Reduced Clone Variation of CHO Cells by Single Targeted Integration

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Introduction

TurboCell™ is a CHO-K1 cell-based production platform with a single targeted-integration site. Stable expression has been shown over long-term cultivation of the Master Cell Bank (MCB) and different ProducerTurboCells (PTCs). As the gene of interest (GOI) is integrated into the same locus clone growth, productivity and product quality are highly similar amongst different PTC clones. Nevertheless, based on natural clonal variance, some differences in PTC process performance can be observed.

In a quantitative proteomics approach using Tandem Mass Tag (TMT) labelling, we compared TurboCell™ to the parental CHO-K1 host cell line and four different PTCs during routine subcultivation and three long-term cultured cell lines. We identified differentially regulated host cell proteins during routine and long-term cultivation and during fed-batch cultivation. Proteins identified by bioinformatics analysis were validated using qRT-PCR and Western Blot.

1. Samples and data analysis

2. Statistical comparison of host cell, MCB and different PTCs

3. Confirmation of proteomics data

4. Pathway regulation during a small scale fed-batch

5. Changes in protein expression during small scale fed-batch

Summary and Conclusions

- 8% of the 4600 identified proteins are differentially regulated between the CHO-K1 and MCB, while only 1% are differentially expressed between PTCs and MCB.
- Hierarchical clustering identifies the CHO-K1 as most distinct from the MCB, the RCB and the PTCs and confirms similarity between TurboCell™ cell lines.
- Differential expression was confirmed for two chosen proteins (Protein 1 and Protein 2) by qRT-PCR and Western Blot.
- Cellular responses to feeding and temperature changes during fed-batch cultivation were observed. Three proteins upregulated by addition of feed were members of a network of proteins differentially regulated during small-scale fed-batch cultivation.

Outlook

- Effects of Protein 1 and Protein 2 on process performance should be further analyzed by overexpression or knockdown in different PTCs. Literature suggests Protein 1 induces proliferation, while Protein 2 is mainly responsible for transport and storage of media supplements, both important aspects during production.
- Literature describes Protein 3 and its downstream targets as anti-apoptotic. Differential expression of Protein 3 will be confirmed and downstream targets activated by Protein 3 assessed.
- Further bioinformatics analysis will be performed on the dataset to gain more insights into the differences in process performance observed for PTCa and PTCd.