1809 Establishment of The Inducible AAV Production Cell Line 293RSS2.0

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The production of adeno-associated virus (AAV) in mammalian cells predominantly relies on the transient triple-plasmids transfection into HEK293-based cells. Four of seven EMA/FDA-approved AAV gene therapy products employ this production approach, capitalizing on its inherent assurance of product quality. Nevertheless, owing to the expenses associated with the transfection reagent and the production of three plasmids, the overall cost of transfection-based manufacturing proves to be high. Conversely, the constrained stability exhibited by the transfection complex during operational durations, coupled with the challenges associated with achieving prompt yet gentle mixing in a bioreactor, poses impediments to the scaling-up of production volumes beyond 500 liters for transfection-based AAV production. The cumulative drawbacks underscore the pressing necessity for the development of a production cell line that can seamlessly integrate all essential elements for AAV production. In our previous work, we have constructed a prototype production cell line, 293RSS1.0, characterized by inducible expression of AAV Rep and Cap proteins, which demonstrated promising potential for inducible AAV vector packaging. A new generation of stable production cell line, 293RSS2.0, was successfully developed with the stable integration of inducible Rep, Cap, and Helper expression cassettes, which only need the GOI plasmid transfection for AAV production.

In 293RSS2.0, further refining the expression ratio between Rep78 and Rep52 was achieved by engineering the inducible Rep expression cassette, which exhibited over a >10-fold enhancement in AAV production yield compared to 293RSS1.0. Lingyi-patented inducible Cap expression cassette and inducible Helper expression cassette were both incorporated in 293RSS2.0 cell line. This 293RSS2.0 production cell line consolidates all essential components necessary for AAV vector packaging, except for the gene of interest (GOI). Efficient AAV vector packaging was achieved via transfection with a single GOI plasmid in conjunction with a small-molecule inducer. In the absence of the inducer, the AAV packaging rate remains exceedingly low, consistent with Western blot results indicating minimal leakage of the essential components required for AAV packaging. The bioactivity of the vector generated through this innovative system was evaluated, demonstrating comparable bioactivity to that of the vector produced via the conventional triple-transfection method.

In conclusion, the 293RSS2.0 cell line facilitates the inducible expression of various components necessary for AAV vector packaging. The comparable yield and quality of AAV production suggest that this stable cell line-based system has the potential to serve as a transformative platform for future AAV vector manufacturing.

1810 The First Coacervate-Based Delivery System for CAR-T Cell Engineering and Manufacturing

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Chimeric antigen receptor (CAR) T cells have revolutionized the treatment of hematological malignancies, but efficient and scalable engineering of primary cells remains a significant challenge in CAR-T cell manufacturing. Coacervates are condensed droplets containing proteins or nucleic acids formed via liquid-liquid phase separation. Here, we report the identification of a mammalian endogenous protein capable of forming coacervates with nucleic acids. These coacervates exhibit an RNA encapsulation capacity 1000-fold greater than lipid nanoparticles (LNPs), efficient cellular uptake, robust cytoplasmic release of nucleic acids, and broad compatibility with diverse primary immune cell types, including T cells, NK cells, and hematopoietic stem cells (HSCs). Building on these properties, we developed the first coacervate-based system for gene delivery (EASY) engineered using human and mouse proteins (PCT/CN2024/124967). EASY supports a wide range of genetic cargoes for gene overexpression, knockout, and targeted integration.

Using EASY, we achieved >90% transfection efficiency of mRNA encoding GFP in primary human T lymphocytes while maintaining 95% cell viability. GFP expression was sustained in 64% of cells for up to 12 days post-transfection, underscoring the platform's capacity for prolonged expression. Using EASY, we successfully generated mRNA-based anti-CD19 CAR-T cells that eliminated tumor growth in NSG mice xenografted with CD19+ Raji cells after a single dose (5×10^6 CAR-T cells). Furthermore, EASY facilitates efficient gene knockout by co-delivering Cas9 mRNA and sgRNAs. For instance, >80% knockout efficiency was achieved in the TRAC gene in primary T cells, enabling precise CAR-T engineering.

To enable gene-specific integration for CAR-T manufacturing, EASY was employed to co-deliver Cas9 mRNA, sgRNA, and single-stranded DNA (ssDNA) templates for homology-directed repair (HDR). This approach achieved >30% knock-in efficiency of an anti-CD19 CAR cassette at the PD locus, generating CAR-T cells with sustained CAR expression and disrupted PD1 function. These integrated CAR-T cells demonstrated potent anti-tumor activity in NSG mice xenografted with CD19+ Raji cells, highlighting their therapeutic potential.

EASY's simplicity, adaptability, and scalability make it ideally suited for clinical applications. The system has been successfully scaled up to deliver mRNA into over 10^9 primary T cells with >90% efficiency, demonstrating its feasibility for large-scale CAR-T manufacturing. Its straightforward preparation and high-throughput transfection capability further distinguish it as a transformative tool for advancing CAR-T engineering. In conclusion, EASY offers an innovative, non-viral, LNP-free, and electroporation-free platform for efficient and scalable CAR-T cell engineering. Its high efficiency, minimal toxicity, and compatibility with diverse genetic modifications position EASY as a next-generation solution for CAR-T manufacturing, unlocking new possibilities for cell therapy development.

1811 Development of a Quantitative Screen to Assess AAV On/Off-Target Binding and Transduction

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Adeno-Associated Viruses (AAV) are a leading vector in gene therapy, with three FDA therapies approved over the last 5 years (1). Continued interest in AAV's shows the promise of this field, however issues including immunogenicity and large-scale manufacturing costs remain limiting factors for advancing AAV-based gene therapies (2,3). Here we describe a novel flow cytometry based dual screening capability for AAV's as a downstream assessment of targets identified using the Charles River Retrogenix® Cell Microarray platform.

Data from the Retrogenix® Cell Microarray technology, consisting of >6,500 full length human cell surface, secreted, tethered secreted, and heterodimeric vectors, and more recently non-human (Cynomolgus) protein targets, each over-expressed in HEK293 cells, is already used extensively in regulatory BLA and IND submissions. To provide further understanding for AAV profiling (4), flow cytometry testing has been developed. This sensitive, multiparametric and quantitative method provides a powerful tool for gene therapy by enabling detection of AAV receptor binding, transduction efficiency, and expression of encapsulated reporter genes in live cells.

Wild Type AAV9 controls were used to develop a flow cytometry binding assay to the known KIAA0319L AAV receptor, along with off targets previously identified using the Retrogenix® platform. In addition, AAV internalization (transduction) methodology was developed by measuring reporter gene expression. To avoid underestimating functional effects of low-level transgene expression, this was recorded as % positive cells, a method typically used in gene therapy (5,6).

Complimenting Retrogenix® Cell Microarray data using an orthologous platform provides a comprehensive insight into primary receptors and potential adverse event liabilities of AAV's. With the recent addition of non-human plasma membrane proteins with 90% human homology in the extracellular domain, our approach of assessing AAV