

Accelerated Performance Enhancement in Cultured Mammalian Cells Using High Throughput Screening Technology

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Abstract

We have applied a high throughput screening (HTS) system to two mammalian cell performance enhancement strategies (media optimization and small molecules screening) to significantly reduce the time and labor requirements of both strategies. Due to the large number of components in media, the determination of each component's optimal level cannot be adequately determined using traditional shaker flasks or TPP bioreactor tubes. In a similar manner, screening a large number of candidate small molecules suffers from the same limitations. Therefore, a microtiter plate-based HTS system was developed to field the relatively large number of conditions required to analyze the cell's performance under these conditions. The efficiency gained was measured by the increase in number of conditions that can be analyzed and a decrease in the time required compared to the use of the more traditional cell culture platforms. Efficiency is not the only benefit in utilizing HTS systems. For example, in media optimization, HTS techniques allow the interrogation of a large number of factors resulting in a robust understanding of media factor concentrations and interactions of those factors. Support activities, such as, LIMS and databases will be discussed.

Introduction

For the past decade the demand for recombinant antibodies has increased rapidly due to their importance as therapeutic agents. To meet this demand, strategies are employed to increase production by enhancing cell performance, specifically improving antibody bioproduction titers. One of the most effective strategies is to improve the media formulations used to grow cultured cells. Cell Sciences and Development (CSD) within SAFC Biosciences develops new cell culture reagents and provides services targeted to customers developing and producing biotherapeutics using cell culture systems. One of the main functions of CSD is to develop medium formulations that can improve cell line productivity. There are two main paths utilized to achieve this goal: 1) determining the optimal level of the media components, and 2) identifying new stimulatory components as potential additives for media. Due to the difficulty of examining a large number of components at different concentrations, or screening a large number of potentially stimulatory compounds, these types of studies cannot be rapidly and efficiently executed using traditional shaker flasks or bioreactor tubes.

In order to accelerate media development and screening activities, a microtiter plate-based high throughput screening (HTS) system was developed to field the relatively large number of conditions required to optimize cell line performance. In addition, with the increased workload and data generation by the HTS system, we also developed a Laboratory Information Management System (LIMS) to manage these HTS system data. Our current LIMS contains plate handling, sample management, and data acquisition and analysis functionalities. This LIMS enables CSD to keep pace with increased data volume and facilitates managing resources. The result of the combination of the HTS system with the LIMS is that we are able to run more complex experiments, generate discoveries and make decisions at a much faster pace. Moreover, our new systems should allow us to gain significant new insights into the effect of media components, their concentrations and their interactions on growth, viability and productivity of various cell lines.

SAFC-Bioscience High Throughput Screening System

Our HTS system was developed to advance the process of media development (media optimization and stimulatory molecules screening) by greatly reducing timelines. The current HTS system was developed based on shake flask and bioreactor tube culture dynamics. It utilizes 96-well plates that mimic cell culture performance and dynamics observed in flasks on shaking platforms and TPP bioreactor tubes in large orbital shakers. The cell density and productivity measurements for several cell lines tested in our HTS system demonstrated good correlation with standard shake flasks and TPP bioreactor tubes (Table 1).

Cell Line	TPP/Shake Flask Peak VCD	SAFCB-HTS Peak VCD	VCD Correlation (R ²)	TPP/Shake Flask Peak IgG Conc.	SAFCB-HTS Peak IgG Conc.	IgG Correlation (R ²)
GS-Clone A	2.9 x10 ⁶	2.5 x10 ⁶	0.95	148 mg/L	141 mg/L	0.97
GS-Clone B	4.1 x10 ⁶	4.0 x10 ⁶	0.95	610 mg/L	600 mg/L	0.95
GX1	4.0 x10 ⁶	4.1 x10 ⁶	0.86	183 mg/L	160 mg/L	0.80
GX2	4.5 x10 ⁶	4.1 x10 ⁶	0.98	659 mg/L	614 mg/L	0.82
DUXB11-Clone A	3.1 x10 ⁶	3.0 x10 ⁶	N/A	298 mg/L	254 mg/L	N/A
DUXB11-Clone B	5.3 x10 ⁶	5.7 x10 ⁶	N/A	250 mg/L	175 mg/L	N/A
SP2/0-Clone A	4.0 x10 ⁶	4.2 x10 ⁶	N/A	N/A	N/A	N/A
SP2/0-Clone B	2.7 x10 ⁶	3.2 x10 ⁶	N/A	37 mg/L	32 mg/L	N/A
DG44	4.2 x10 ⁶	4.4 x10 ⁶	0.97	143 mg/L	126 mg/L	0.99

Table 1: Cells Tested in SAFC-Bioscience HTS System

Our HTS system can be divided into three parts:

1. Liquid handling:

- Tomtec Quadra 4™ – A liquid handler that is responsible for initial cell inoculation and subsequent culture sampling for growth, viability and productivity measurement.
- BioMek 3000™ – A liquid handler that is responsible for mixing and manipulation of media formulations.

2. Incubation:

- Orbital Shaker – Orbital shakers that create a vortex in the liquid in the wells of a 96-well plate in a manner similar to TPP bioreactor tubes or shake flasks.
- Forma Environmental Chamber – An incubator that can maintain temperature and humidity while incubating multiple plates on multiple shakers.

3. Detection:

- ForteBio Octet™ Biolayer Interferometer System – An IgG measurement system with high sample throughput capability.
- IntelliCyt HyperCyt™ Cell Counting System – A high throughput cell density and viability detection system with modified procedures to meet high throughput needs.

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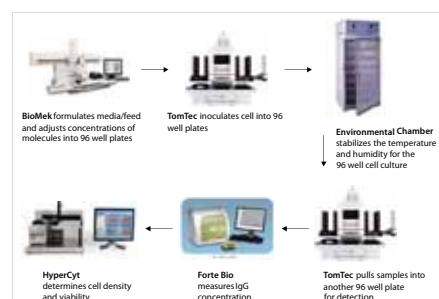


Figure 1: Workflow of the SAFC Biosciences High-Throughput System

SAFC Biosciences Laboratory Information Management System

Historically, paper documentation and spreadsheet-based software packages are routinely used for data capture and analysis, recording important inventions and archiving information in laboratories. However, the increased data generated by the HTS system resulted in a need for a LIMS to provide global access and enterprise visibility for cross-functional groups to leverage the large amounts of data, thus increasing operational efficiencies. Our LIMS was developed based on the Sapphire™ architecture to support the high throughput screening workflow. The Java-based Lab Vantage Sapphire technology was adapted because of its easy accessibility, high throughput lab-oriented customization, and flexibility and scalability to accommodate new instruments and new lab techniques. The architect of our LIMS is summarized in Figure 2.

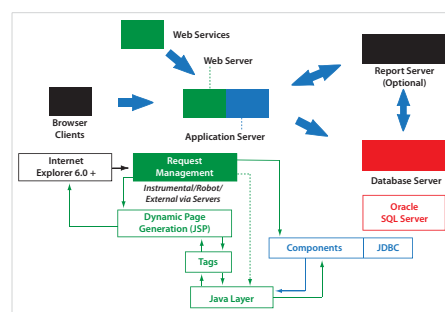


Figure 2: The Architecture of SAFC Biosciences LIMS

The SAFC Biosciences LIMS features the following:

- Data tracking – tracks samples, plates, and well positions while maintaining hierarchical relationships.
- Integration with the instrumentation and automation equipment export files.
- Rapid search, retrieval and analysis of data.
- Easily accessible and presentable data via web.
- Stock compound management – compound storage and stock maintenance information.

The SAFC Biosciences LIMS can be divided into three parts:

- Compound Screening Database – Data storage, tracking, analysis and retrieval of potential bioactive compounds that were tested in the HTS system.
- Media Formulations Screening Database – Data storage, tracking, analysis and retrieval of media formulations that were tested in the HTS system.
- Experiment Searchable Database – Raw data generated in both HTS and non-HTS experiments.

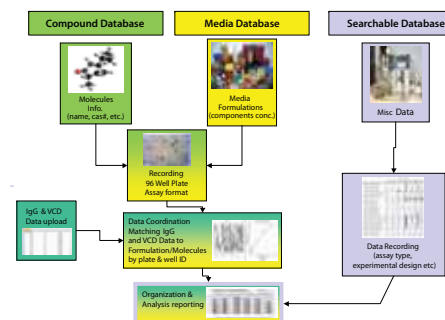


Figure 3: Workflow of the SAFCB LIMS

Media Optimization

In order to improve the productivity of an Sp2/0 cell line, we attempted to identify an optimal batch formulation for this cell line. Using the BioMek 3000, we mixed 30 parental media into 330 different formulations. The Sp2/0 cell line was cultivated in these 330 formulations to identify an optimal batch medium. The workflow of this experiment is summarized in Figure 4. We found that five media formulations improve the productivity by more than 50% (Figure 5).

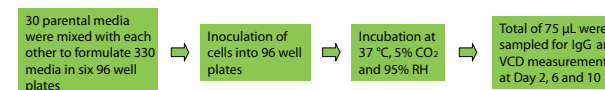


Figure 4: Experimental Summary of the Media Optimization Experiment

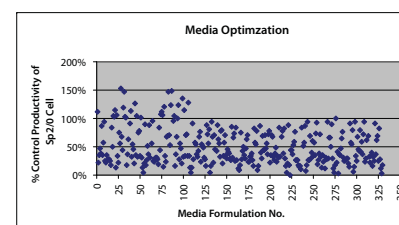


Figure 5: Summary of the Media Optimization Experimental Result

Compound Screening

In order to improve the productivity of a recombinant antibody producing CHO GS cell line, we decided to screen small organic molecules for stimulation of IgG productivity. All chemicals were purchased from Sigma-Aldrich. Chemical stocks were added into the cell culture at final concentration of 20 µM (0.25% DMSO). The GS cell line was cultivated in CHO CD fusion media (SAFC Biosciences Cat. No. 14365C). The workflow of this experiment is summarized in Figure 6. We found that several compounds appeared to stimulate the productivity by more than 100% (Figure 7).

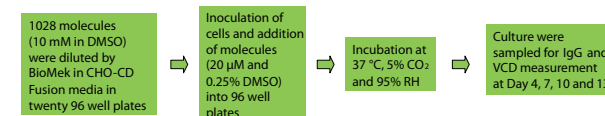


Figure 6: Experimental Summary of the Molecules Screening Experiment

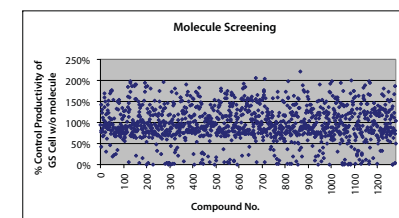


Figure 7: Summary of the Molecules Screening Experimental Result

Result and Discussion

The HTS improves throughput, efficiency and hands-on bench time over traditional shake flask/TPP bioreactor methods. Our HTS system for cell culture gives us a significant advantage over the traditional system in terms of throughput (Figure 8).

Maximum numbers of samples can be measured per day per person

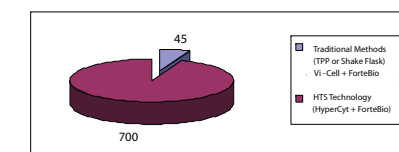


Figure 8: Increased Throughput by HTS

The efficiency improvement of the HTS system is more obvious when large numbers of conditions are being screened. This is illustrated in Figure 9. It should be noted that the traditional methods will take approximately 3 years to finish the screening of 1600 samples, while it will take less than a month for the HTS system to accomplish a similar task.

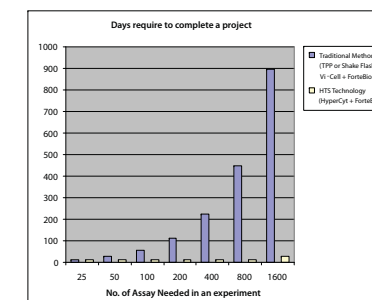


Figure 9: Improvement of Efficiency in Screening Completion

The HTS system also significantly reduces hands-on bench time. Thus, the HTS system saves on labor costs indirectly. Scientists now have more time to come up with other innovative ideas and solutions. Figure 10 demonstrates the significant reduction of hands-on experimental time when running 1600 samples.

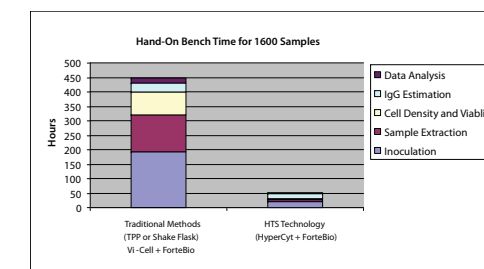


Figure 10: Reduction of Hands-On Experiment Time When Running 1600 Samples in an Experiment

The compound screening and media optimization experiments above illustrate the efficiency improvements possible with the HTS system. Figure 11 shows that our current HTS can reduce the time for a 1028 compound screening experiment from 450 days to 30 days. Similarly, the HTS system can reduce a 98 day media optimization experiment to 17 days (Figure 12).

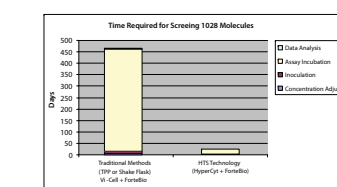


Figure 11: Time requirement for screening 1028 compounds using different techniques

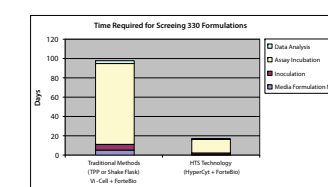


Figure 12: Time requirement for screening 330 formulations using different techniques

Conclusion

Here we demonstrate that our HTS system has much higher throughput than traditional TPP bioreactors tubes/shake flask methods, and that it significantly accelerated the performance enhancement process for cultured cells. Traditional media optimization strategies require step-by-step linear experimentation that limits throughput. Our current HTS system enables us to investigate relatively large numbers of conditions simultaneously. Thus increases the probability and shortens the time requirement for enhancing the productivity of a cell line. Thus, efficiency is not the only benefit in utilizing the HTS system. It also allows scientists to have a more robust understanding of media component concentrations and their interactions relevant to cell line productivity.

Acknowledgement

The successful completion of any task would be incomplete without thanking the people who made it possible. I would like to thank all scientists and managers of CSD and the entire SAFC Biosciences team for their constant guidance and help at every stage of the project.

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