TurboCell™ – A Fast Way to Stable Production Cell Lines

Britta Reichenbächer, Brigitte Anic, Katharina Köther, Janet Köhler, Jadranka Koehn
Rentschler Biopharma SE, Laupheim, Germany, britta.reichenbaecher@rentschler-biopharma.com

Introduction

One of the most important criteria for the successful production of biopharmaceuticals is the availability of a stable production cell line expressing the target product in a suitable quantity and quality. In general, the generation of a suitable production cell clone by the traditional random gene integration is a costly process and requires a time-consuming clone stability study.
Targeted integration is an innovative, fast tool in cell line development. Based on CHO-K1 cells, Rentschler Biopharma developed its Master TurboCell™ for a single copy site-directed integration of genes of interest (GOI) into a stable, pre-tagged and characterized hotspot of the CHO-K1 genome by recombinase-mediated cassette exchange (RMCE). Due to the integration of GOI into a known locus, the resulting clones show low variations concerning genetic stability, growth and protein quality characteristics, which minimalizes the total screening effort.

Results

1. From DNA to RCB – the TurboCell™ CLD Process

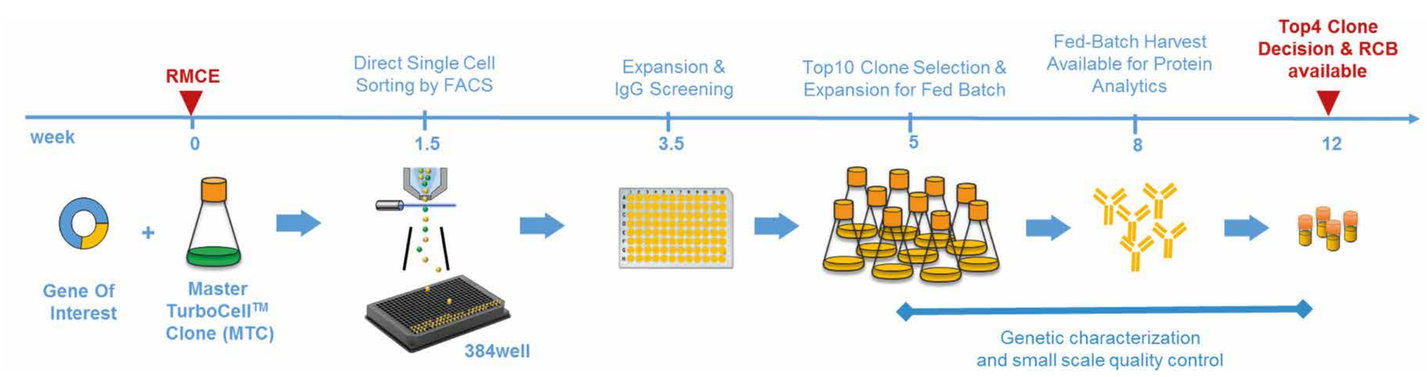


Figure 1: Targeted integration (RMCE) of a gene of interest (GOI) is followed by direct single-cell sorting of the resulting producer cells about 1.5 weeks later. The monoclonal status of the cells is then verified before subsequent expansion two weeks later. Clones are screened for titer (IgG) and growth during the expansion phase. The Top4 clones are selected based on the results of a screening fed-batch cultivation, protein analytics and genetic characterization, which takes place 10 weeks after RMCE. RCBs are prepared after Top4 clone selection.

2. Stability of the Master TurboCell™

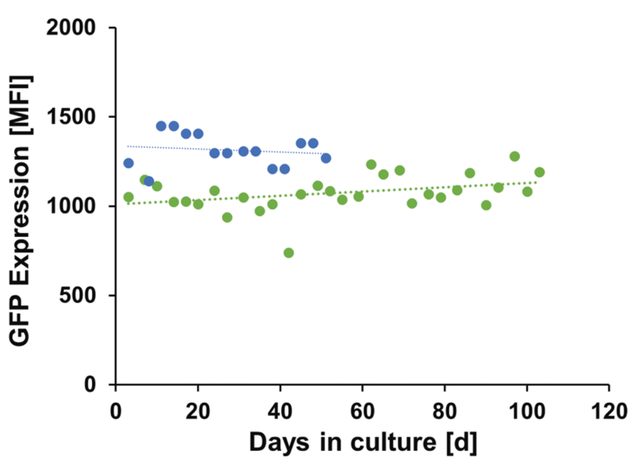


Figure 2: The Master TurboCell (MTC) originates from CHO K1, tagged with a GFP cassette which is flanked by recombinase-recognition sites for cassette-exchange. The mean fluorescence intensity (MFI) for GFP expression is plotted against the days in culture for two different MTC thaws and their corresponding cultivations. There is no significant loss in GFP intensity for up to 103 days in culture.

3. Verification of stability for different antibody producer clones

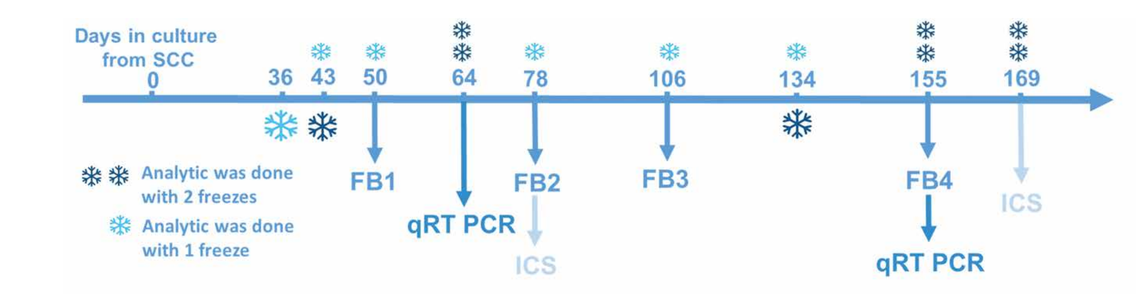


Figure 3: Origin of the cells. Producer TurboCell™ clones (PTC) for IgG1A and IgG1B were generated in parallel by TurboCell™ technology and subsequently frozen (with 36 days in culture after single cell cloning). After selection of the Top4 clones, selected cells were thawed for stability study with different fed-batch cultivations. In addition to this, freeze vials from the beginning (day 43) and end (day 134) of the previous cultivation were thawed. Based on this second revitalization, further assays on stability were done. For most of the assays, IgG1A and IgG1B reference clones were used in parallel.

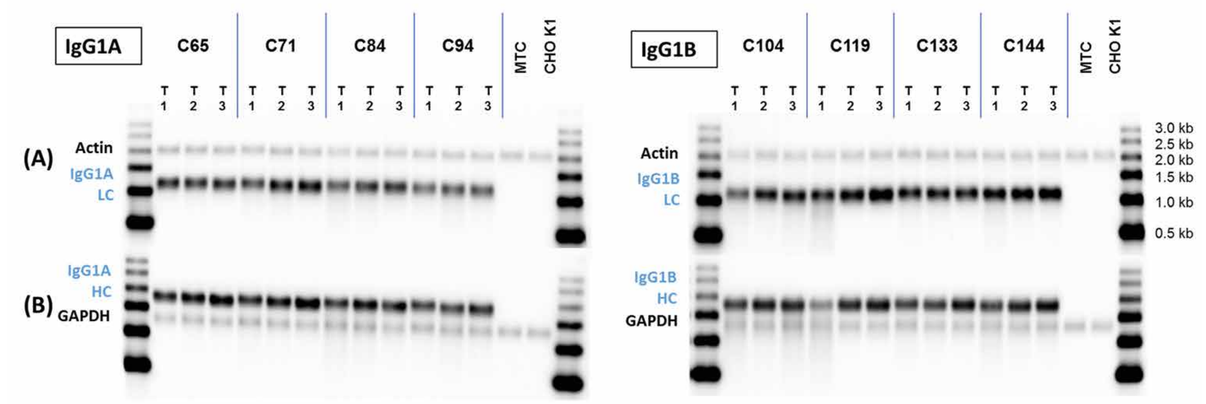


Figure 4: Northern Blot analysis. RNA was prepared from Top4 producer cells of both IgG1 constructs with 50 (T1), 78 (T2) and 106 (T3) days in culture. Northern Blot analysis was done with light- and heavy-chain-specific probes with a suitable house keeping gene probe (Actin vs. LC (A) and GAPDH vs. HC (B)). No significant decrease in light- or heavy-chain signal was detected over time.

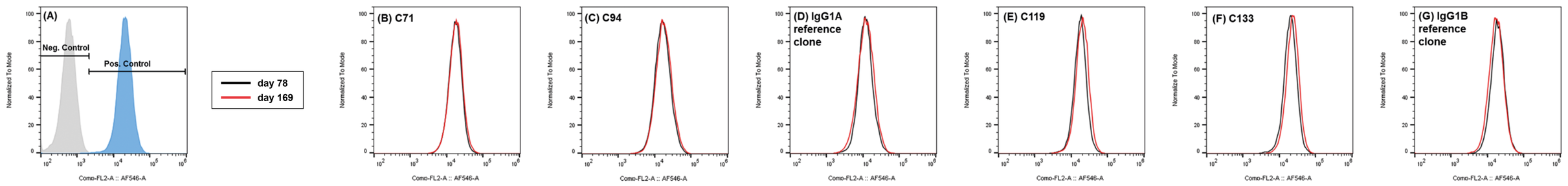


Figure 5: FACS analysis. Intracellular staining with an Alexa Fluor®546 goat anti human IgG-detection antibody and subsequent analysis by flow cytometry was used to examine the expression of the target antibody. A non-antibody producer clone was used as a negative control (A, grey) and a reference antibody producer clone was used as a positive control (A, blue), as visualized by the significant shift to the right on the AF546 fluorescence axis. In histogram (B)-(G) an overlay of day 78 (black) and day 169 in cultivation (red) is shown for four different exemplary TurboCell™ producer clones. As curves of both timepoints largely overlap, no loss in antibody expression is detectable.

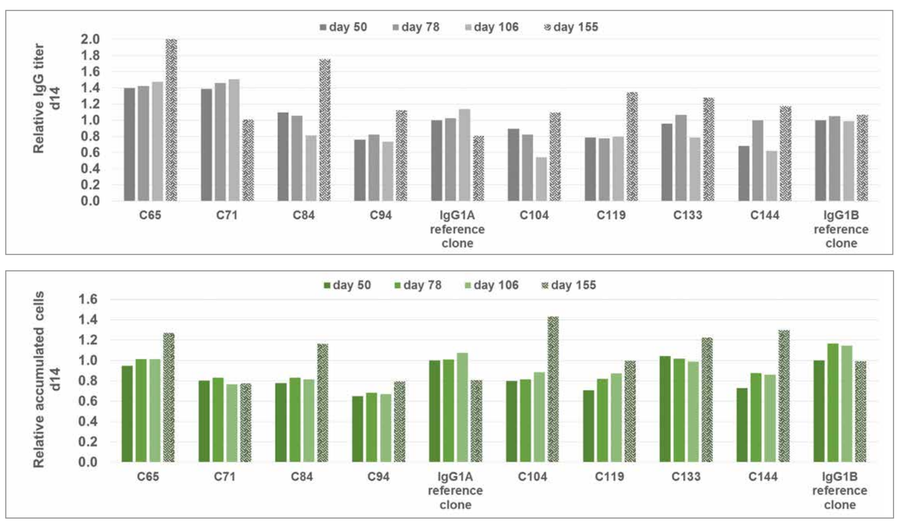


Figure 6: Fed-batch analysis. All values were normalized to their reference clones day 50. In FB4 (day 155) a significantly higher final d14 titer is detectable for most of the clones. This is related to the higher final accumulated cells and is caused by changes made to the fed-batch process. No significant loss in productivity and cell growth is detectable over time for the generated producer and reference clones of IgG1A and IgG1B.

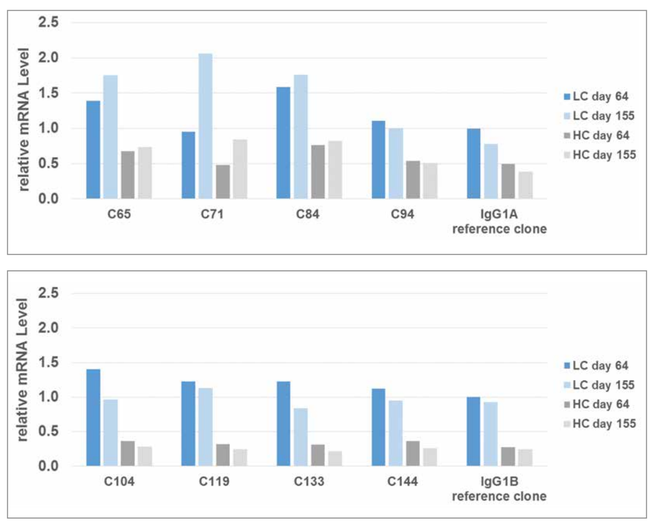
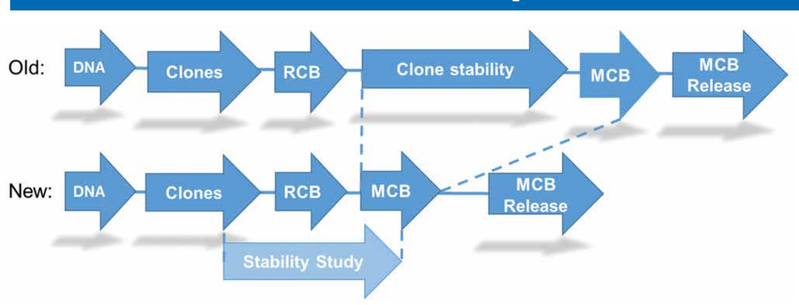


Figure 7: qRT PCR analysis. All results were normalized to their reference clone day 64. qRT PCR was performed on samples of day 64 and 155, representing the middle and the end of a long-term cultivation (n=2). No loss in RNA expression is visible over time for IgG1A clones (upper graph). IgG1B clones showed a slight decrease in LC and HC expression level (lower graph) between day 64 and 155, which is not significant for most of the clones.

Conclusion and Prospects



Using the TurboCell™ line, producer clones with different target molecules can be developed in parallel from DNA to the final RCBs within 12 weeks. A comprehensive stability study showed reproducible and stable producer clone performance, confirmed by the mRNA level, the productivity and the cell growth, using methods such as ICS, fed-batch analysis, Northern Blot and qRT PCR. Based on the stable growth and GFP expression of the Master TurboCell™ for more than 103 days, the resulting producer TurboCell™ clones were shown to have high inherent genetic stability, eliminating the need for extensive clone stability studies. These results open up new possibilities for reducing the time-consuming cell line stability testing process prior to GMP cell banking, because it provides the opportunity to go directly from RCB to GMP cell banking at low risk. To minimize the remaining risk, a stability study can be done starting from an early point in clone generation, because additional freezes have no influence on stability.

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