Novel Cell Engineering Platform for Increasing Specific Productivity of CHO Cells for Therapeutic Antibody Production in Fed-Batch Cultures

Kathy Ngo[1], Lawrence Chasin[2], Larry Forman[1]

[1]CHO Plus, Inc., South San Francisco, CA, [2]Columbia University, New York, NY

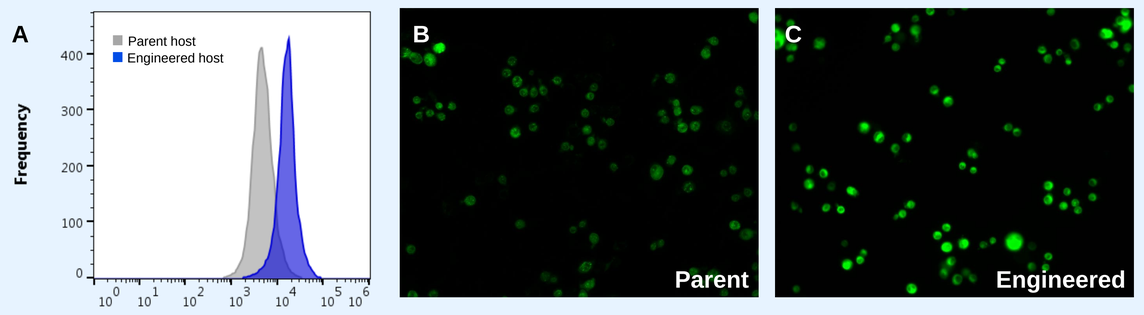
CHO+Plus

TECHNOLOGY PLATFORM

EXCEEDING BIOLOGICAL LIMITS

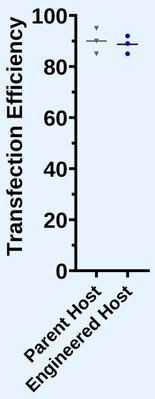
Experimental Approach: Repeated **h**omotypic fusions of CHO cells result in genome shuffling and amplification of whole chromosomes. Cells were selected for high expression of proteins associated with endoplasmic reticulum (ER). Fused cell hybrids advantageously selected for enhanced ER (up to >10-fold higher) were evaluated for recombinant protein production by transient and stable transfection of heavy- and light-chain genes coding for a recombinant human therapeutic monoclonal antibody (mAb).

10x HIGHER ER

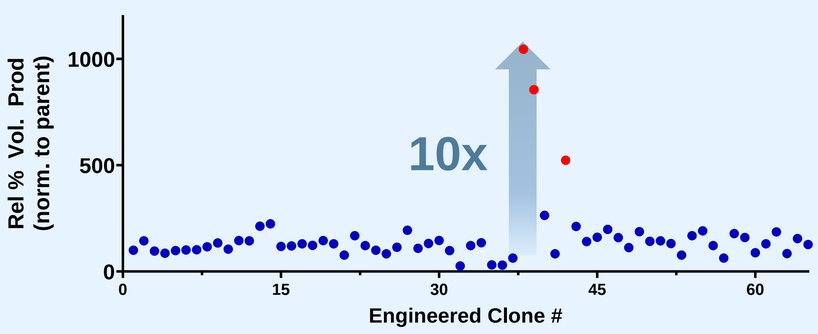


A - Flow cytometry histogram showing distribution of a fluorescently-labeled protein expressed in the endoplasmic reticulum (ER) in original parent CHO host (gray) and engineered host cells, pooled (blue). **B, C -** Fluorescent microscopy images of CHO cells (parent host in **B**; engineered host in **C**), labeling protein expressed in the ER. The same exposure time for parent host and engineered host was used for image acquisition.

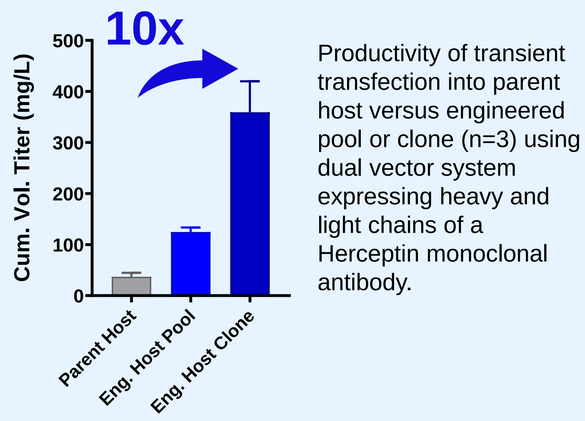
IMPROVEMENT OF TRANSIENT PRODUCTION OF THERAPEUTIC ANTIBODIES



Transfection efficiency n=3), measured using a mammalian expression vector driving expression of a fluorescent protein.



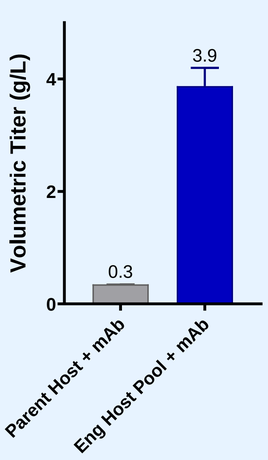
Distribution of volumetric productivity for 60 engineered host clones transfected with a human IgG1 expressed via a single vector. Volumetric productivities have been normalized using volumetric productivity of the parent host (100%). Red indicates top host clones with the highest transient productivities.



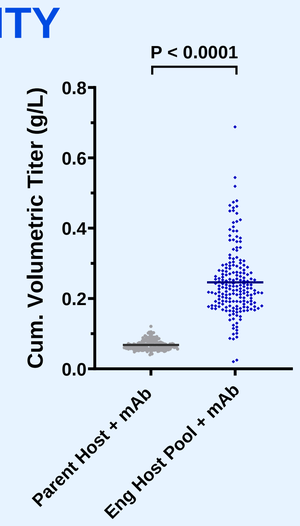
Productivity of transient transfection into parent host versus engineered pool or clone (n=3) using dual vector system expressing heavy and light chains of a Herceptin monoclonal antibody.

IMPROVEMENT OF STABLE PRODUCTION OF THERAPEUTIC ANTIBODIES

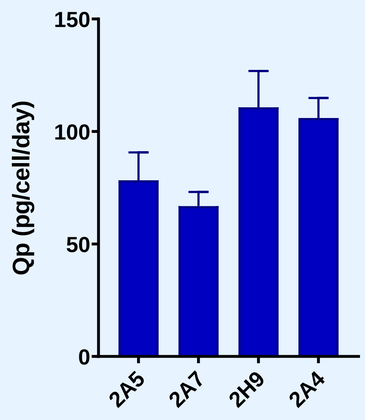
10x PROTEIN PRODUCTIVITY

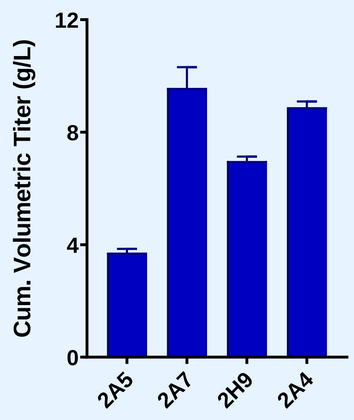


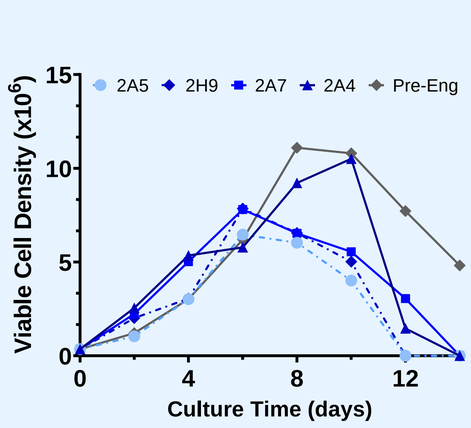
125-mL shake flask fed-batch productivity for parent and engineered **host pools** stably transfected using transposase technology.



Clonal volumetric productivity derived from the stable pool of parent host (left) compared to engineered stable pool of engineered host (right) grown in **96-well plates**, static culture.

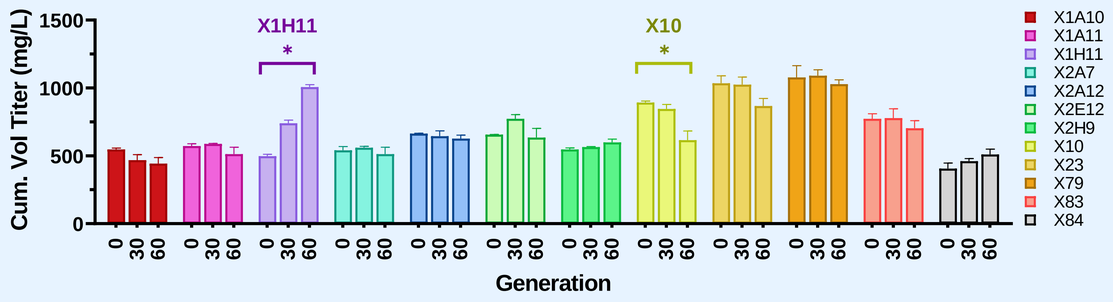






Specific productivity (Qp), cumulative volumetric productivity, and growth curve of fed-batch runs of 4 representative lead clones (from B) derived from engineered host (pools) stably transfected using transposase technology.

STABLE CLONES



Host Chinese hamster ovary cells (CHO) cell were engineered using CHO Plus proprietary technology and stably transfected with vector expressing heavy and light chain antibody genes. Single cell clones expressing monoclonal antibody are stable after 60 generations. Cumulative monoclonal antibody (mAb) concentrations for each sample were measured in 5 replicates after 7-day production run at Generation 0, 30 and 60 (G0, G30, and G60; n=5). With the exception of cell lines X1H11 and X10, no statistical significance observed between batch productivity at G30 and G60 compared to G0. mAb concentrations of harvested supernatants were measured using Biolayer Interferometry. For all samples, statistical significance was calculated using the Kruskal Wallis test at a confidence level of 0.05. * denotes cell lines that showed statistical significance (P<0.05) between G30 or G60 compared to G0.

CONCLUSIONS

We have demonstrated a disruptive cell-engineering platform to significantly enhance CHO cell culture manufacturing capabilities:

- \+ Up to a **10-fold increase in specific productivity** (117 pg/cell/day) for engineered CHO cells producing a therapeutic mAb via transient and stable transfections
- \+ Up to 9.5 g/L and 117 pg/cell/day demonstrated for single-cell clones producing a therapeutic mAb at shake flask scale
- \+ Synergistic productivity enhancement in engineered cells when **Sleeping Beauty Transposase** technology is used

www.CHO-Plus.com

WE'RE HIRING!