Improving CHO-Cell Biomanufacturing with a Whole-Genome CRISPR Screening

with Jamie Freeman

On 18 October 2018, BPI presented a free "Ask the Expert" webinar with Jamie Freeman, a bioproduction product manager at Horizon Discovery. His company is developing a whole-genome screen using clustered regularly interspaced short palindromic repeats (CRISPR) for improving the capacity of Chinese hamster ovary (CHO) cells in biomanufacturing.

Freeman's Presentation

Freeman described the screening approach that Horizon developed to improve its own CHO cells. It also may be used to improve other such cell lines for biomanufacturing. Founded 11 years ago based on cell-line engineering using recombinant adenoassociated virus (rAAV), this company now works on all aspects of genome engineering and its applications to the use of cell lines in drug discovery, development, and manufacture.

Regulator-approved cell lines using the glutamine synthetase (GS) selection system have provided established mechanisms for generating clones at commercially viable and relevant levels. But users have been frustrated with licensing/financial restrictions related to accessing the industry standard glutamine synthetase (GS) selection system. In particular, there are restrictions on which culture media can be used with those cells, on which contract manufacturing organizations (CMOs) can use them, and on the modifications they can undergo. In addition, full sequence information was unavailable for GS-CHO cells.

Conversely — and together with accessible financial terms — Horizon has a flexible approach to the use of its cellsand actively encourages (and assists with) modifications of its cell lines. Freeman said that the GS selection system is good for identifying rapidly the best-expressing clones from a stable pool, and it is the best metabolic selection system available. But it does not fundamentally change the biology of CHO cells to increase their expression capacity. To address that, Horizon wants to build on that platform: e.g., increasing the amount of product that can be generated, the specific productivity of individual cells, and/or viable cell densities.

The company is using CRISPR in research and development to screen and validate genetic targets that significantly affect the performance of CHO cell lines. Once those genetic targets are validated, Horizon then returns to its rAAV platform to generate cells for use in commercial manufacturing. Although that has lower efficiency and throughput than the GS system, CRISPR's clear intellectual property (IP) position allows the company to generate cell lines with freedom to operate in biomanufacturing without the GS restrictions.

For a successful CRISPR screen, you need three moving parts working in parallel: a good library, a protocol to introduce that library to cells, and an assay that can show which genes have an effect. Freeman said that previously available sequence information for CHO lines was of insufficient quality for generating a high-fidelity CRISPR library. By sequencing the CHO genome, Horizon overcame that challenge to generate a high-quality, whole-genome CRISPR library that initially targets 20% of the genome for proof-of-concept. A full genome will be ready soon. The company has generated and validated a flourescence-based assay that is compatible with whole-genome CRISPR screening.

Proof-of-concept screens using two different assays against both monoclonal antibodies and difficult-to-manufacture architectures have identified a number of potential targets that are currently being verified and validated in secondary assays. The outputs from these screens also have enabled analysis of different cell biological pathways that can be targeted to identify synergistic interactions to generate cell lines with multiple edits that are significantly improved over anything else available.

Freeman said that his company is the fastest growing provider of new manufacturing cells to the biopharmaceutical industry. In addition to offering its own high-quality cell line that is being improved with genome engineering, Horizon can improve clients' cells using the same techniques. Licensing terms are flexible, and licensing costs make the service accessible.

Questions and Answers

What growth media and/or supplements work best with your cell lines? We have recommended protocols. The cells were originally adapted to suspension growth in OptiCHO media from Thermo Fisher Scientific, and we currently recommend the use of FortiCHO media for standard culture. We have partnered with a media development company to develop a formulation that is specific to our cells, but that won't be released until the second half of 2019. Although we provide recommended protocols, some process optimization is still encouraged to get the optimal performance.

Can you share the name or the publication of the CRISPR design algorithm that you discussed? It is proprietary to Horizon and unpublished, so unfortunately I can't share that information at this time.

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