New Cell Engineering Method for Increasing Recombinant Therapeutic Antibody Productivity Up To 10-Fold in Batch CHO Cultures

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

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

ABSTRACT

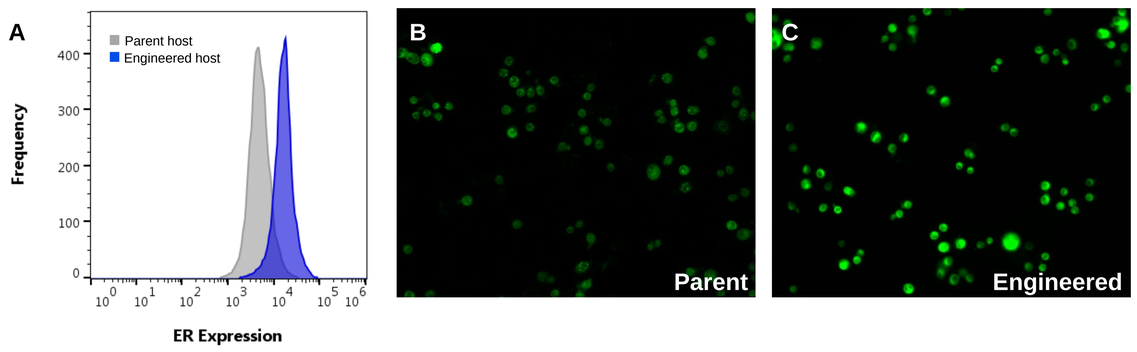
Background and Novelty: Despite decades of cell culture process improvements and cell engineering methods development, there remain intrinsic bottlenecks to higher recombinant protein productivity using Chinese hamster ovary (CHO) cells. These bottlenecks limit CHO cell-based manufacturing potential. Here we disclose a directed evolution strategy to genetically engineer and select for CHO cells with enhanced protein-production machinery. CHO Plus's innovative approach circumvents natural limitations present in CHO cells, positioning it to become a new and vastly improved manufacturing paradigm for recombinant therapeutic protein production.

Experimental Approach: Homotypic fusions of CHO 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).

Results and Discussion: CHO cells engineered using our approach exhibited enhanced transient specific and volumetric productivities for a human mAb of up to 4-fold compared to un-engineered parental cells in batch cultures. For stable transfection, up to 10-fold increases were observed for specific and volumetric productivities for engineered cells expressing a human mAb at the transfected-pool stage, with clones producing mAb concentrations of up to 9.5 g/L in shake flask cultures.

CHO Plus has demonstrated that our cell engineering technologies, leveraging directed evolution methods, represent a novel and disruptive platform for vastly improving CHO cell manufacturing capabilities.

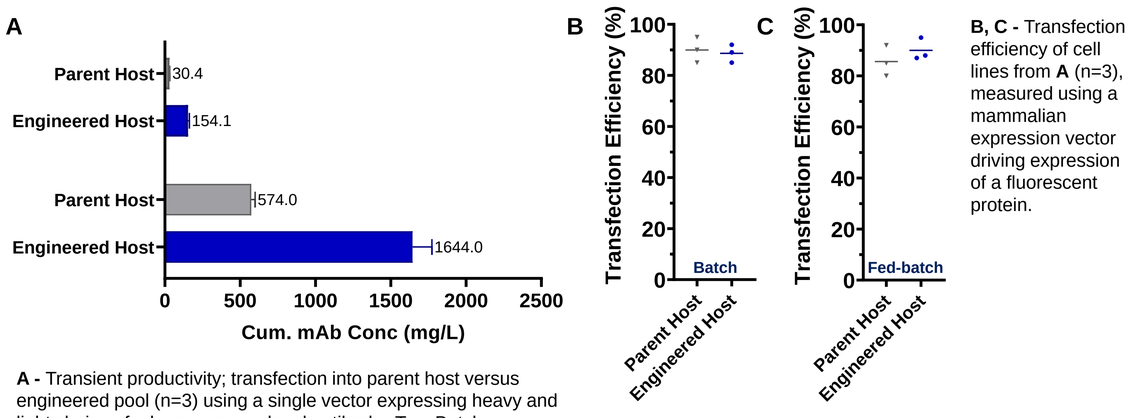
CHO Plus technologies can be used to engineer CHO cells to contain ~10-fold higher ER



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.

Improved transient production using engineered CHO host cells

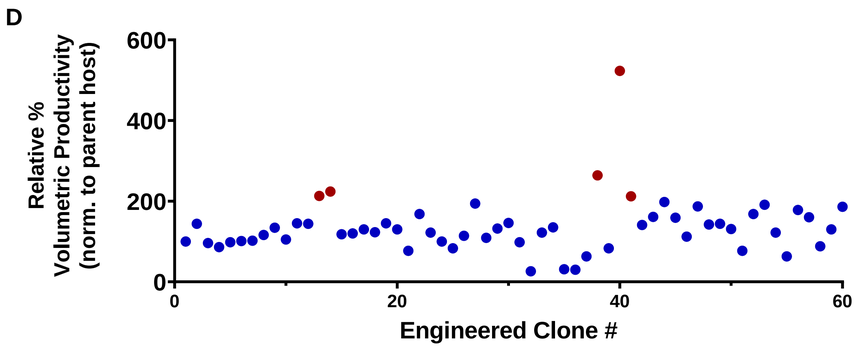
Pool Production, Shake Flasks



A - Transient productivity; transfection into parent host versus engineered pool (n=3) using a single vector expressing heavy and light chains of a human monoclonal antibody. Top: Batch process; Bottom: Fed-batch process.

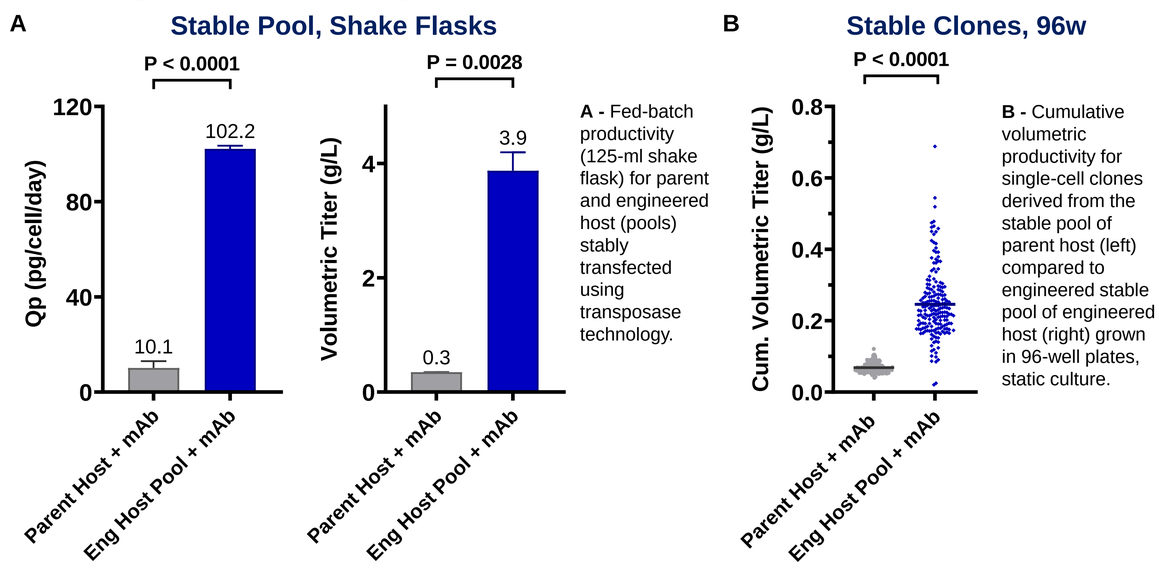
B, C - Transfection efficiency of cell lines from **A** (n=3), measured using a mammalian expression vector driving expression of a fluorescent protein.

Clone Production, 24-well, shaking cultures



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

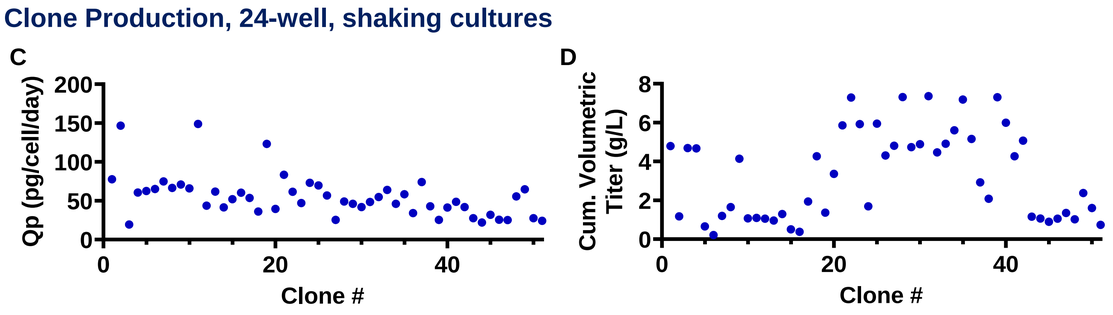
Synergistic stable productivity enhancement when transposase transfection technology is used with engineered parent cells



A - Fed-batch productivity (125-ml shake flask) for parent and engineered host (pools) stably transfected using transposase technology.

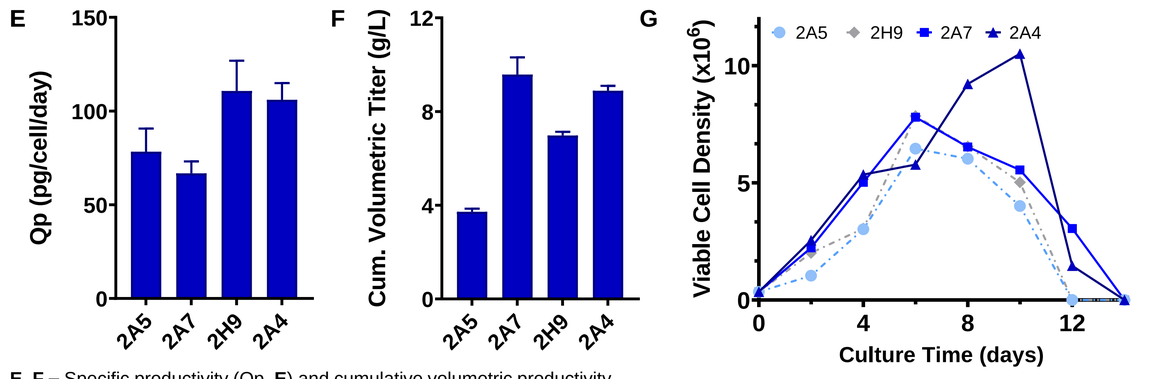
B - Cumulative volumetric productivity for single-cell clones derived from the stable pool of parent host (left) compared to engineered stable pool of engineered host (right) grown in 96-well plates, static culture.

Clone Production, 24-well, shaking cultures



C,D - Fed-batch productivity (24-well) of single cell clones derived from engineered host (pools) stably transfected using transposase technology. Distribution of specific productivity (left panel) and cumulative volumetric productivity (right panel) are shown for individual clones.

Clone Production, 125-ml shake flasks



E, F – Specific productivity (Qp, **E**) and cumulative volumetric productivity (**F**) of fed-batch runs of 4 representative lead clones (from C, D) derived from engineered host (pools) stably transfected using transposase technology.

G - Viable cell density from the same fed-batch run from **E**, **F** of 4 representative lead clones.

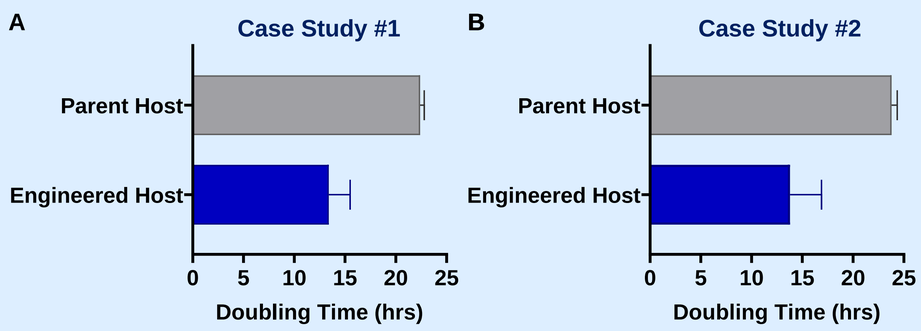
CONCLUSION

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

+ Synergistic productivity enhancement in engineered cells when used with transposase technology
+ Up to 10-fold increase in productivity via transient and stable transfections
+ Up to 9.5 g/L volumetric productivity demonstrated for single-cell clones at shake flask scale
+ Additional phenotypes, such as faster growth, can be engineered into host cells
+ Cells can be engineered for faster growth; growth rate increased by 50% while maintaining initial cell-specific productivity

OTHER CAPABILITIES

The CHO Plus cell engineering platform can be used to engineer-in additional advantageous phenotypes, such as faster growth, while maintaining productivity of the un-engineered cells:



A, B - Doubling time of parent and engineered host grown in 125-ml shake flasks. Doubling time was averaged after 5 successive generations (n=5). As a proof-of-concept, two different CHO hosts were used as a starting point.

www.CHO-Plus.com