TurboCell™ Line Development 10 Weeks from DNA to Top4 Clone Decision

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Introduction

Targeted integration is an innovative, fast tool in cell line development today. Based on CHO-K1 cells, Rentschler developed its Master TurboCell™ for a single copy site-directed gene integration into a pre-tagged and characterized hotspot of the CHO-K1 genome by recombinase-mediated cassette exchange (RMCE). Due to integration of the gene of interest (GoI) into a known locus, the resulting clones show low variations concerning genetic stability, growth and protein quality characteristics, which reduces the screening effort to a minimum.

Using TurboCell™ line development, RCB generation of 4 different human IgG antibodies of different subtypes (IgG1, IgG2, IgG4) was done in parallel. The timeline of the existing TurboCell™ process from DNA to the Top4 clones was reduced to 10 weeks by using modern FACS and screening technologies. The use of direct single cell sorting into 384-well plates instead of a combination of pool sort and subsequent limiting dilution cloning reduced the cell line development (CLD) time by 4 weeks. High sensitivity batch IgG titer screening further reduced the timeline for clone selection and expansion by 2 weeks.

The Top10 clones of each antibody construct were compared regarding cell growth, viability, and productivity in fed batch cultivations (shake flask). Harvest material was purified using a generic small scale ProtA purification. Protein quality attributes such as monomer content, purity, intactness, charge variants and glycosylation were analyzed using SE-HPLC or CE methods. In parallel, a genetic characterization to confirm successful RMCE and exclude random GoI or recombinase integrations was performed on genomic DNA. In parallel, RNA integrity was confirmed for all clones (see poster by K. Köther).

Based on genetic profile, growth and productivity Top4 clones were selected and thawed from backup vials for expansion and RCB preparation.

Finally, performance of some Top4 clones was monitored over additional fed-batch cultivations at different stages of subcultivation, and after different freeze-thaw cycles to analyze process and clone robustness. Pending on those results, an additional reduction of timeline for TurboCell™ line development from DNA to final RCB in 10 weeks is possible.

Conclusion and Prospects

- Direct single cell sorting and sensitive batch titer screening reduced the time to clone decision to 10 weeks
- Targeted integration resulted in homogenous clone performance
- Clone performance was reproducible and stable over 6.5 weeks and zero to two freezing cycles
- Previous studies have shown both the Master TurboCell™ and all derived subclones to be genetically stable over 14 weeks
- Thus a further reduction of the TurboCell™ line development timeline of two weeks by seamless RCB preparation from an ongoing cultivation is possible.

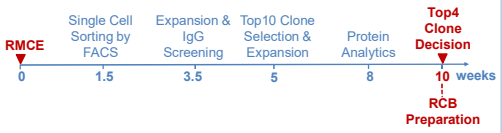
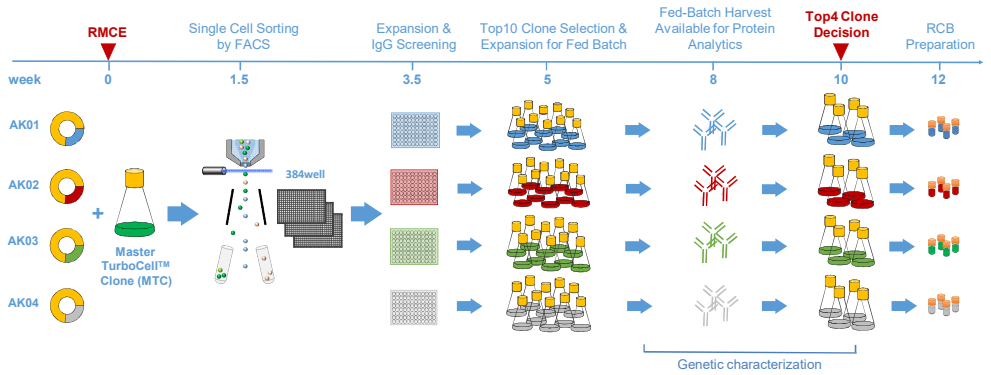


Figure 4: Optional reduced timeline for TurboCell™ line development. From DNA to RCB preparation in 10 weeks.

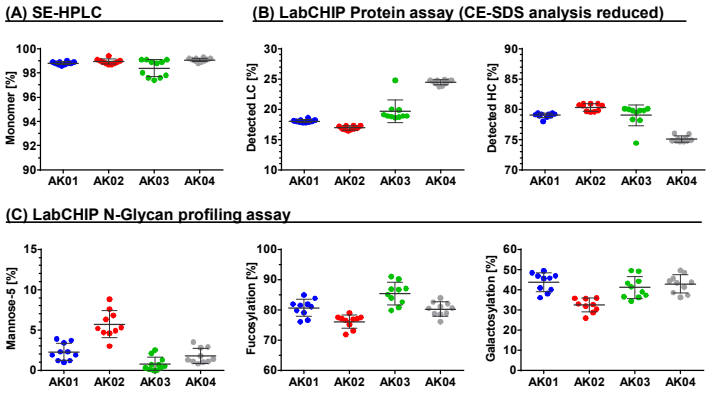
Results

Figure 1: TurboCell™ CLD process from RMCE to the Top4 clone decision in 10 weeks



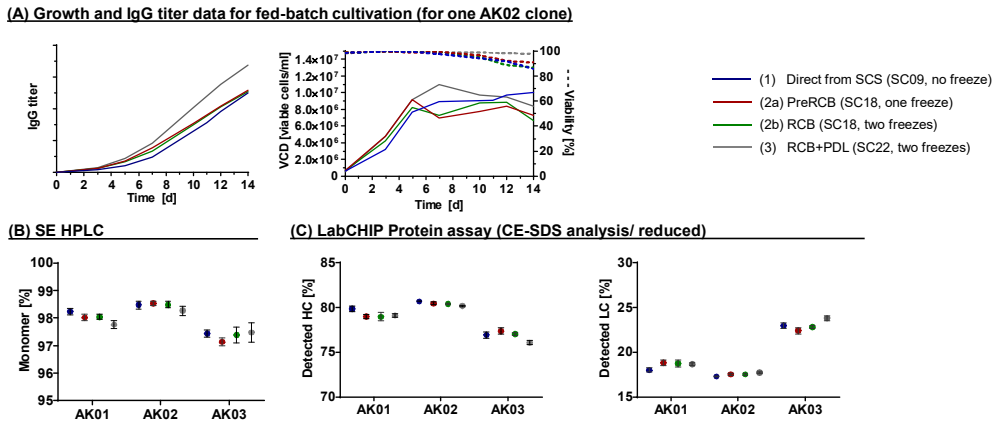
Targeted integration (RMCE) of different antibodies representing different IgG subtypes (AK01- AK04) into the Master TurboCell™ was followed by direct single-cell sorting of the resulting producer cells 1.5 weeks later, monoclonal status of cells was verified and followed by subsequent expansion. Clones were screened for IgG titer (GyroLab) and growth during expansion phase two weeks later. The Top4 clones were selected for each antibody subtype based on results of the screening fed-batch cultivation, protein analytics and genetic characterization 10 weeks after RMCE. For preparation of final RCBs, clones were thawed from an early backup-freeze and frozen two weeks later (week 12).

Figure 2: Homogenous product quality from TurboCell™ producer clones



Secreted antibodies of the Top10 clones were purified by ProtA and analysed. (A) Monomere content, as determined by SE-HPLC, showed a homogenous pattern for each construct. (B) Capillary electrophoresis (CE) under reducing conditions showed difference in LC and HC content, for the different antibody subtypes but high homogeneity of the clones of the same IgG subtype. (C) N-glycan profile of the purified antibodies was detected by LabCHIP analysis (Perkin Elmer). The distribution of fucosylated, galactosylated forms, and the mannose-5 content depended mainly on the antibody subtype with some clonal variation observed.

Figure 3: Comparability of data from different points in the TurboCell™ CLD process



Fed-batch cultivation was repeated for two clones of AK01, AK02 and AK03 at later stages of the cell line development process. Data from the fed-batch production after clone expansion prior to freezing (1) was compared to a later fed batch (2) performed with clones thawed and expanded prior to RCB preparation (2a) and on growth controls of the RCB (2b). A third fed batch (3) was performed on the RCB after additional 4 subcultivations (SC). Growth behavior, titer (A) and product quality (B, C) were comparable showing no influence of the number of freeze-thaw cycles or the additional subcultivation. Therefore, RCBs could be prepared from running maintenance culture directly at Top4 decision date to reduce the CLD timeline by two additional weeks.

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