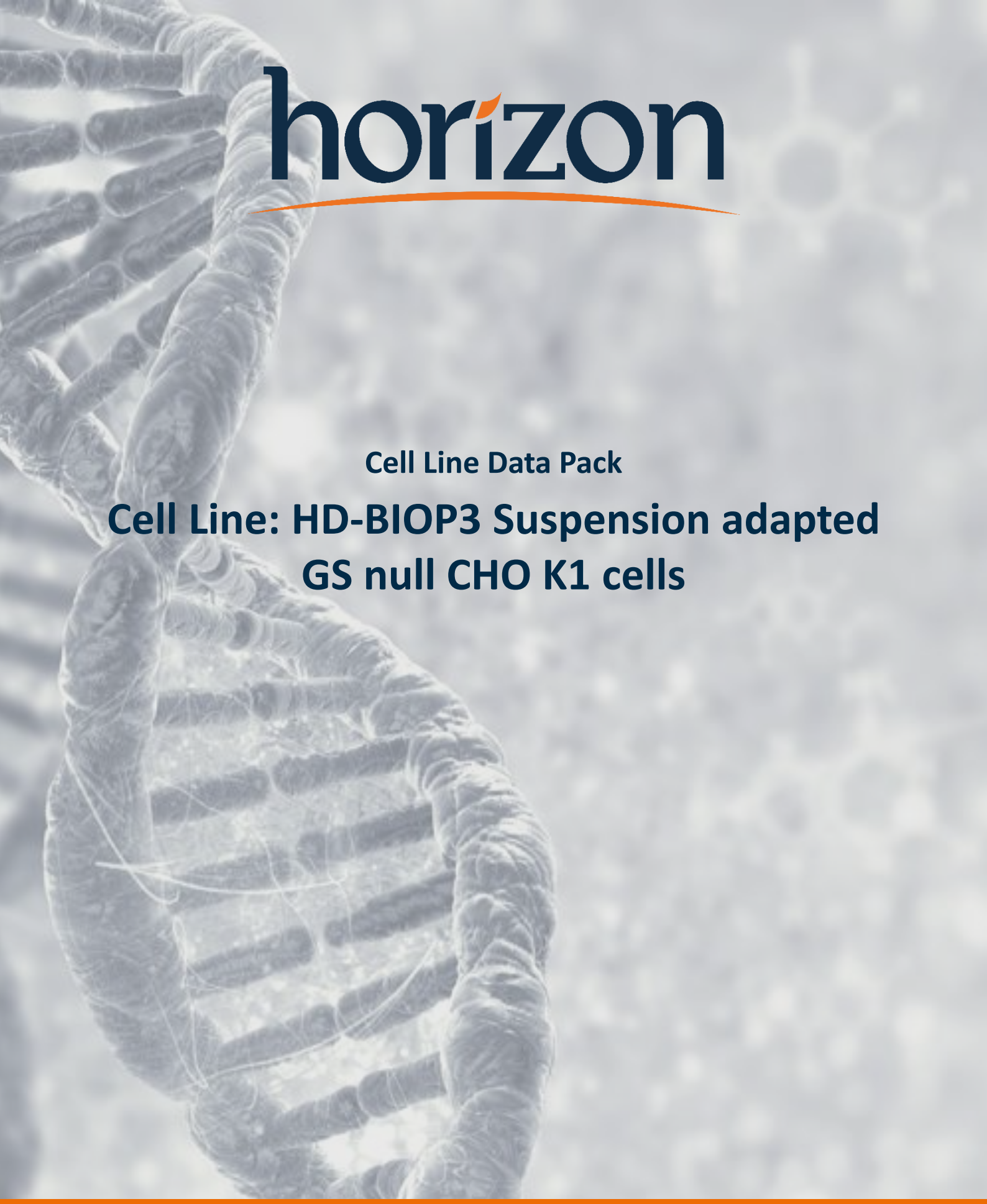

Cell Line Data Pack

Cell Line: HD-BIOP3 Suspension adapted GS null CHO K1 cells

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f + 44 (0)1223 655581
e info@horizondiscovery.com
w www.horizondiscovery.com
Horizon Discovery, 7100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

GLUTAMINE SYNTHETASE SELECTION

- Glutamine is an amino acid required for cellular viability
- Glutamine Synthetase (GS) is an enzyme that catalyses the production of glutamine from glutamate and ammonia
- This is the only source of glutamine production in the cell
- In the absence of functional GS, the cell is dependent on exogenous glutamine for survival
- Expression of an exogenous GS gene overcomes this requirement for glutamine in the media
- Vectors expressing recombinant protein and GS can be stably transfected into target cells with no endogenous GS activity
- This allows metabolic selection of successful transfectants using media lacking glutamine

GLUTAMINE SYNTHETASE KNOCKOUT

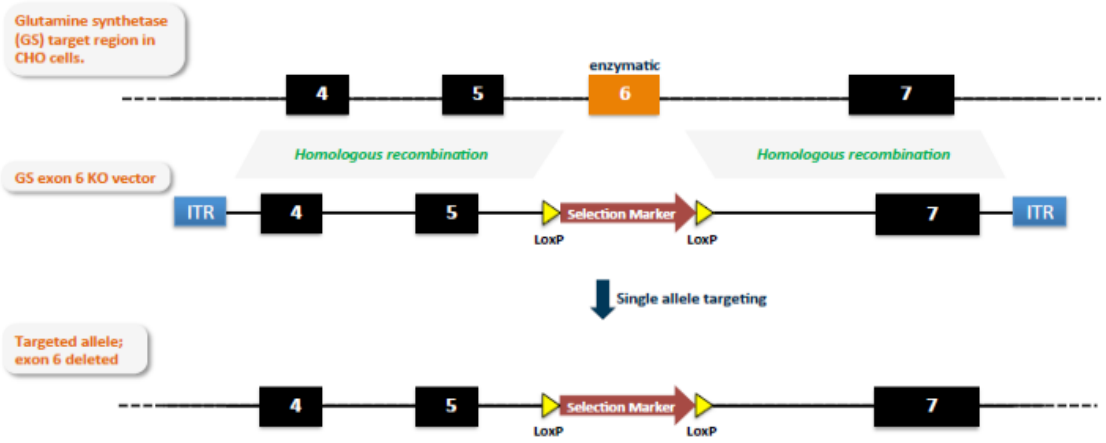
- Historically GS has been inhibited by Methionine Sulphoximine (MSX)
- Gene amplification associated with glutamine selection leads to increase yield
- However MSX is toxic and cannot be used in drug production
- Using parental cells lacking GS have been shown to increase selection efficiency over MSX selection
- Using parental cells lacking GS have been shown to increase bulk culture productivity over MSX selection
- Using GS null cells allows for single step selection of high producing clones
- GS selection is currently the industry accepted method

SPECIFICATION

- Cells originated from ECACC CHOK1 line
- Banked under cGMP conditions
- Exon 6 of GS gene (required for enzymatic activity) removed by rAAV mediated recombination
- Adapted to suspension growth in commercially available chemically defined, animal component free media
- Sequence specific
- No off target mutations
- Documented traceability
- Production history
- Genetic testing
- Sterility testing
- Comprehensive testing for human and rodent viruses

VECTOR DESIGN

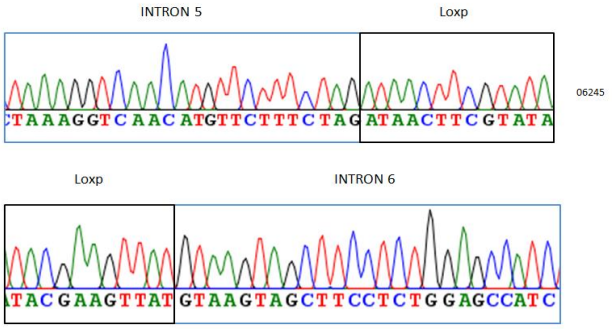
Exon 6, required for the enzymatic activity of Glutamine Synthetase , was removed from CHOK1 cells by targeted recombination.



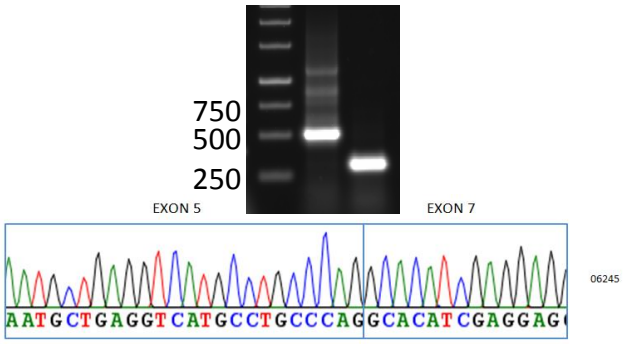
MOLECULAR BIOLOGICAL VALIDATION OF KNOCKOUT

Knockout of exon 6 was confirmed by PCR and sequencing.

Genomic PCR across GS locus

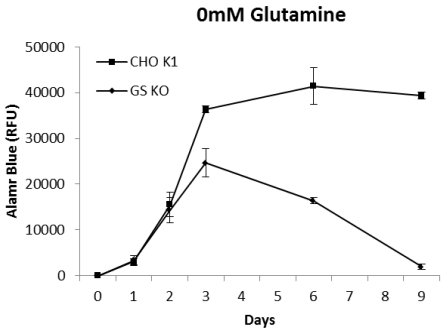


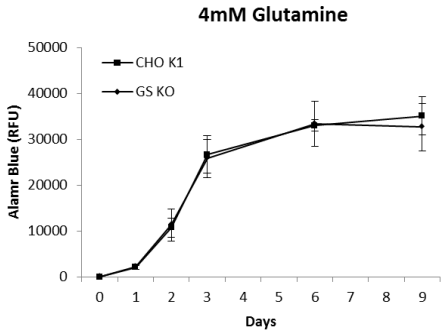
RT-PCR across GS transcript



FUNCTIONAL VALIDATION OF KNOCKOUT

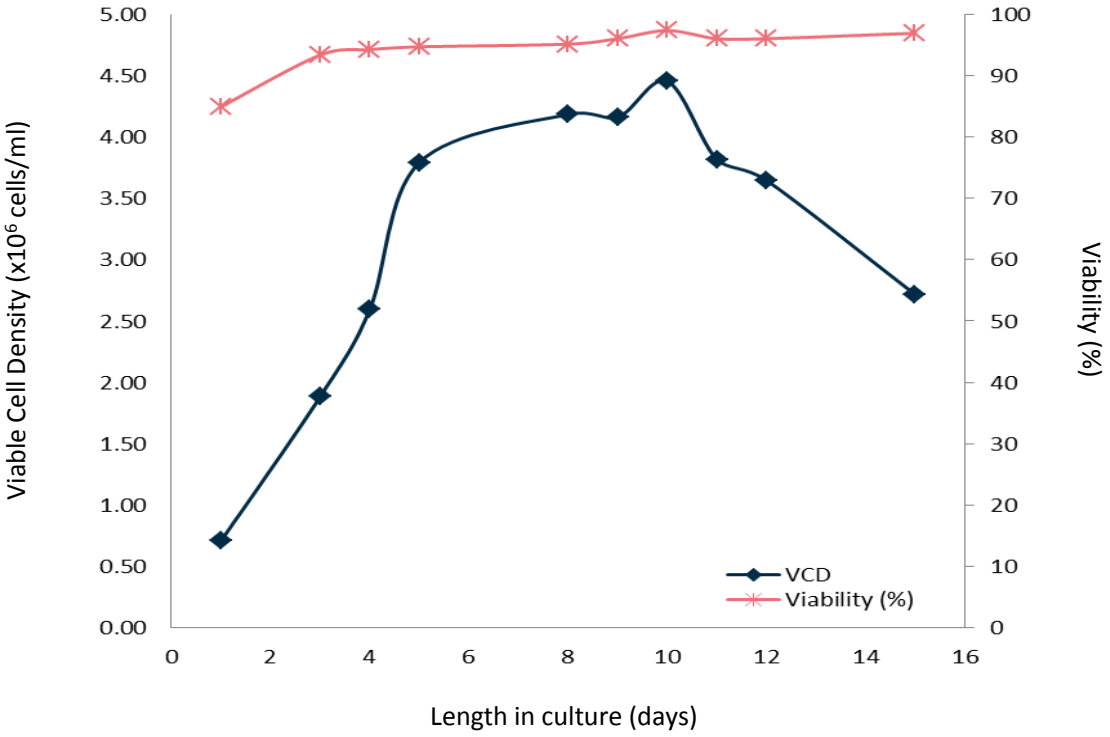
When transferred to media lacking glutamine, the wild type CHO cells remain viable whilst the GS-/- cells are unable to survive. However, when transferred to media supplemented with 4mM glutamine, both cell lines grow at comparable rates.





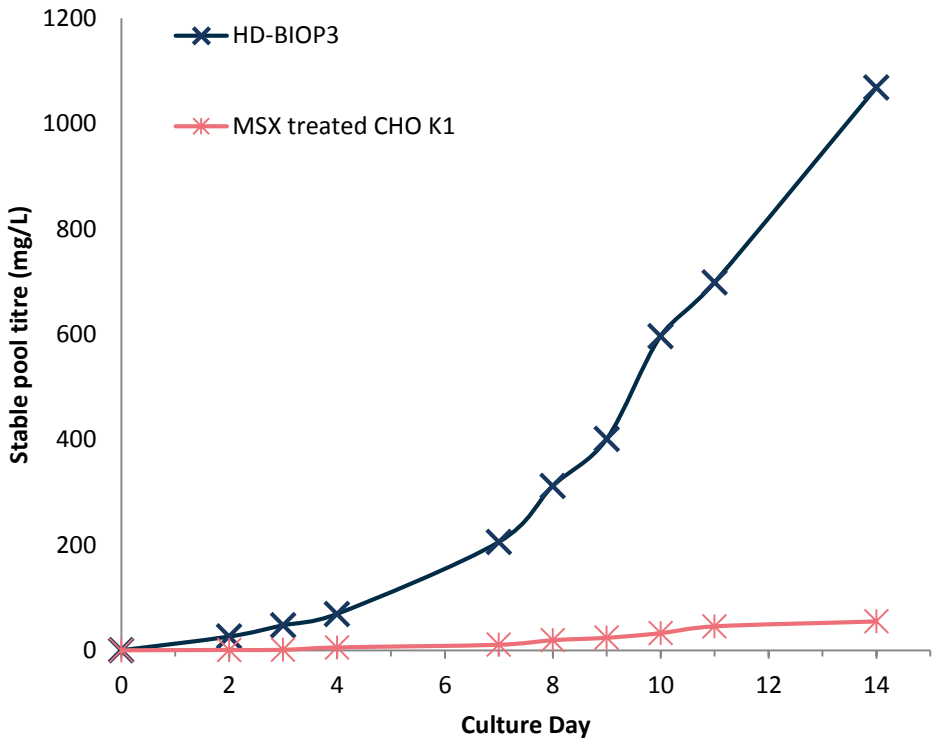
ADAPTATION TO SUSPENSION CULTURE

The cells were adapted to suspension culture in animal component free, chemically defined media. In the example below, the cells reached a Viable Cell Density of over $4x10^6$ cells/ml when expressing a Glutamine Synthetase expression vector. A high viability was maintained for over 14 days in a 5L bioreactor batch culture.



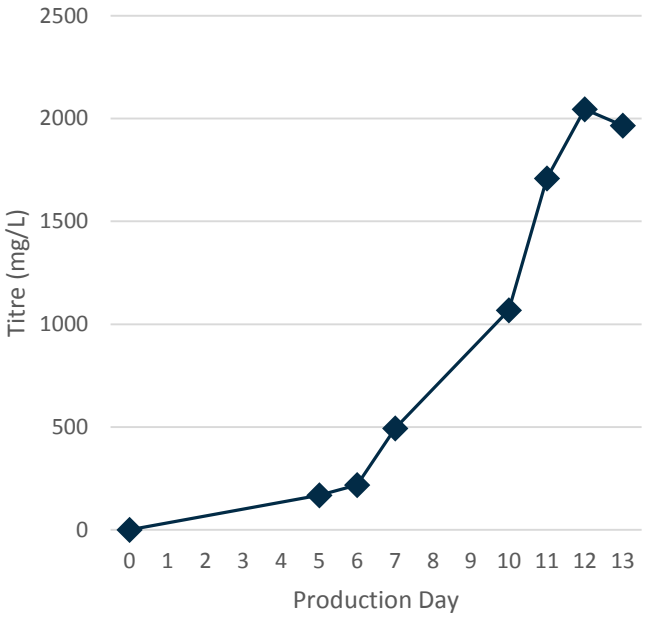
EXPRESSION OF REFERENCE PRODUCT

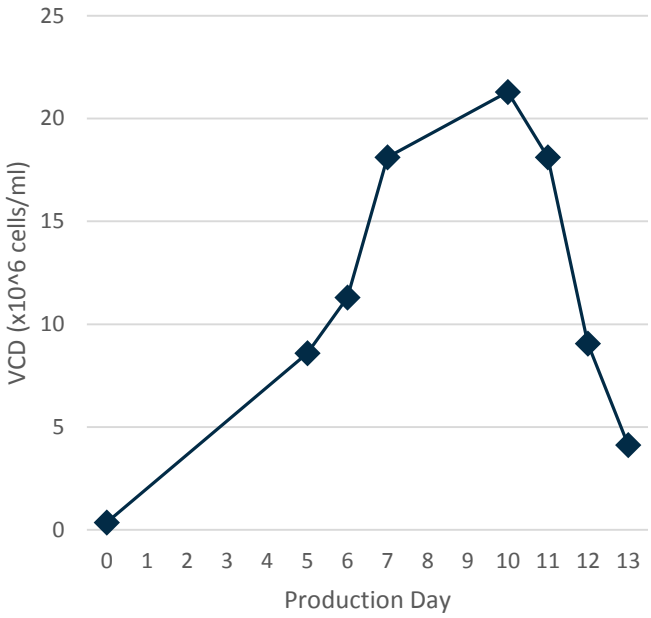
After being transfected with a GS selection vector containing an IgG product and placed under selection in glutamine free media, the cells expressed significantly higher titres of protein than the reference system comprising MSX treated wild type CHO K1 cells.



STABLE POOL EXPRESSION

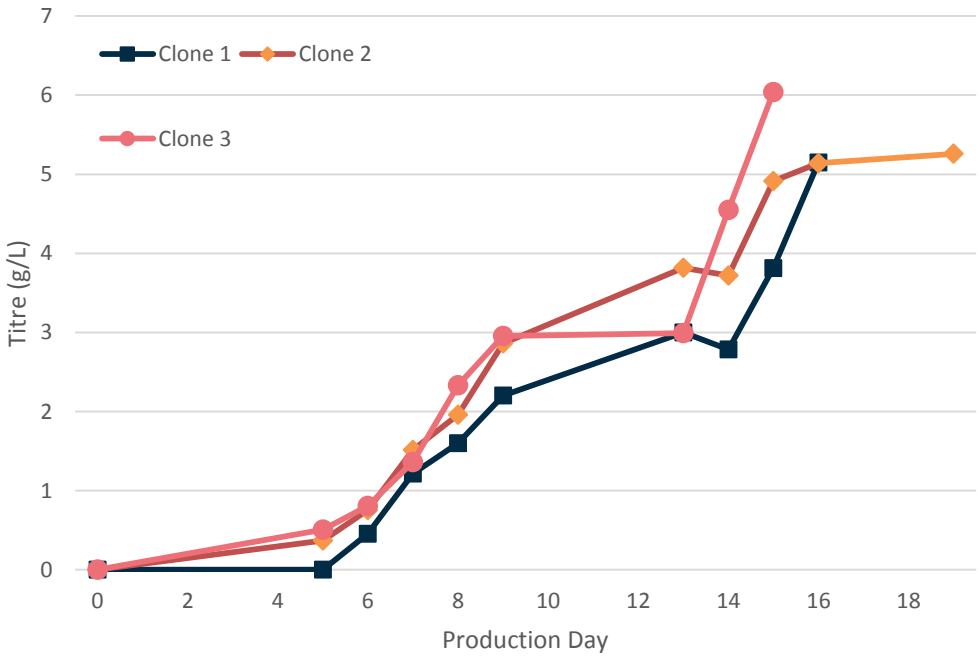
As part of an evaluation of different vector technologies, the cells were transfected with a construct expressing an IgG in a vector containing Unic™ elements from ProteoNic.
Fed-batch culture of the stable pools extended the culture time of the cells while maintaining a good VCD. Titres in excess of 2g/L of a biosimilar IgG were achieved at stable pool stage.





CLONAL EXPRESSION

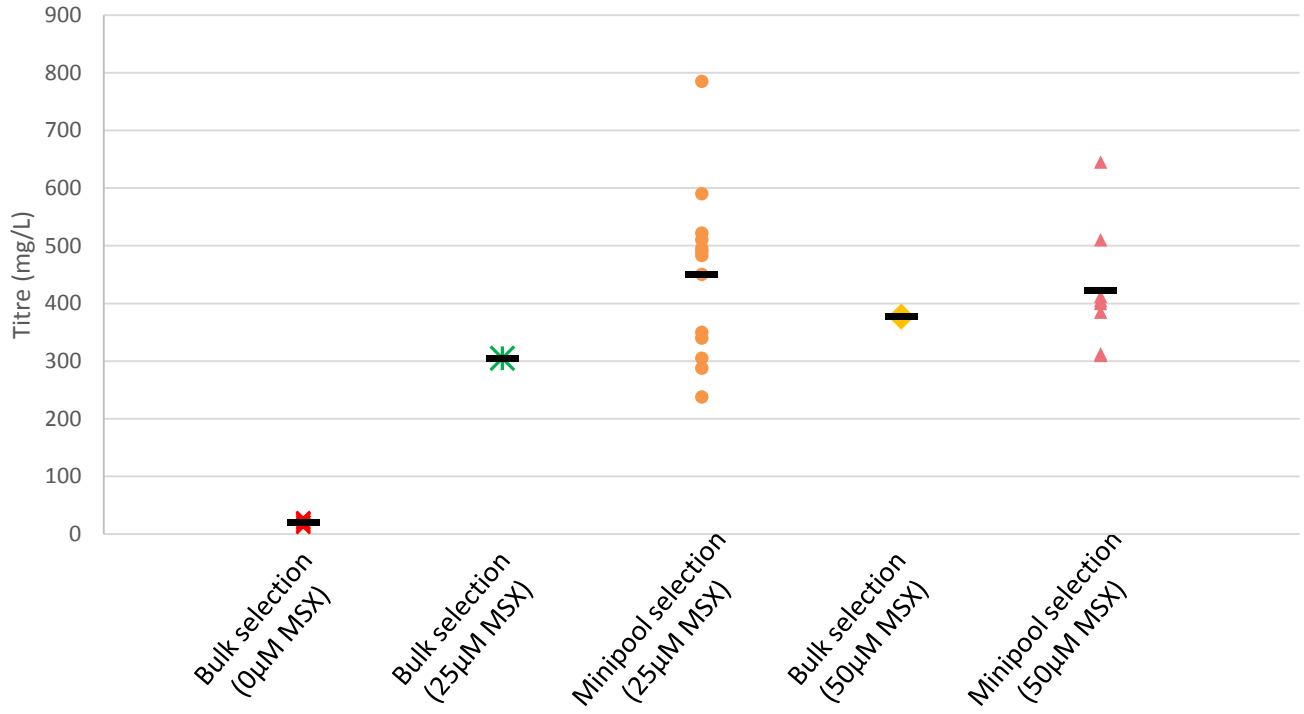
Four hundred clones from the stable pool above were screened and assessed for productivity. Three clones expressed at >5g/L. This is currently an un-optimised process, with further advances expected.



ProteoNic

STABLE POOL EXPRESSION

As part of an evaluation of HD-BIOP3, a Biopharmaceutical company in China developed stable pools using a vector provided by DNA 2.0. They assessed the impact of using bulk selection compared with generating minipools through applying selection to 500 cells. Together with this, they evaluated the impact of using MSX with this vector. **Data shown is after 5 days of shake culture**.



STABLE POOL EXPRESSION

As part of an evaluation of HD-BIOP3, a Biopharmaceutical company in the USA developed stable pools using a vector provided by DNA 2.0, together with increasing concentrations of MSX. **Data shown is after Protein A purification.**