Yield, Yield, Yield

Yield is still an area that requires significant improvement for many promising recombinant protein and antibody products under development in the fight against human disease. Batavia Biosciences has created STEP® which enables the rapid generation (<12 weeks) of stable (>60 generations in absence of antibiotics), mammalian CHO cell lines, able to provide at least 10-fold more product per cell as compared to a commercially available benchmark.

How STEP® works

On a single fully synthetic DNA plasmid, STEP® combines newly discovered potent genetic enhancer elements with an extremely stringent, yet adaptable cell selection system. In brief (see figure for a graphic representation of the STEP® plasmid), a potent promotor (CMV) drives transcription of one mRNA as all elements transcribed are genetically linked. From the single mRNA both the protein of interest, derived from the gene of interest (GOI), as well as a functionally impaired Zeocin antibiotics selection marker (FI-Zeo) are translated. The GOI and FI-Zeo are linked through an internal ribosomal entry site (IRES) and a stretch of DNA (spacer).

STEP® vector

The FI-Zeo was purposely mutated by our scientists such that the resulting enzyme is less able to neutralize the antibiotic as compared to wild type Zeocin marker. In addition, the spacer sequence reduces translational efficiency and thereby decreases the amount of FI-Zeo protein produced. Thus, a cell needs to produce enormous amounts of mRNA to have sufficient marker protein to survive antibiotics selection and as such produces enormous amounts of the GOI product. This stringent cell selection cassette was subsequently flanked by newly discovered potent enhancer elements (EE) that allow extreme cell survival.

In addition, our scientists developed three STEP® plasmids, each identical in design except for different mutations carried by the FI-Zeo which results in more or less potent Zeo selection stringency.

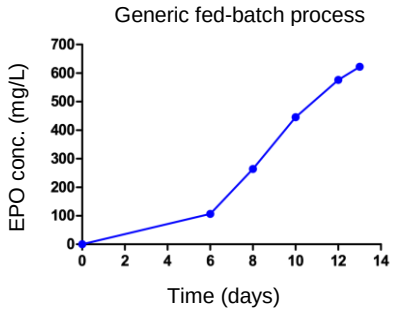
The Erythropoietin case study

One of the many proteins expressed with STEP® technology is Erythropoietin (EPO), a recombinant protein used in the treatment of anemia, and known to require extensive post-translational modification in order to be biologically active and retain correct pharmacokinetics profile. Stable STEP® CHO cell lines were successfully obtained in less than 10 weeks. As shown in figure 1, selected EPO cell lines demonstrated over 600 mg per liter EPO production in a 14-day, 10 liter bioreactor fed-batch process using a generic non-optimized feed.

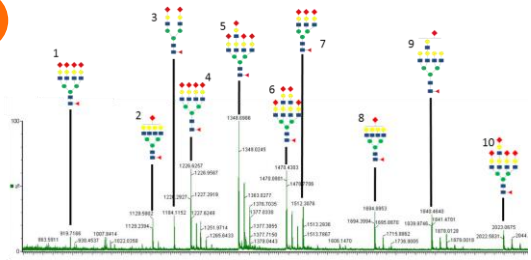
Biological activity was confirmed on UT-7 cells and proved comparable to the marketed EPO product called EPREX®. In addition, the EPO protein produced with STEP® technology proved identical in regard to sialylation and glycosylation patterns to the EPO biological reference standard (figure 2). Finally, cells proved stable as witnessed by maintaining high specific productivity over 60 cell generations in serum-free medium in the absence of antibiotic selection pressure (figure 3).

This study cased showed that high expressing stable CHO cell clones were obtained in less than 10 weeks and that cells proved rapidly scalable to 10 liter bioreactor. The STEP® process only yielded few very high expressing cell line and thus time consumption and cost in manufacturing stable, high producing cell lines are significantly reduced as compared to other available stable cell line generation technologies.

1

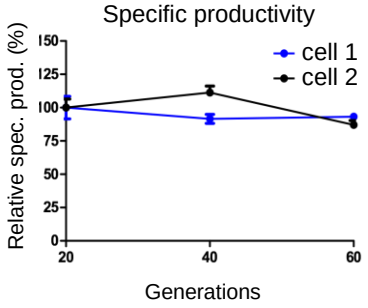


2



Glycan Profiling

3



STEP® forward

- Highly stringent, flexible and adaptable mammalian expression platform
- Delivers high expressing stable CHO cell lines with at least 10-fold higher yield
- Correct glycosylation pattern, biological activity, and pharmacokinetics profile
- Most rapid and cost-effective stable cell line process available

for more information: **www.bataviabiosciences.com**