



Bioprocessing TechNote

Rapid Identification of Production Strains

Accelerating Protein Concentration and Functional Analyses to Improve Efficiency

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Accurate protein quantitation is critical to the selection of expression strains for development and optimization of bioreactor titers in production. Traditional analytical methods include ELISA, HPLC, nephelometry, and densitometry. Drawbacks to these methods include long analysis times, lack of specificity, labor-intensive protocols, and imprecision.

Label-free assays offer an ideal solution for reducing bioprocessing bottlenecks and ensuring high product quality and improved efficiencies.

This tutorial describes the use of ForteBio's (www.fortebio.com) Octet instruments and Dip and Read™ biosensor assays in the protein expression workflow at Pfenex (www.pfenex.com). Previously a business unit of Dow Chemical, Pfenex was recently spun-off as a separate entity, specializing in protein expression strain engineering and process development using high-throughput, parallel processing that is

robotically enabled and relies upon Pfenex Expression Technology™, a *Pseudomonas fluorescens*-based system.

ForteBio's Octet family of instruments provides analysis of antibodies and other therapeutic proteins (Figure 1). Its analytical capabilities have applications where existing methods such as HPLC and ELISA have limitations in throughput, performance, time-to-result, and ease of use.

With full-plate protein quantitation in

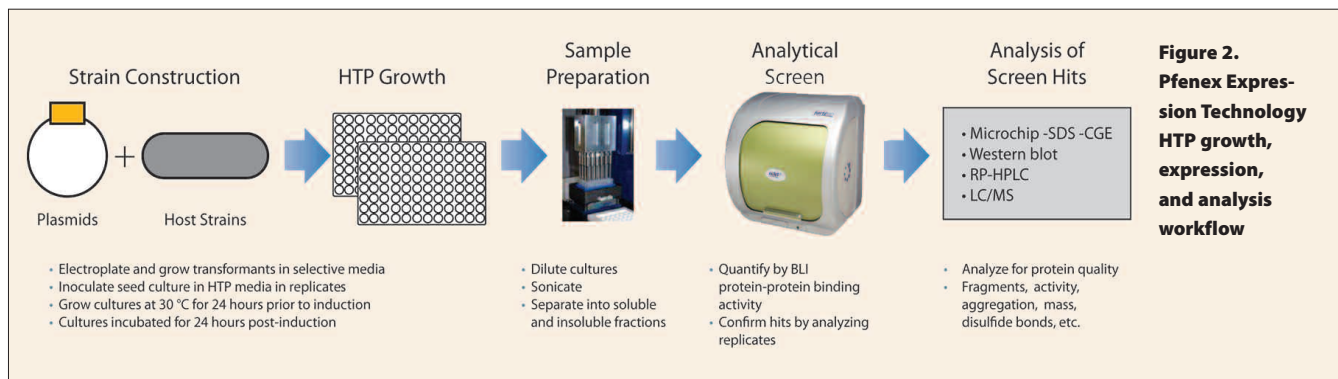
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15 to 30 minutes, Octet systems provide direct label-free quantitation of antibodies or other proteins that are critical to early cell culture screening, protein purification, cell-line selection for optimization of antibody production, and biologics manufacturing.

Octet systems utilize BioLayer Interferometry (BLI) technology to monitor



Figure 1. The Octet platform consists of Octet RED384 and QK384 systems that provide 16-channel simultaneous read-out, two microplate positions, 96- and 384-well plate formats and automation compatibility, and Octet RED, QKe, and QK systems that provide eight-channel simultaneous read-out and 96-well microplate format.



the binding of proteins and other biomolecules to their partners directly, in real time. The binding interaction is continuously monitored by measuring the change in thickness of the protein layer on the tip of a biosensor. The method does not require labeling of the protein with fluorescent or chromogenic tags, thus eliminating interference issues.

A variety of Dip and Read biosensor chemistries are available off-the-shelf such as protein A, antihuman and antimurine IgG, antipenta-HIS, streptavidin, and custom biosensors specific for a program or analyte that can facilitate the rapid development of assays for IgG and other proteins. These biosensors measure protein concentration in the presence of extraneous matter such as cell debris, which is typically present in cell lysates and cell culture supernatants, thus providing rapid titer measurements without laborious sample preparation.

Octet software provides multiple curve fits, exports reports to Excel, and automates batch-mode data analysis.

While the Octet platform can enable fast titer measurements, Pfenex Expression Technology can help accelerate development of protein leads into clinical studies by assuring first time, significant production of virtually any aglycosylated protein in a scalable system within five weeks. Specific components of the plat-

form were designed to ensure soluble and active expression through the avoidance of proteolytic clipping and post-translational modifications along with enhancements to solubility through protein-folding improvements.

The Pfenex Expression Technology platform has been constructed to assess the performance of thousands of host strain/plasmid combinations in parallel to rapidly identify production strains for proteins that cannot be expressed in any other system. Host strains include those with one or more deletions in protease genes, co-expression of folding modulator proteins, proteins involved in disulfide bond creation, along with combinations of these.

Genetic control elements on expression plasmids (promoters, ribosome binding sites, secretion leaders) have been combined to generate >100 off-the-shelf, unique, rapid cloning vectors, allowing control of protein expression and quality.

Predicting which components will be crucial in successful expression of any given protein cannot be determined from intrinsic information such as amino acid sequence. This means that the combination of critical factors is empirical for each individual target protein. Accordingly, Pfenex has developed a high-throughput, robotically

enabled screening work process in 96-well format. This process is coupled to high-throughput analytical methods such as the Octet system, which allows the parallel evaluation of hundreds of unique host strains containing a variety of expression strategies for a specific gene (*Figure 2*).

How it Works

As one example of how the screening process works, Pfenex came up with a high-producing strain for expression of soluble granulocyte colony stimulating factor (G-CSF) for low-cost production of filgrastim as a biosimilar in the therapeutic protein market. The non-glycosylated form of G-CSF is currently produced in *E. coli* as an inclusion body that requires solubilization and refolding.

The first stage in developing a production process for G-CSF was to screen Pfenex Expression Technology strains for expression of soluble, correctly folded G-CSF. The Octet system with G-CSF receptor immobilized to Dip and Read streptavidin biosensors was used for titer measurements (*Figure 3*). The assay demonstrated sufficient range and sensitivity for detecting soluble, functional G-CSF directly in crude cell extracts with minimal sample preparation.

Expression strains were screened in parallel using the Octet assay following

the process outlined in *Figure 2*. Plasmids were constructed carrying the G-CSF gene fused to 12 *P. fluorescens* secretion leaders, targeting the protein to the periplasm, and these plasmids were transformed into 20 different host strains with varying phenotypes to create 240 unique expression strains. The soluble fractions of replicate culture lysates were analyzed for G-CSF receptor binding using the BLI method. The resulting screen identified several dozen strains that exhibited soluble expression of G-CSF. Notably, the type of secretion leader had a significant impact on the level of G-CSF expression in each host strain. Six secretion leaders showed low relative G-CSF expression, two showed medium expression, and three secretion leaders showed the highest relative G-CSF expression. One secretion leader did not exhibit any soluble G-CSF expression. From this ini-

tial screen, the highest producing strains were characterized further and selected for fermentation development.

The entire process to identify a strain took less than five weeks. The Octet Dip and Read assay provided both a means to assess the amount of soluble G-CSF produced by strains as well as an indication of the functional quality of the material by its ability to bind to the G-CSF receptor. The same G-CSF Dip and Read assay was used at Pfenex during fermentation optimization and downstream process development stages of development.

Several Octet assays have been developed by Pfenex and implemented in expression screens for different classes of proteins including mAbs, antibody derivatives (Fab, Fab', single-chain antibodies, etc.), growth factors, cytokines, and vaccine antigens. The streptavidin biosensor can be used to load the appropriate cap-

ture reagent to allow the analysis of all of the above targets. The Octet platform provides a method for measuring protein titer and verifying functionality in a simple and direct binding assay format that is broadly applicable across a variety of protein types.

Development of therapeutic proteins requires rigorous measurements of kinetic and thermodynamic binding properties of the protein to its receptor for candidate optimization and clinical dosing strategies. Octet systems monitor binding interactions in real time and measure kinetic constants k_{on} and k_{off} and affinity constant K_D .

The ability to measure concentration as well as kinetic parameters in a single assay makes the Octet platform a useful tool for various stages of the bioprocessing workflow, as shown in this G-CSF example with Pfenex Expression Technology. GEN

