Novel Directed Evolution Platform for Creating High Productivity Cells for Therapeutic Protein Production, Virus Production, and Other Processes

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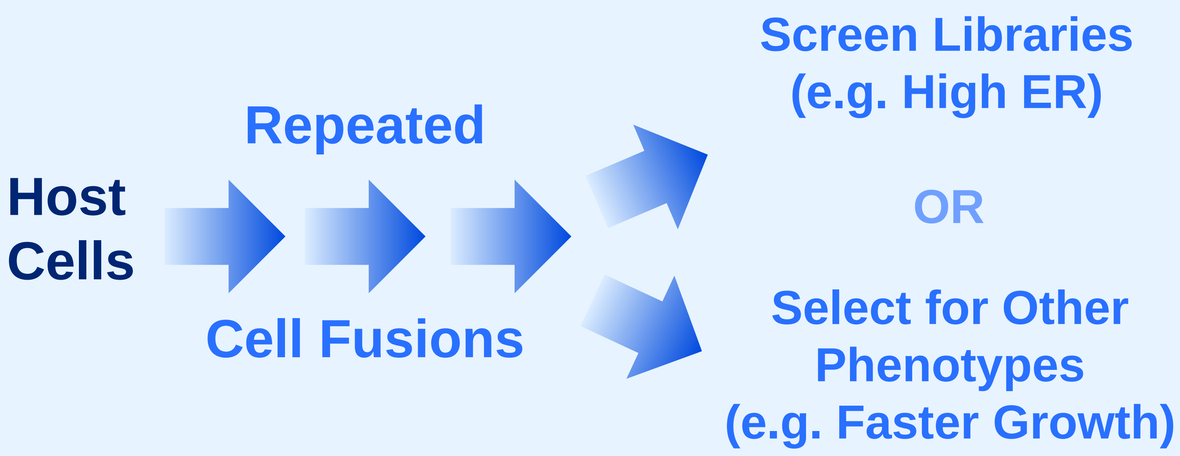
CHO+Plus

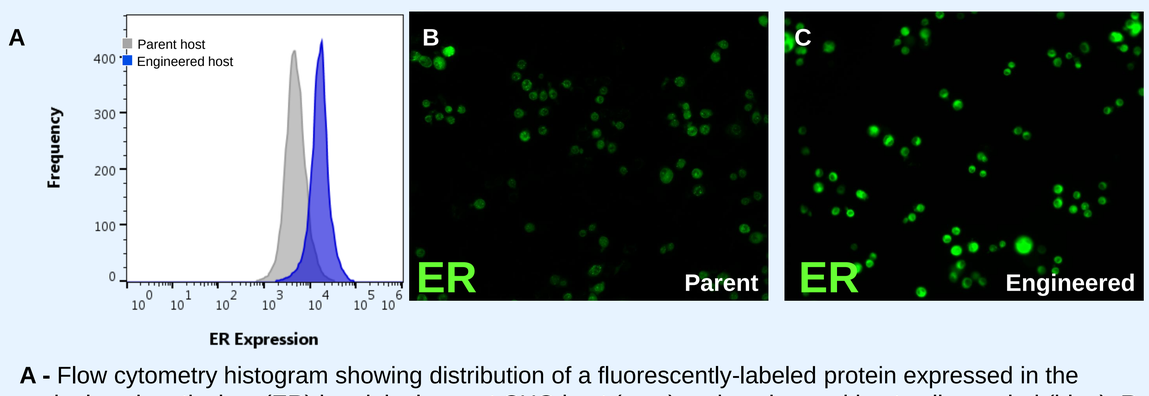
TECHNOLOGY PLATFORM

EXCEEDING BIOLOGICAL LIMITS

Experimental Approach: Repeated homotypic fusions of CHO or HEK-293 cells result in genome shuffling and amplification of whole chromosomes. Cells can be screened or selected for desirable phenotypes that lead to enhanced manufacturing capabilities. Fused CHO cells have been screened for (~10-fold) higher endoplasmic reticulum (ER) for enhanced therapeutic mAb production, or selected for faster growth. Fused HEK-293 cells have been screened for enhanced AAV productivity including higher titer, higher capsid percent-full, and/or higher infectivity.



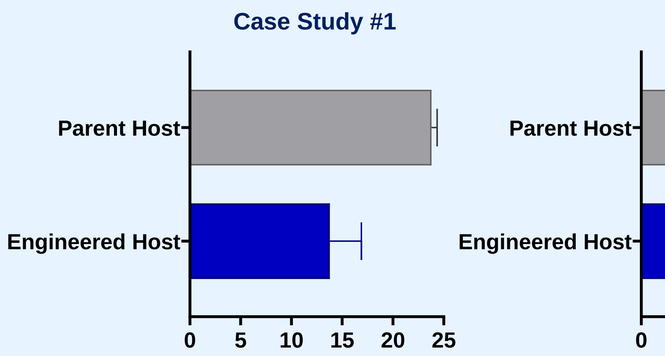
High ER



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.

50% FASTER GROWTH

...While maintaining productivity of the un-engineered cells:

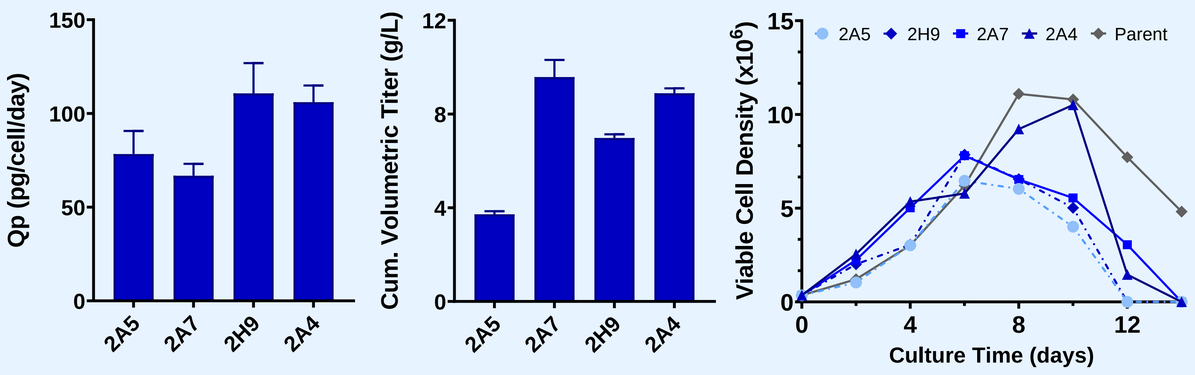


Doubling time of parent and engineered host grown in 125-ml shake flasks. Doubling time was averaged after 5 successive generations (n=5). As a proof-of-concept, two different CHO hosts were used as a starting point.

OUR PLATFORM CAPABILITIES

10x HIGHER PROTEIN PRODUCTIVITY

Up to 117 pg/cell-day and 9.5 g/l volumetric productivity

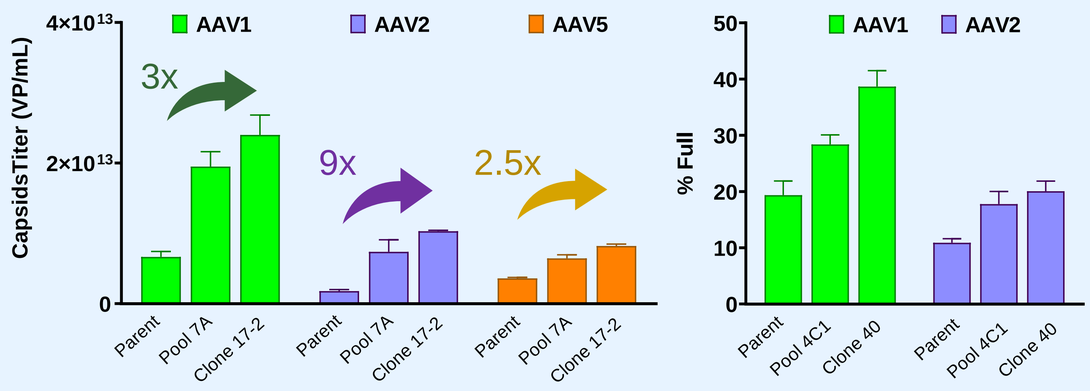


Specific productivity (Qp) and cumulative volumetric productivity of fed-batch runs of 4 representative lead stable clones transfected using **Sleeping Beauty Transposase** technology.

Viable cell density from the same fed-batch run from **left** of 4 representative lead clones.

9x HIGHER VIRUS PRODUCTIVITY OR FULL CAPSIDS

Cells can be selected for higher productivity or higher full-to-empty ratio



Left - AAV1, AAV2, AAV5 titer in HEK 293 parent, engineered pool (7A) and clone (#17-2). 3-fold, 9-fold, and 2.5-fold increase compared to parent host, respectively. **Right** – Engineered pool (4C1) clone (#40) selected for advantageous phenotypes showed a 2-fold increase in full-to-empty ratio.

CONCLUSIONS

See our PROTEIN poster (#50) and our AAV poster (#181) for more details

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

- \+ Up to **10-fold increase in protein productivity** 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
- \+ Additional phenotypes, such as **faster growth** and **higher virus productivity**, can be engineered into host cells
- \+ CHO cells can be engineered for faster growth; growth rate increased by 50% while maintaining initial specific productivity for a therapeutic mAb
- \+ HEK-293 cells can be engineered for up to 9-fold higher AAV productivity; 2-fold higher full-to-empty