New Cell Engineering Method for Increasing Productivity of Recombinant Therapeutic Antibodies Up To 10-Fold in Fed Batch Cultures

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ABSTRACT

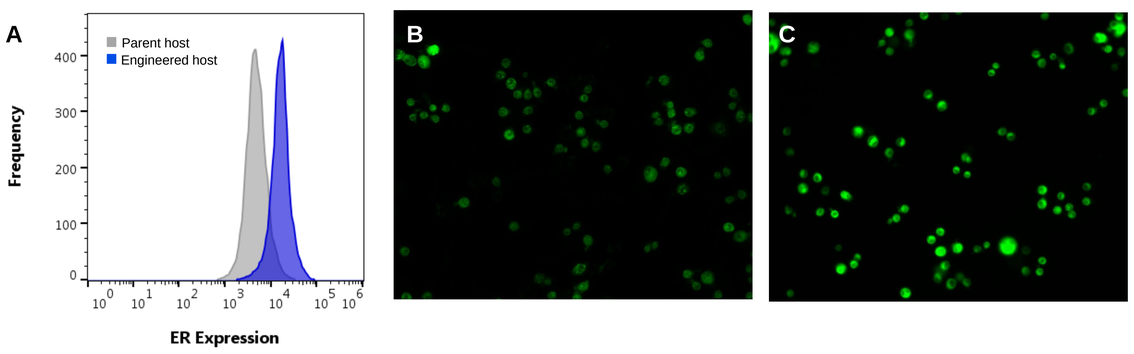
Background and Novelty: Despite decades of cell culture process improvements and cell engineering methods development, there remain intrinsic bottlenecks to higher recombinant protein productivity using Chinese hamster ovary (CHO) cells. These bottlenecks limit CHO cell-based manufacturing potential. Here we disclose a directed evolution strategy to genetically engineer and select for CHO cells with enhanced protein-production machinery. Our 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 ER) were evaluated for recombinant protein production by transient and stable transfection of a recombinant human therapeutic monoclonal antibody (mAb).

Results and discussion: CHO cells engineered using our approach exhibited enhanced transient cell 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 recombinant human mAbs at the transfected-pool stage.

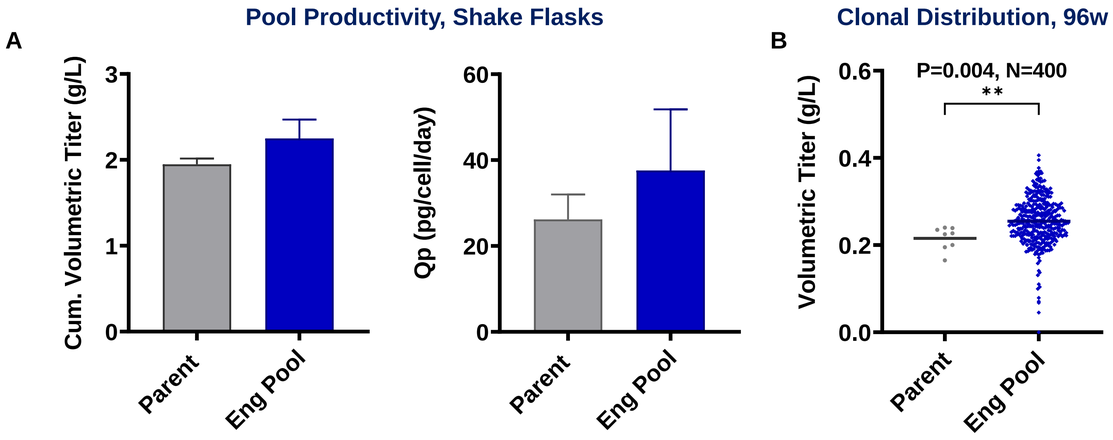
We have demonstrated that our cell engineering technologies, leveraging directed evolution methods, represent a novel and disruptive platform for vastly improving CHO cell manufacturing capabilities.

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

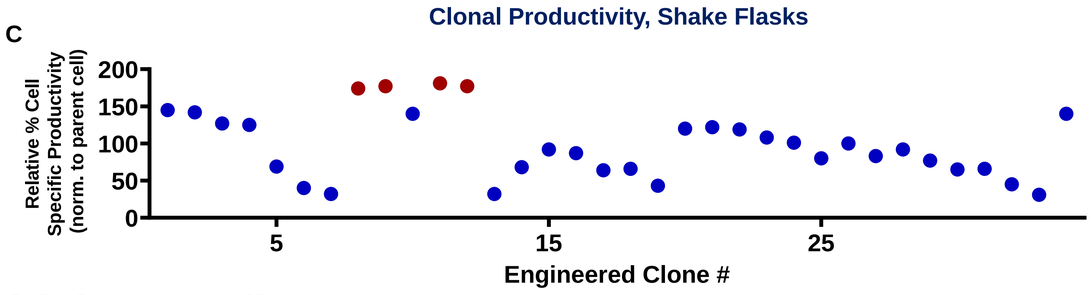


A - Flow cytometry histogram showing distribution of a fluorescently-labeled protein expressed in the endoplasmic reticulum (ER) in original parent CHO host (gray) and engineered host cells, pooled (blue). **B, C -** Fluorescent microscopy images of CHO cells (parent host in **B**; engineered host in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

Our technology can improve productivity of a pre-existing manufacturing cell line producing a therapeutic monoclonal antibody



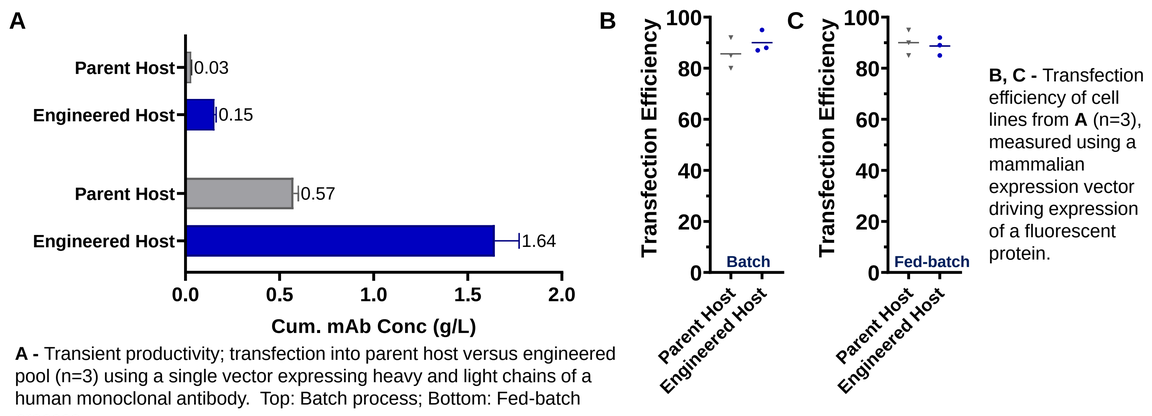
A - Fed-batch productivity (125-ml shake flask) of a manufacturing cell line and a manufacturing cell line pool engineered using CHO Plus technology. **B -** Distribution of volumetric titer in 96-well, static cultures of single cell clones derived from parent and engineered pool from **A**, demonstrating that the volumetric productivity difference between clones derived from a parent manufacturing cell line and the engineered pool is highly significant (performed using Student's t-test).



C - Fed-Batch productivity (125-ml shake flask) of single-cell clones derived from pools of an engineered manufacturing cell line. Relative cell specific productivities were normalized to the parent manufacturing cell line (100%), performed in the same experiment. Red indicates top producers.

Improved transient production using engineered CHO host cells

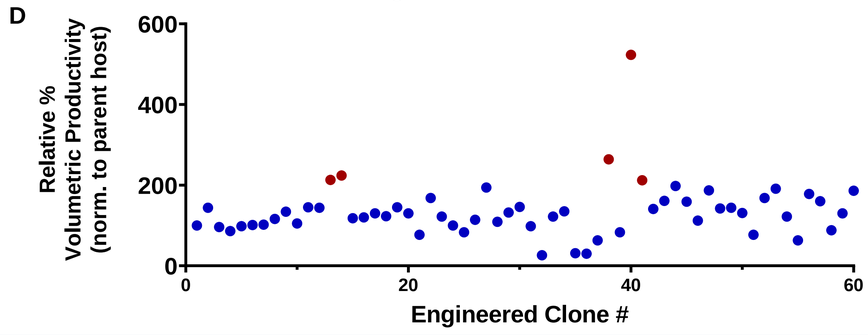
Pool Production, Shake Flasks



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.

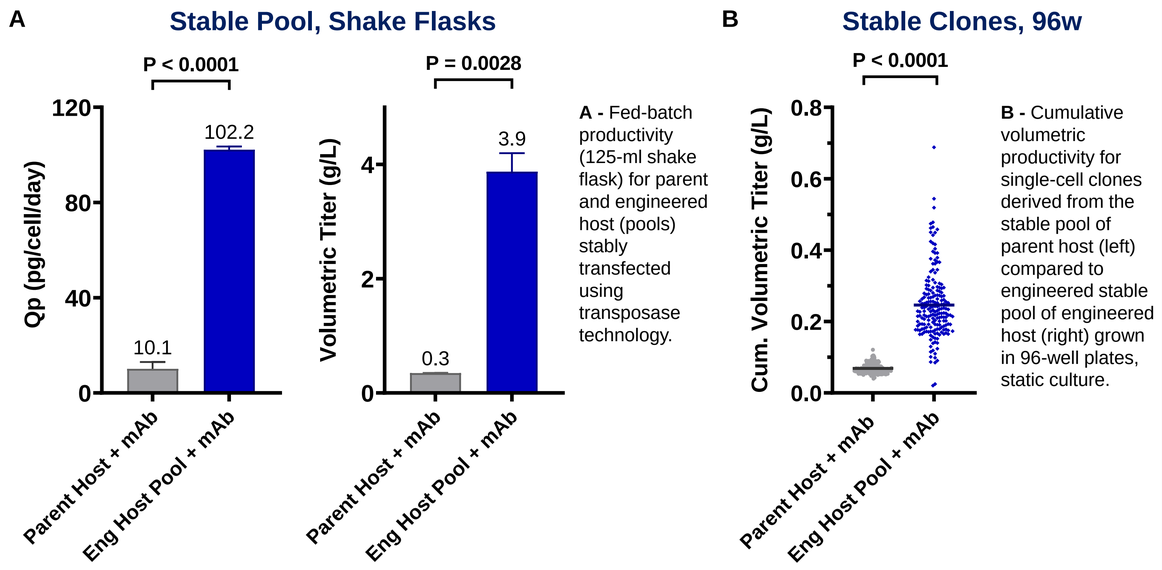
B, C - Transfection efficiency of cell lines from **A** (n=3), measured using a mammalian expression vector driving expression of a fluorescent protein.

Clone Production, 24-well, shaking cultures



D - Distribution of volumetric productivity for 60 engineered host clones transfected with 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.

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

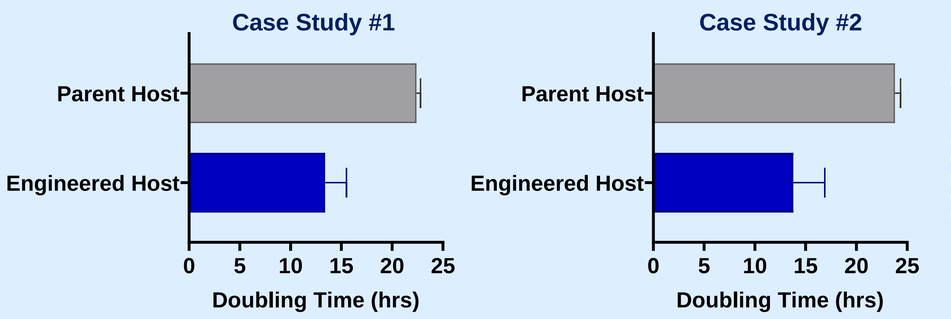


A - Fed-batch productivity (125-ml shake flask) for parent and engineered host (pools) stably transfected using transposase technology.

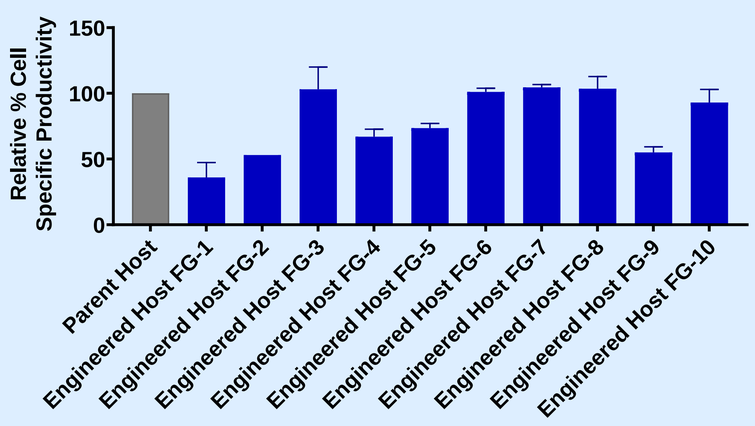
B - Cumulative volumetric productivity for single-cell clones derived from the stable pool of parent host (left) compared to engineered stable pool of engineered host (right) grown in 96-well plates, static culture.

DISCUSSION

CHO Plus platform can be used to engineer-in additional advantageous phenotypes, such as faster growth, while maintaining productivity:



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.



Transient productivity (batch process); transfection into parent host versus engineered, fast-growing clones (n=10) using a single vector expressing heavy and light chains of a human monoclonal antibody; normalized (parent =100%).

CONCLUSION

We demonstrate a disruptive platform to enhance CHO manufacturing capabilities:

+ Synergistic productivity enhancement in engineered cells when used with transposase technology
+ Cell growth rate increased by 50% while maintaining initial cell-specific productivity
+ Up to 10-fold increase in productivity via transient and stable transfections
+ Up to 4 g/L cumulative volumetric productivity demonstrated for stably-transfected pools
+ Additional phenotypes, such as faster growth—and others, can be engineered into host cells

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