Novel Cell Engineering Platform for Improving Production of AAV for Gene Therapy Applications

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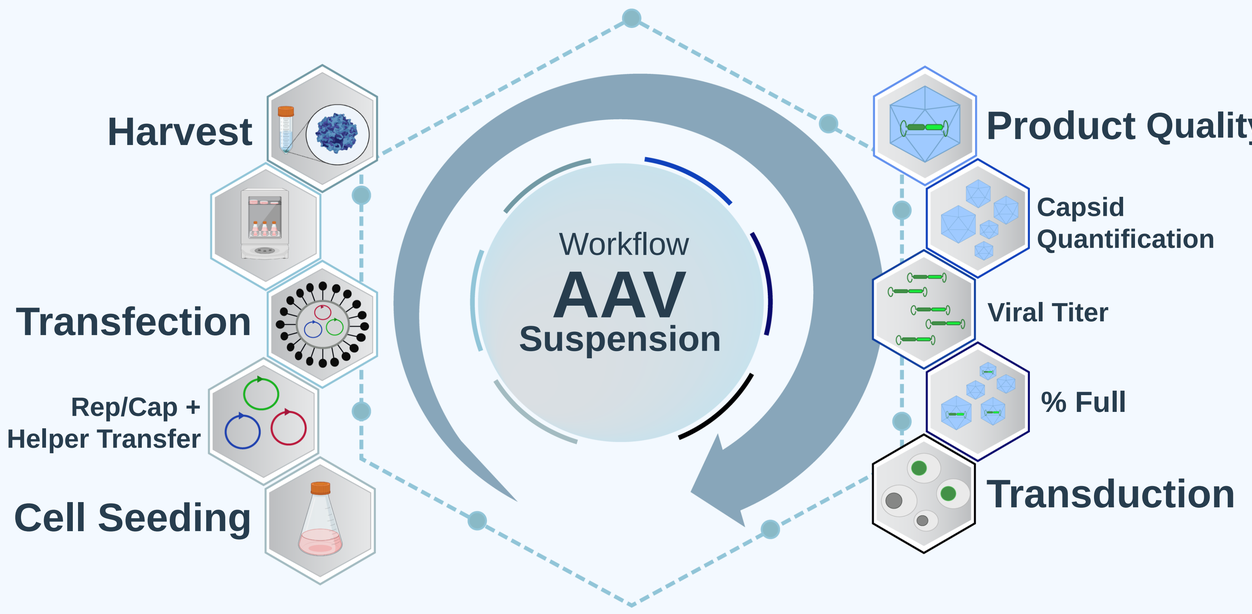
TECHNOLOGY PLATFORM

EXCEEDING BIOLOGICAL LIMITS

Experimental Approach: Repeated homotypic fusions of HEK-293 cells result in genome shuffling and amplification of whole chromosomes. Cells were screened for desirable phenotypes that lead to enhanced manufacturing capabilities. Fused cell hybrids were selected for enhanced AAV productivity including higher titer, higher capsid percent-full, and/or higher infectivity.

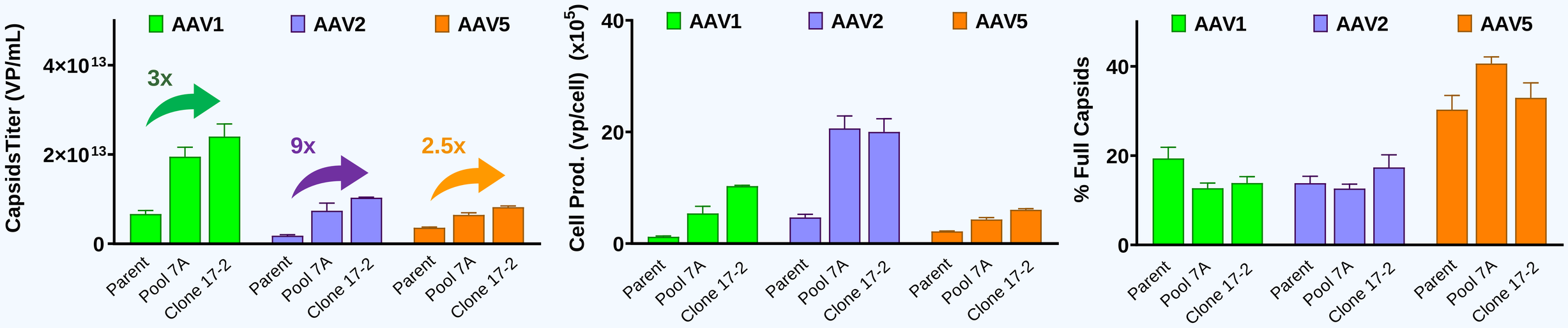
9x VIRUS



Workflow for production of AAV using suspension HEK 293 host. Left – cell seeding, transfection and harvesting of AAV particles from lysed cells. Right – assessment of product quality: capsid quantification, viral titer (or capsid concentration), % full capsids, and functional titer by transduction.

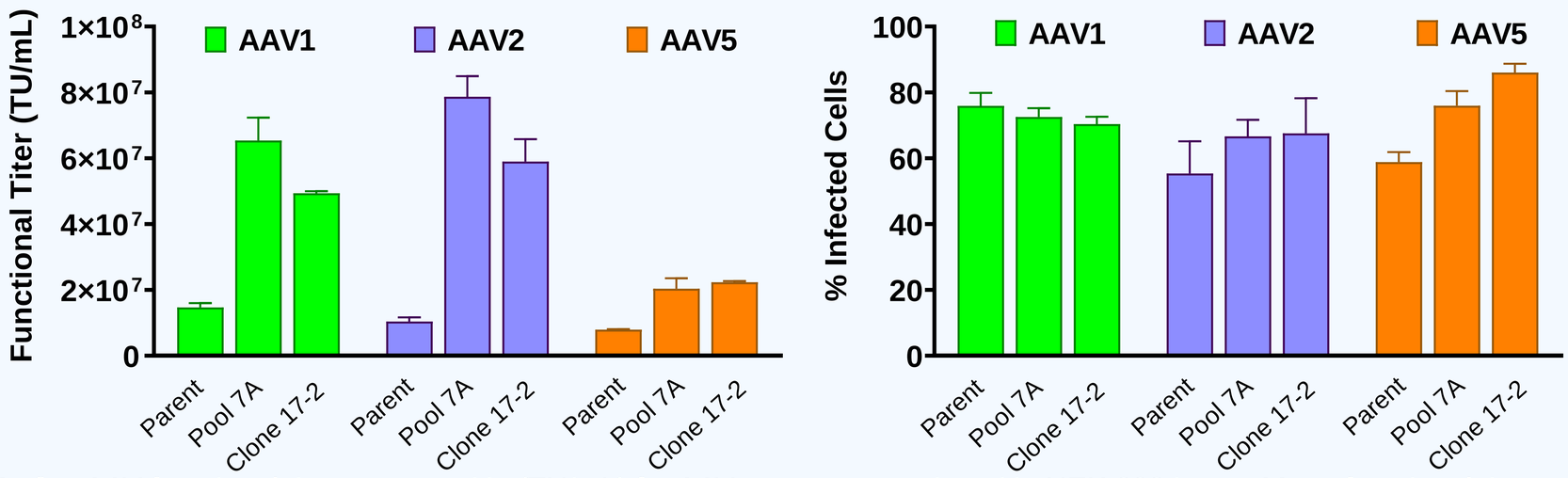
IMPROVEMENT OF AAV PRODUCTION FOR MULTIPLE SEROTYPES

HIGHER PRODUCTIVITY AND COMPARABLE % FULL CAPSIDS



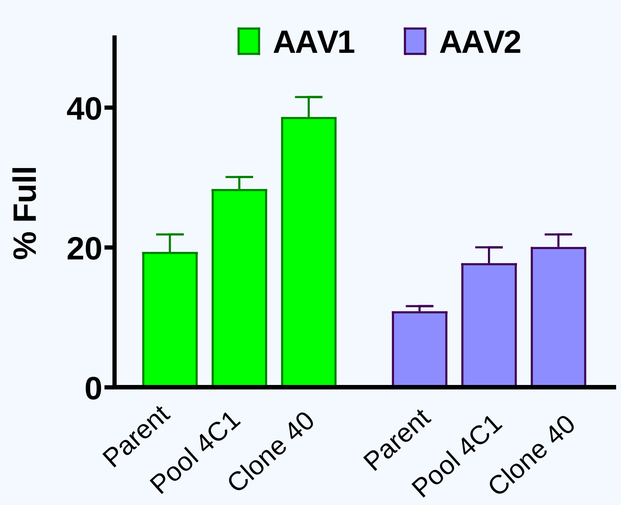
Cumulative capsids productivity (**left**), cell specific productivity (VP/cell; **middle**), and % full capsids (**right**) of AAV1, AAV2, AAV5 of HEK 293 parent, engineered pool (7A) and clone (#17-2). Engineered clone (17-2) showed 3-fold, 9-fold, and 2.5-fold increase compared to parent host, respectively.

HIGHER FUNCTIONAL TITER



Left – AAV functional titer measured in (TU/mL) for AAV serotypes produced in HEK 293 hosts. Mean functional titer were calculated at three different virus concentrations (dilutions ranging from 1:25 to 1:300). **Right** – Cell infectivity measured by % cells exhibiting GFP fluorescence (AAV transfer vector transgene) at 3 different multiplicity of infection (MOI) ranging from 500 to 50,000 (serotype dependent)

2x % FULL IMPROVEMENT



Engineered pool (4C1) clone (#40) selected for advantageous phenotypes showed a 2-fold increase in full-to-empty ratio.

CONCLUSIONS

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

- \+ HEK-293 cells can be engineered for up to 9-fold higher AAV productivity for multiple serotypes
- \+ AAV critical quality attributes (CQAs) such as full-to-empty ratio and functional titer is not compromised using our platform technology.
- \+ Capsid percent-full can be increased, with further improvement possible

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