Development of Cost-Effective and Scalable Recombinant Baculovirus Production Process for the Manufacturing of AAV

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Manufacturing Overview

cGMP Facility Supports TN-201, TN-401 and Future Tenaya or Partner AAV-Based Gene Therapy Programs

48K square foot facility with ~50K square feet for expansion

~45 FTE in-house team conducting Process Development, Analytical Development, Quality Control

Non-GMP thru cGMP Productivity

- IP and know-how to enable scale from starting materials to large (> 5000L) bioreactors
- Maintenance of high potency from small to large volumes
- Consistently high purity vector production

Analytical and Assay Development

- Robust internal development of assay to support DS, DP release
- FDA supports Tenaya CMC strategies (Type B meeting 2021)

Ongoing Optimization Efforts

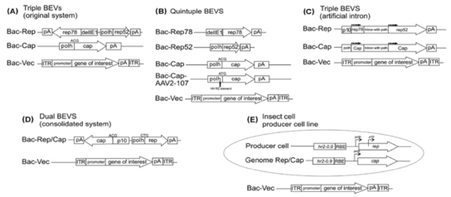
- Development and validation of proprietary technologies to increase yield in Sf9 and HEK293 systems

Background

Insect cell, Baculovirus Expression Vector (BEV) platforms have been utilized for decades to produce recombinant proteins by academics and industrial ventures for biologics and therapeutics because of their high yield and recombinant protein bioactivity in mammals. Baculovirus inherent safety having from having a narrow host range, being commonly ingested by humans and the limited scope of adventitious agents in producer insect cell lines. As a result of insect cell/BEV safety profile, versatility, high yield, and relatively low COGS, BEV's are utilized in the manufacture for a variety of commercially available therapies including, immunotherapy PROVOGENE® (FDA 2010) and several vaccines, for example, Cervarix® (EMA 2007) and Flublock® (FDA 2016). Numerous approved AAV gene therapies have also been produced in BEV platforms with the first regulatory approval for Glybera® (EMA 2012) and recent approval of ROCTAVIAN™ (EMA 2022) and HEMGENIX® (FDA 2022; EMA 2023) continue to ignite interest in BEV expression and manufacturing technology development for AAV Gene Therapy.

Highly efficient recombinant Baculovirus Expression Vectors are commercially available including developments for BEV-AAV production since the 2002 publication (Urabe et al.) describing a novel system to produce high-titer AAV culture, but scale-up due to genome instability of the BEV has prevented large volume and high quality baculovirus Primary Virus Seed Stock (PVSS) for AAV production. Prevalence for instability is innate, effecting the ITR-Cassette (Transgene) as well, but investigators have largely focused on overcoming the major limiting factors to BEV-AAV productivity, notably Cap and Rep overlapping open reading frames of wtAAV expressing the 3-Capsid structural proteins and 4-Rep proteins.

Figure 1. Insect Cell-based Recombinant Adeno-Associated Virus Production: Molecular Process Optimization
Lubelski et al., Bioprocessing Journal, 2014



These expression-based approaches include mutation of strong AUG start codons and have been successful for the expression of Cap proteins, but Rep78/68 and REP52/40 remained as an issue of splitting the overlapping sequences and producing duplicate homologous sequences increasing BEV instability. This was improved in utilizing a 2nd generation BEV-AAV platform with alternate codon usage only expressing Rep78 and Rep52 (Kotin et al. 2009). An artificial intron recognized by insect cells introduced to the BEV platform (Chen 2008) eliminated the requirement for homologous sequences though instability is less it is not eliminated.

Novel bioprocess iterations have also been developed alongside the molecular approaches to mitigate genome instability. In one instance, the invention of baculovirus infect insect cells (BIIC) by infecting an insect cell culture with a plaque and banking the infected cells to be used in larger dual BIIC infection to produce AAV. The advantage of BIIC is still limited in linear scale up of AA productivity to 200L (Kotin 2011).

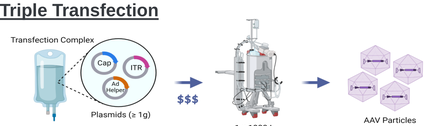
Abstract

After the early success of Zolgensma® and Luxturna®, AAV gene therapy is entering a mature stage and expanding its therapeutic potential to serve broader patient populations suffering from more prevalent genetic diseases. At Tenaya, our Gene Therapy platform uses AAVs to deliver genes to specific cells in the heart to correct or compensate for functional defects. Genetic root causes of hypertrophic cardiomyopathy (HCM) and arrhythmogenic cardiomyopathy (ARC) have the potential to be readily corrected *in vivo* using the delivery of AAVs. However, due to the prevalence of HCM and ARC, the conventional triple transient HEK293 process, which is limited by yield, scalability, and high cost of goods (COGS) is not economically feasible as a manufacturing platform to produce sufficient quantity of AAV drug products (DPs). Tenaya has developed a proprietary manufacturing platform process using Sf9/recombinant baculovirus (rBV), which overcomes the limitations of yield, scalability, and COGS. As part of this Sf9/rBV manufacturing process, Tenaya has also developed a scale-independent recombinant baculovirus production process that has consistently produced high quality recombinant baculovirus seedstock which maintained productivity, quality, purity, safety, and potency of AAV DPs at 1,000L scale.

AVV Production Platforms

HEK293T Triple Transfection

- ❑ Effective expression system for ultra-rare indications
- ❑ Difficult to maintain specific yield in manufacturing scale >500L without mammalian helper virus
- ❑ High manufacturing cost



Tenaya Sf9/BEV

- ❑ Readily scalable beyond 1kL for indications beyond orphan drug designation
- ❑ Consistent AAV DS productivity and product quality
- ❑ 6-10x Higher productivity & less than 30% cost of HEK293 triple transfection process

PVSS Scale-Up & Discussion

Scaling to STR | **Product Recovery** | **Long-term Storage** | **PVSS & AAV Productivity**

- Gassing Strategy
- Foam Control
- Cell Stress
- Surfactants
- Culture Viable Cell Density
- Agitation
- Filtration Train Formats and Media

- GMP Manufacturing Volume Aliquoting, Freezing and Thawing Operations
- Infectious Titer recovery
- Genome Stability
- AAV Productivity

Effect of Anti-Foam on PVSS Productivity

Cell culture in a stir tank reactor (STR) will indefinitely increase bubble foam formation due to a variety of reasons, (e.g. gassing strategy, protein content, etc.). Foam may prevent efficient gas exchange, cause cell damage and filter clogging resulting in lower yield or termination. Assessing the effect of anti-foam proactively will assist scaling to adequate production volume and address potential adverse impacts on product quality.

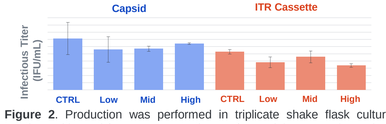


Figure 2. Production was performed in triplicate shake flask cultures, treating with anti-foam concentrations of a minimum and maximum leveraged from prior studies for Sf9 AAV production development. This study confirmed there is a tolerable level of impact to PVSS infectious titer to account for throughout development.

Harvest clarification by depth filter is guided by production culture and product composition. A variety of filter media are available from organic solutions (e.g. diatomaceous earth and regenerated cellulose), synthetics, resins, and pore size. Appropriately selected media and membrane size maintain consistent operation throughout scale-up with minimal impact to product quality and yield.

Figure 3. Recoverable infectious titer was assessed with PVSS ITR-cassette against a control centrifugation method. Two formats were selected from this study for additional development. In contrast to AAV clarification PVSS products and culture were developed for intact cell removal and is a budded enveloped virus with potential increased affinity to common filter compositions and mechanical shear.

Tenaya's PVSS Product Stability

Supports Consistent High Quality AAV Drug Product for Indications with Large Patient Populations

Storage and recovery is a formidable task with sensitive and unstable products at large volumes. For AAV Drug Product consistency both in quality of the therapy and scale independent high-yield platform, PVSS must achieve a greater level of extended stability to serve the patient populations that Tenaya's product pipeline is seeking to address. The PVSS method we developed supports multiple AAV GMP manufacturing campaigns and multiple pipeline products that utilize platform PVSS (e.g. AAV RepCap) of ≥12 x 1000L reactors from a single Lot of PVSS. Critical to consistency for Drug Product is not only maintaining a predictable recoverable infectious titer, but the genome stability of our therapeutic and expression platform components, which has long plagued insect cell baculovirus platforms for AAV manufacturing.

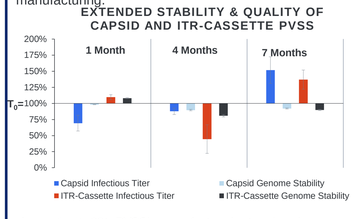


Image: Blast Freezer completing freeze cycle with PVSS harvests stored in ultra-cold storage containers.

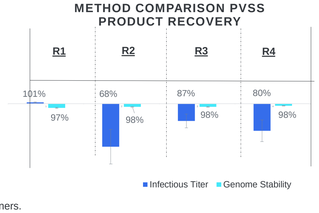


Figure 4. TN-201 PVSS's manufactured, stored and recovered per GMP manufacturing operations at time points relevant to supporting 1000L TN-201 drug product campaign. Characterization of PVSS for >6 months maintains ≥81% Genome Stability and Infectious titers within acceptable variation (cell-based assay) of T_0 recovery. AAV production in 200L pilot scale utilizes the T_0 Infectious titer for Multiplicity of infection (MOI) calculations and produced high-yield AAV Products throughout the 7-month assessment period.

Figure 5. An additional Lot of TN-201 ITR-Cassette PVSS was stored and recovered per modified methods relevant to GMP manufacturing. All recovery characterization were analyzed against sample format for GMP-Quality Control submission. Method R1, observed to have the most comparable product quality, but was only stored in a short-term environment for manufacturing use. Methods R2 through R4 observed comparable stability to each other and preserved critical quality attributes.

Figure 6.

Tenaya Sf9 AAV Therapeutic Safety	
Bioburden	Not detected
Sanger sequencing	100% conforming to reference (4x Bi-directional coverage)
Mycoplasma spiroplasma	Not detected
Invitro adventitious agents	Not detected

- ❑ Product Safety testing consistent over 4x 200L AAV Drug Product
- ❑ Tenaya AAV purification process achieves >20 cumulative Log-Reduction Values (LRV), providing a robust level of safety margin

Summary

- ❑ **Tenaya has successfully developed and manufactured PVSS process addressing both Capsids and ITR-Cassettes**, which achieve and retain high Infectious Titer and Genome Stability
- ❑ Insect cell expression platforms productivity is between 6x to 10x greater than HEK293 triple transfection at significantly lower costs for development and manufacturing while consistently producing high quality AAV Drug Product
- ❑ Our proprietary process makes it possible to develop therapies for non-orphan drug indications supporting ≥12x 1000L AAV productions spanning over half a year from a single PVSS production lot
- ❑ Tenaya's scale-independent PVSS process is flexible to maintain accelerated timelines for clinical development and reduces cost, in contrast to other manufacturing approaches requiring multiple lot releases of PVSS resulting in variable quality or limited scalability
- ❑ **The combination of Tenaya's AAV purification process with highly efficient viral clearance enables our GMP manufacturing teams to supply drug product with consistent yields, quality and purity which may result in greater safety for patients**