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Scientific Poster

From Screening to Small Scale GMP Biomanufacturing:
Exploring a simple, unified platform strategy for handling a range of cell culture needs

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Summary

This poster discusses a process for a 12-week clone development process. The process starts in silico, and progresses through screening and clone selection in 96-deep-well plates up to five liter shaker flasks. Machine learning and protein engineering make it fast and easy to generate large numbers of high expressing stable cell lines. Such cell lines are moving biomanufacturing from mega-scale towards smaller efficient processes. To select the best of many candidates, tools for clone screening, selection and scaling up have become more important than ever.

The ready availability of high-expressing cell lines makes it possible to use incubation shakers instead of bioreactors for many bioproduction needs. Incubation shakers are already standard issue for cell line screening and development. They therefore provide a simple and easily validated path from clone screening all the way to cGMP manufacturing.

Cell Line Development Process

Candidate Generation
The cell line development process starts with in silico optimization combining codon optimization algorithms, a secretion signal toolbox, flexible expression vector configurations and high productivity CHO-K1 cell lines. Machine learning combined with *in vitro* screening is used to consider the end product and final process from the onset of the project. Process development can be done in parallel with clone development, thus reducing the number of steps and shortening the overall project time.

Screening
Screening is performed using 96-well plates in an automated platform and either transient HEK cells or stable CHO cells.

Multitron incubation shakers configured for 96-well plates are used (3 mm shaking throw/1000 rpm mixing). The shakers control all critical running parameters (agitation, temperature, CO_2) and have active humidification to minimize evaporative losses. This ensures consistent and reproducible results and scaling across different working volumes. Each shaker has a capacity of up to 80 plates (7,680 wells). This makes it possible to test large numbers of clones and running conditions in triplicates, and to generate statistically significant data in every screening pass. Following initial screening in 96-well plates, the most promising clones are transferred to 24-well deep plates and further evaluated prior to scaling up (Fig. 1).

Down Selection
After initial screening and down selection of stable CHO cell lines, the top candidates are ranked and selected for productivity. The 8 best clones are then cultured in 125 mL flasks for 14 days in a Multitron with 25 mm orbit at 150 rpm (Fig. 2).

Scale-Up and Production
Following down selection the cell culture can be scaled up further using incubation shakers. One Multitron incubation shaker can hold 7 Thomson 5 Liter flasks, each with a working volume of 2.4 L. This translates to 17 liters per shaker and 50–85 g of a typical IgG1. A triple stack of Multitron shakers has the capacity for 50 L of cell culture. In other words it is possible to produce 150–255 grams of antibody in a normal 10 to 14 day campaign using three incubation shakers. This is sufficient materialfor both pre-clinical studies and early clinical work.

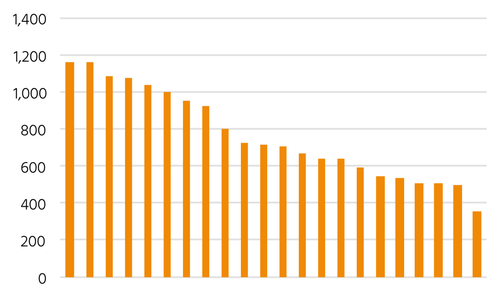

Figure 1:
Productivity of the top 22 clones derived from stable transfection of a transposon-based IgG1 expression construct in HD-BIOP3 GS null CHOK1 cells (Horizon Discovery). Cells grown 7 days in a 24-well deep well plate under nonoptimized conditions.

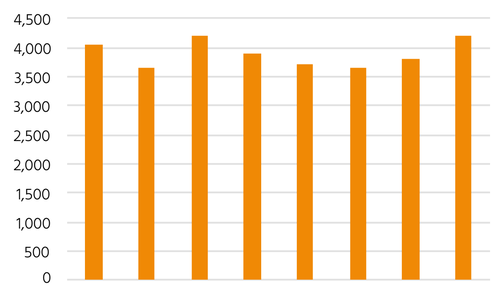

Figure 2:
Productivity of the 8 most productive clones derived from stable transfection of a transposon-based IgG1 express on construct in HD-BIOP3 GS null CHOK1 cells (Horizon Discovery). Cells grown in non-optimized 14 day fed batch 125 ml shake flask culture. The specific productivity of clonal isolates was >40 picograms/cell/day.

The Cell Culture Platform

Significant demands are placed on a platform used to develop and scale a cell line from screening in 96-well plates to 5 Liter flasks. Requirements change as working volumes change as the process moves from screening to development and to production. For example, cell culture in 96-well deepwell plates requires far higher agitation and mixing than work in shake flasks or 24-well plates. A system for 96-well plates uses a 3 mm shaking throw with 1000 rpm agitation, while work in shake flasks and 24 well plates is done using a 25 mm shaking throw and 100-150 rpm.

In order to compare results from different scales and across multiple instruments growth conditions must be kept uniform, and parameters influencing cell growth and productivity controlled. The Multitron incubation shaker allows complete control of mixing, temperature, and CO_2 concentration. It is also equipped with active humidification. This ensures a consistent growth environment from 1 milliliter volumes in 96-well plates to 5 L flasks.

96-Well Plate Cell Culture: Considerations

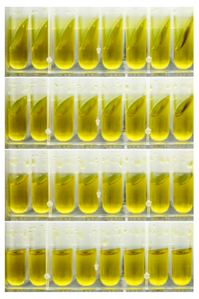

Figure 3:
Mixing in 96-well deep well plates using a Multitron 3 mm orbit shaker. 500 µm/well. Efficient mixing starts at 800 rpm and peaks at 1000 rpm.

Mixing: Standard incubation shakers are not configured for work with 96-well plates. For efficient oxygen transfer and optimal cell growth in small wells high speed mixing and small shaking orbits are required (Fig. 3). An earlier study (Ref.) shows that a 3 mm shaking throw and 1000 rpm mixing gave higher protein expression and CHO titers as much as 3,000% higher compared to plates in standard 25 mm orbit shakers and lower speeds. The same study also showed that efficient mixing in plates does not start until 800 rpm.

Throughput and Automation: In ATUM's laboratory the switch to 3 mm shakers and microtiter plate based screening allowed a 17 fold increase in throughput and enabled automation of the protein purification, simultaneously freeing up resources and reducing variability due to human error (Tab. 1).

Evaporation: Evaporative losses must be considered because of the small volumes used in plates, and the extended process times (10–14 days). Active humidification is employed to limit such losses over the course of the experiment.

Uniformity: When selecting clones grown in triplicates across up to 80 96-well plates stacked and distributed across a large shaking surface tightly controlled uniform conditions are of critical importance (Fig. 4). The accompanying 3D temperature map of the interior of the Multitron illustrates its ability to keep a consistent temperature spread throughout.

	25 mm orbit shaker	3 mm orbit shaker
Culture volume	10 ml	1 ml
Average Yield	55 mg/l	66 mg/l
Growth time	7 days	7 days
Cultures/shaker layer	112	1,920*
Purification	Human	Automated
* 4 layers of plates possible		

Table 1:
Comparison of expression of human IgG1 in HEK293 cells from 1 ml and 10 ml cultures grown under different agitation conditions. Codon optimization was performed using ATUM's GeneGPS. Expression vectors were not optimized using ATUM's VectorGPS.

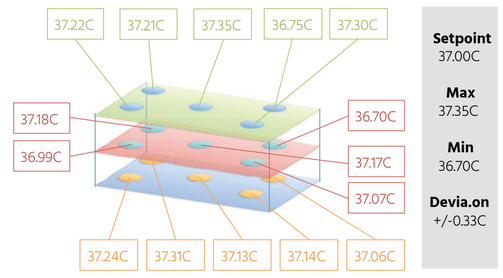

Figure 4:
This 3D temperature map of the interior of a Multitron shaker illustrates how even the temperature is all across the tray.

Scaling-Up

Qualification: The Multitron shaker can be qualified for validated processes. FAT and SAT documentation is available, as is IQ/OQ support. To further mitigate risk to the cell culture several system modifications are available. Those modifications include antimicrobial coating, UV sterilization of the air flow path, and hygienic steam humidification.

Traceability: To allow for interaction with building monitoring systems the Multitron shaker can be equipped with analog outputs for all process parameters. Alternatively the shakers can be controlled using the eve bioprocess software. This will provide traceability on par with a bioreactor, and allow both control of complex processes and integration of 3rd party devices. eve can also be qualified for GMP use.

Conclusions

A machine learning process, combined with protein engineering and efficient screening facilities is capable of yielding stable high-expressing cell lines, e.g. a 3-5 g/L IgG1 clone, in approximately 12 weeks.

Screening: With up to 7,680 data points (80 microtiter plates) per shaker it is possible to manipulate multiple factors while performing all experiments in triplicates. This allows for the generation of statistically valid data for design of experiments (DoE) while also considering quality by design (QbD) as stable clones are generated and optimized.

Scaling: Stable systems, expressing hundreds of grams of protein, can be developed in weeks.

Validation: Systems and process parameters can be qualified and validated for GLP and cGMP work.

Traceability: When combined with the eve® bioprocess control software package the traceability of an incubation shaker is comparable to a bioreactor's. eve® also allows full third party device integration.

Reference

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