Establishment of a CLD platform for generation of high titer CHO cell lines

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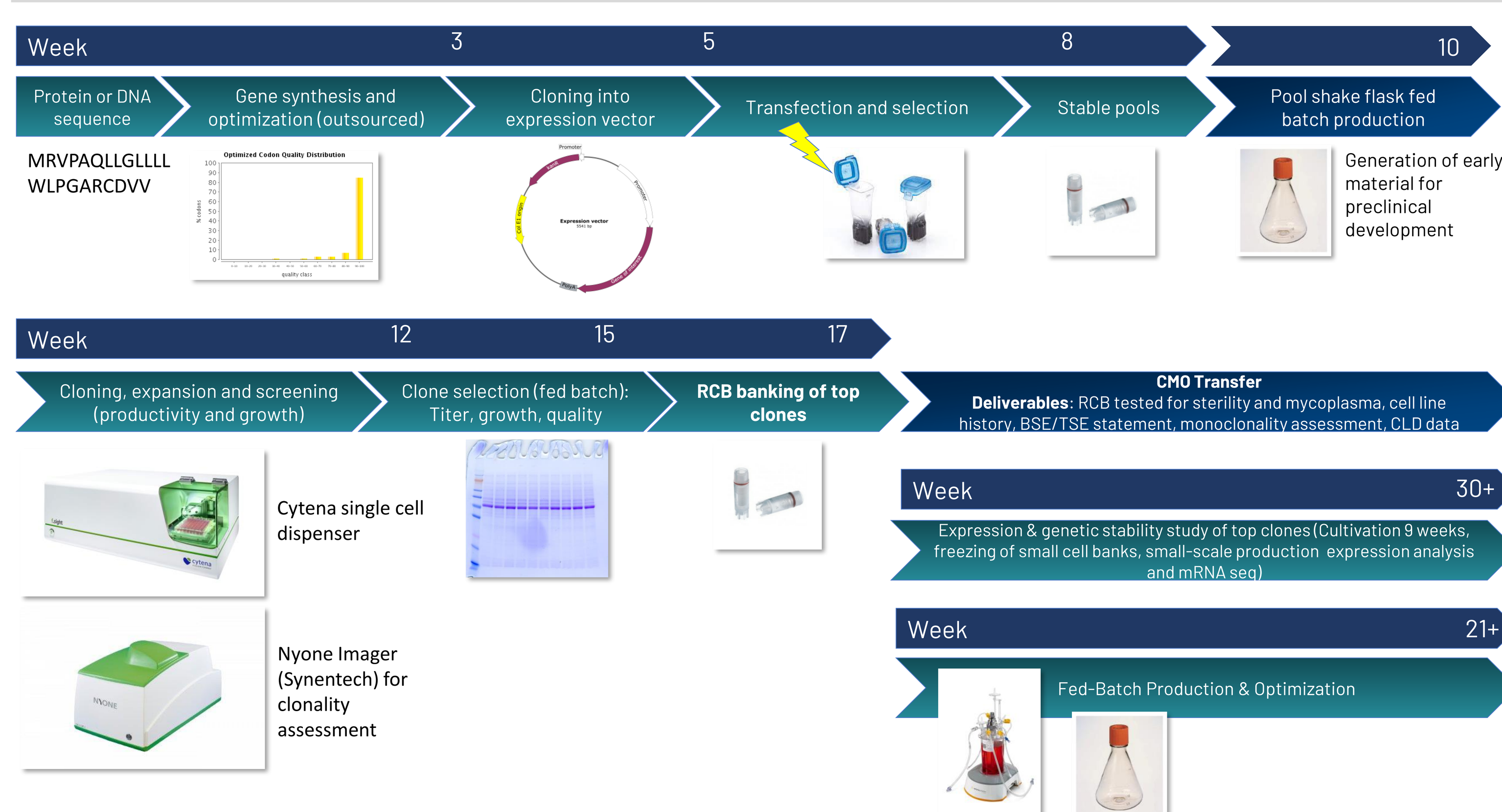
INTRODUCTION

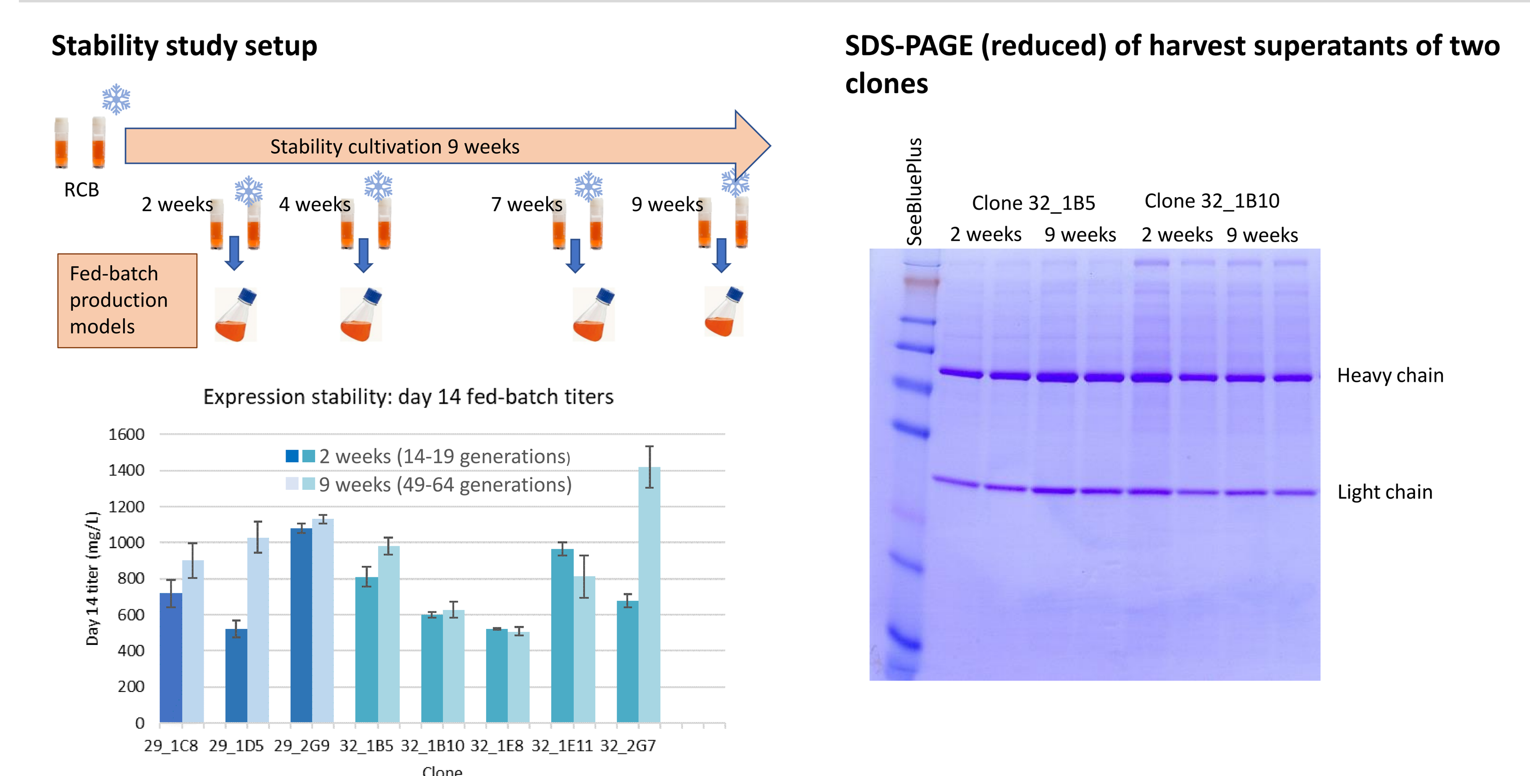
In order to extend our customer services in the recombinant protein area, we have developed a cell line development workflow for generation of stable CHO production cell lines. Our expression host cell line is DG44 which is DHFR -/-. It allows metabolic selection and thus omits the use of antibiotics for selection. Our newly established BION-CHO™ expression vectors carry a DHFR selection marker and cis-regulatory elements which increase product expression. Here, we compare the performance of these new vectors with a commercially available vector system.

Cell line development workflow



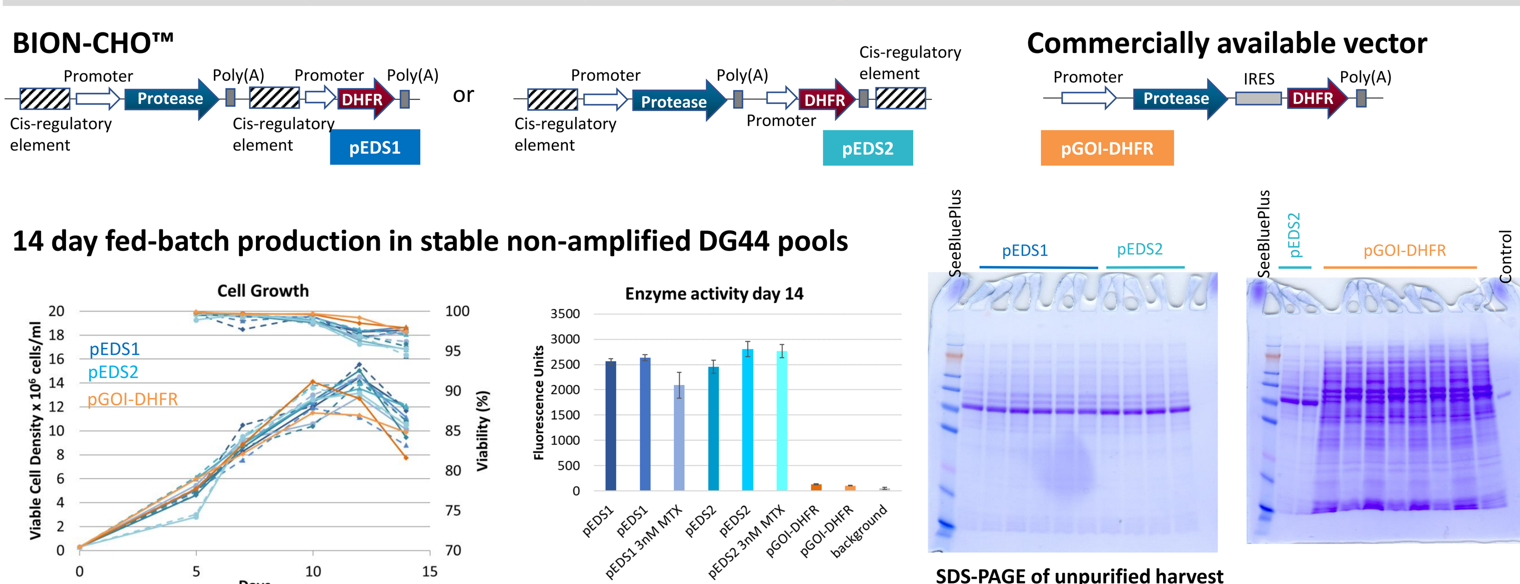
In our cell line development workflow research cell banks (RCBs) are established 12 weeks after transfection. If MTX amplification is added to the workflow, the timeline is extended by 3-4 weeks. The RCBs are provided with the documentation needed for CMO transfer.

Cell line stability



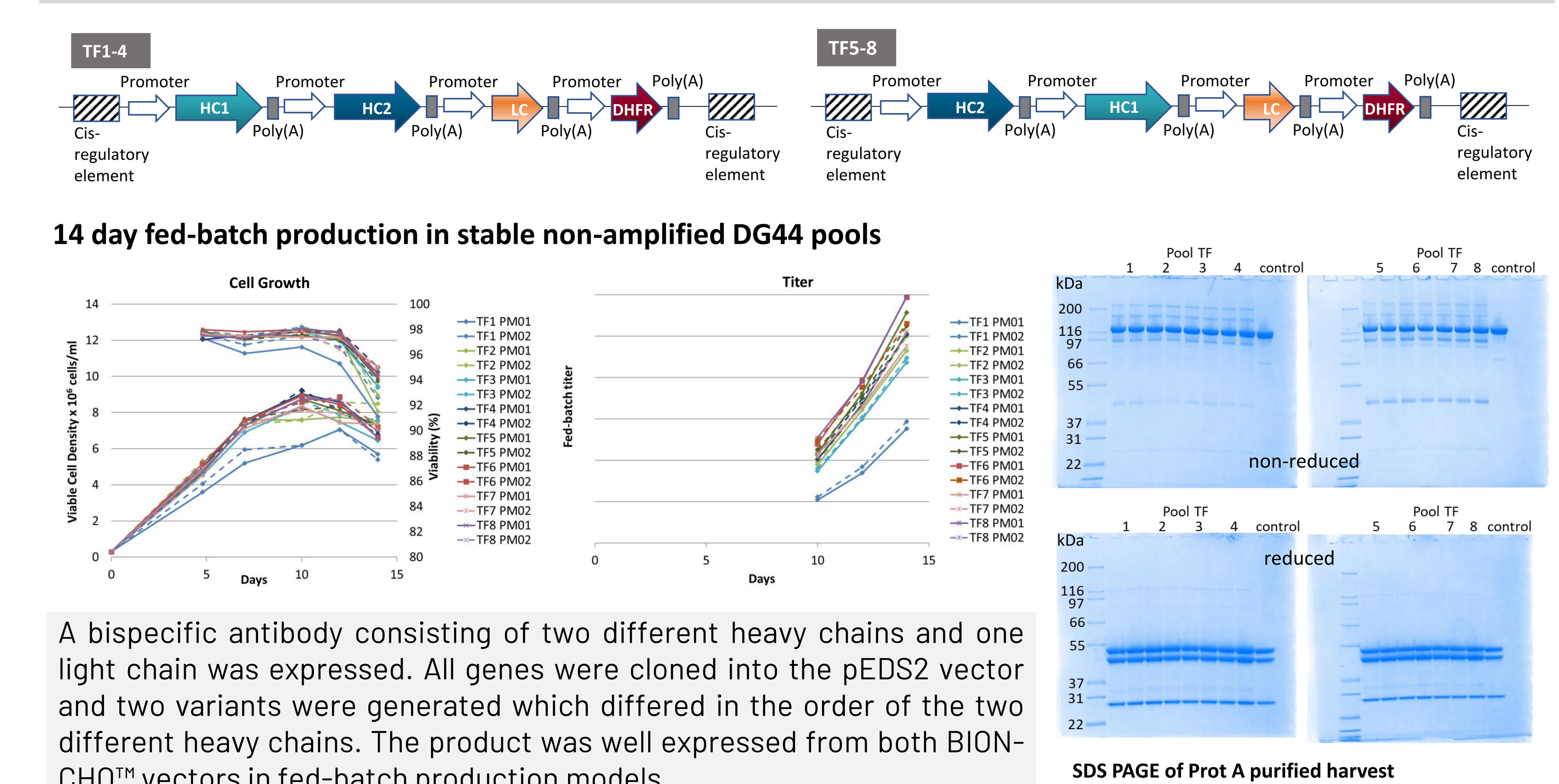
For eight of the clones expressing a mAb from BION-CHO™ vectors a stability study was performed. During the stability cultivation for 9 weeks, cell banks were cryopreserved after 2, 4, 7 and 9 weeks and the fed-batch titer of these cell banks was compared. 6 of the 8 clones showed stable expression with titers not changing more than 16% between week 2 and 9. No aggregation or product degradation was experienced during the stability study.

Protease expression in pools using BION-CHO™ vectors



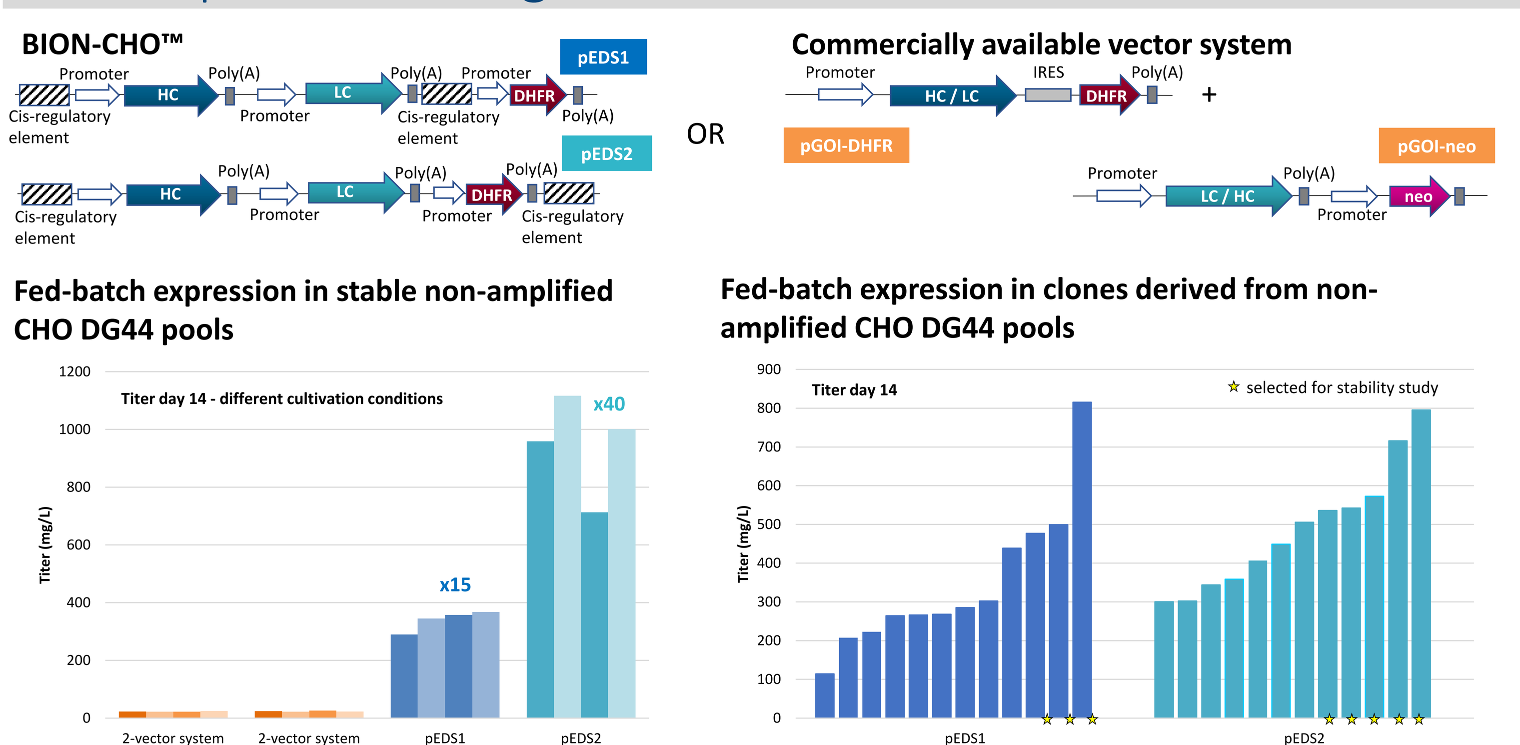
A protease was expressed in DG44 pools stably transfected either with a variant of the BION-CHO™ vectors or with a commercial vector. The Bioneer vectors pEDS1 and pEDS2 differ in the position of the second cis-regulatory element. Enzyme activity in harvest supernatants from fed-batch production models was 20x higher for both BION-CHO™ vectors despite comparable cell growth. The higher protease concentration compared to the commercial vector was confirmed with SDS-PAGE.

bsAb expression in pools using BION-CHO™ vectors



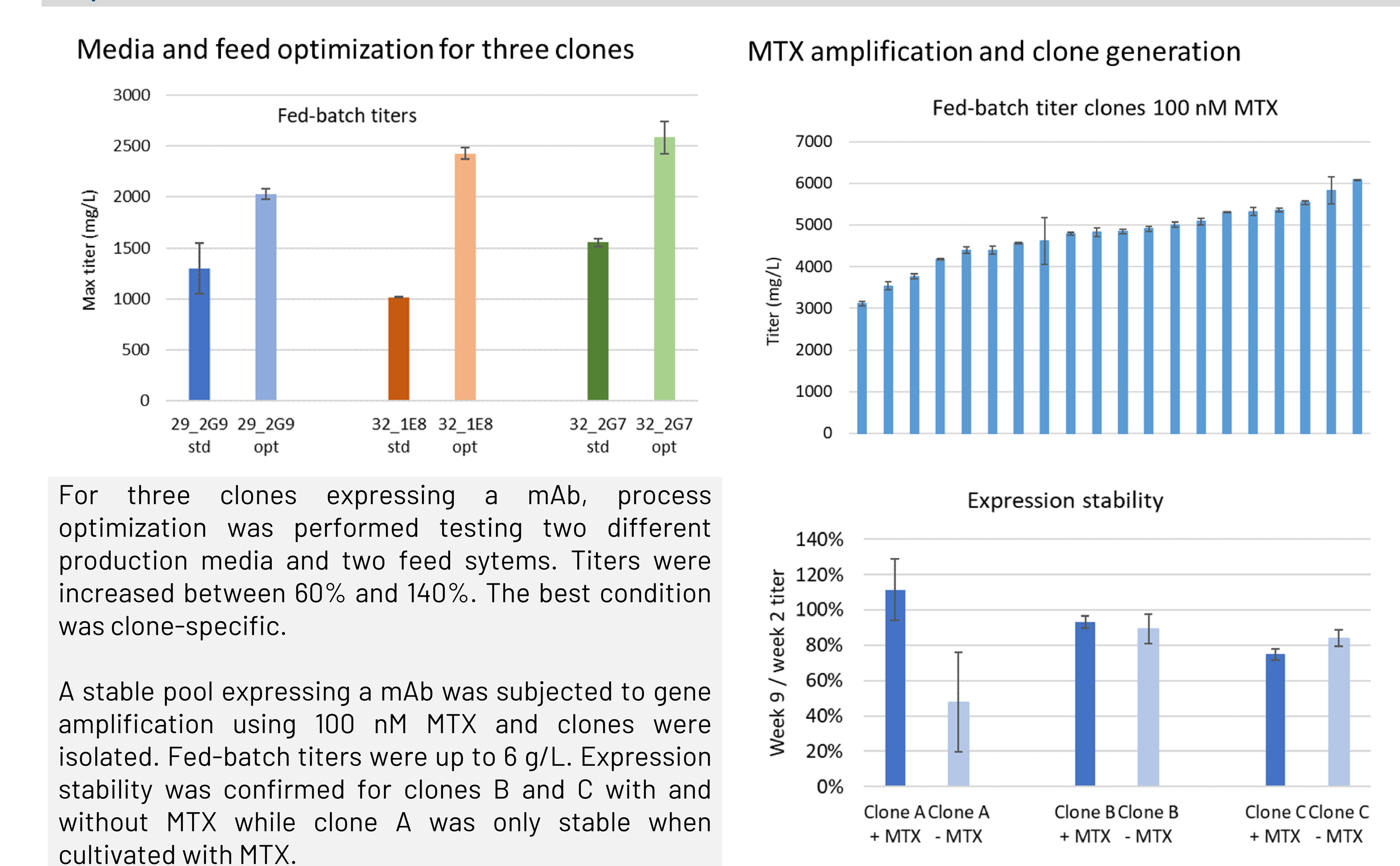
A bispecific antibody consisting of two different heavy chains and one light chain was expressed. All genes were cloned into the pEDS2 vector and two variants were generated which differed in the order of the two different heavy chains. The product was well expressed from both BION-CHO™ vectors in fed-batch production models.

mAb expression using BION-CHO™ vectors



The expression of a monoclonal antibody was compared between two variants of the BION-CHO™ vectors and a commercially available vector system consisting of two expression plasmids for expression of heavy and light chain. Fed-batch titers were 15 x higher for pEDS1 and 40 x for pEDS2. Clones were generated from the BION-CHO™ pools with titers up to 0,8 g/L.

Optimization of clone titers

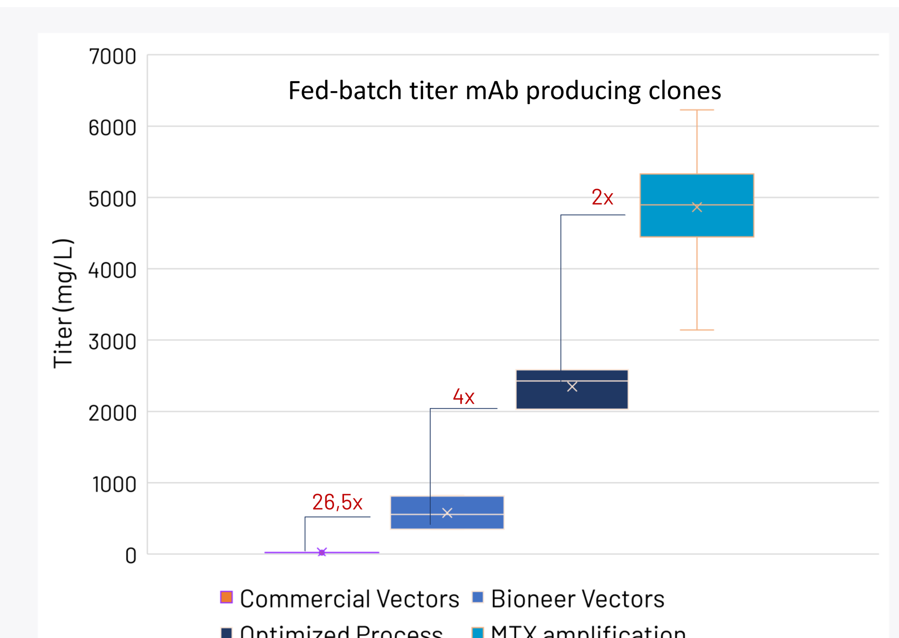


For three clones expressing a mAb, process optimization was performed testing two different production media and two feed sytems. Titers were increased between 60% and 140%. The best condition was clone-specific.

A stable pool expressing a mAb was subjected to gene amplification using 100 nM MTX and clones were isolated. Fed-batch titers were up to 6 g/L. Expression stability was confirmed for clones B and C with and without MTX while clone A was only stable when cultivated with MTX.

CONCLUSION

We have successfully expressed products consisting of one to three protein subunits using the BION-CHO™ vectors. Titers were 15 to 40 x higher compared to a commercially available vector system. Initial fed-batch clone titers for expression of a mAb were 0,8 g/L and increased after process optimization and MTX amplification to 6 g/L.



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