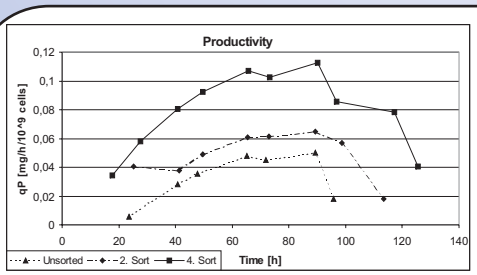


Figure1: Productivity of unsorted, twice and four times sorted cells during the spinner cultivations. The highest productivity could be seen between 60 to 90 h while the cells where in the stationary phase.

Results: Cell sorting

The productivity of the sorted and unsorted cells was determined via triple batch cultivations for each cell population in 100 ml spinner flasks. Cell density and antibody concentrations were determined twice a day. During every cultivation, the product concentration in the supernatant increased the whole time.
The productivity was calculated only for the exponential and stationary growth phases, which is very short with this cells. Assuming that the dying or dead cells later in the cultivation would discharge the earlier produced antibodies from their inside into the suspension, giving false productivity results. The calculations were done with the differential method using the equation shown below. Here the differential of the 4th polynom was used to calculate the productivity. The results are shown in figure 1.
The productivity could be increased through the 4 sortings from about 0,047 to 0,107 mg/h/10^9 cells. This is an increase of productivity during just four sortings of more than 125%.

$$qP_t = \frac{P'_t}{x_t}$$

Equation used for the calcuation of the productivity.

Conclusion

The results obtained in this research clearly show that cell sorting after an affinity matrix staining is a suitable method to increase the product yield of hybridoma cell culture processes. The use of a cell sorter ensures that the selection of an high amount of high producing cells is fast and therfore not as time consuming as the single cell dilution method using 96-well plates. It is important to exclude the dead cells from the sort because they give a high fluorescence signal which can be assumed to be high-producing cells. The batch cultivations and the calculation of productivity after each sort ensures that the results are reliable and not dependend on the cell density as often during the first selection steps using the single cell dilution method. The productivity could be increased even further doing more sortings.