Strategy for AAV packaging of multiple snRNAs with genome integrity

Greg Nachtrab, Rea Lardelli Markmiller, Derek Reid, Rachel Adams, Katy Stein, Claire Geddes, Daniela Roth, Maciej Bozejewicz, Gustavo Villegas, Lynn Nguyen, Oscar Diago, Daniel Knowland, Bryan Almaguer, Yesol Go, Amanda Tran, Aaron Berlin, Ranjan Batra, John Leonard

Introduction

snRNA AAV constructs can be packaged with multiple snRNA cassettes to enhance expression and potency by targeting multiple splicing regulatory sequences. We designed a series of scAAV constructs containing multiple snRNAs and used nanopore-based full molecule sequencing to characterize packaged genomes. We observed truncations and deletions, which significantly reduced the proportion of full-length genomes. Repeated packaging of the same construct resulted in diverse patterns of genomic rearrangements, emphasizing the potential for significant batch-to-batch variability. We hypothesized that the scAAV snRNA packaging aberrations were not caused by the snRNA structure but from the sequence homology between the expression cassettes. We generated >50 snRNA constructs with varied snRNA promoter and terminator sequences. Reducing sequence homology nearly eliminated unintended truncations and deletions with greater than 90% of the genomes being the intended scAAV genome. Varying promoters and terminators also increased AAV yields to 2-6 times that of a repetitive design. The productivity and genomic integrity were maintained from adherent to multiple liter suspension production, indicating that large-scale manufacturing can be accomplished without concerns about batch-to-batch variability. Thus, we have defined and corrected one of the main obstacles that has been preventing the expansion of AAV snRNA gene therapy.

Conclusions

- **Sequence homology and not structure is the major barrier to packaging multiple snRNAs.**
- **Locanabio has developed a strategy for packaging up to 4 snRNAs in an scAAV that maintains genomic integrity.**
- **This strategy eliminates batch-to-batch variability concerns in both 2x and 4x snRNA scAAV preparations.**
- **This strategy also works in a scalable suspension process.**
- **Thus, this strategy enables the production of high quality AAV snRNA vectors for clinical use.**

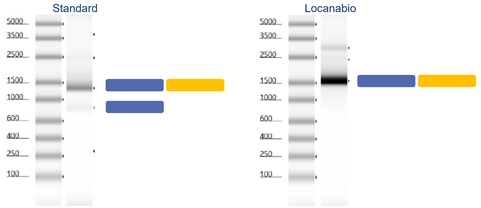
Results

Near uniform packaging of 2 snRNAs in scAAV with mixed promoter and terminator elements

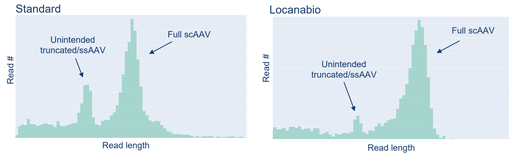
Standard 2x design

Locanabio 2x design

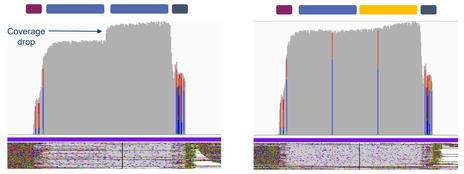
Tapestation of extracted AAV genomes shows smaller genome band in standard designs that is absent from Locanabio design



Nanopore sequencing read length analysis confirms increased proportion of truncated or ssAAV genomes in standard design



Loss of a snRNA cassette is the reason for the shorter sequencing reads in the standard 2x snRNA design

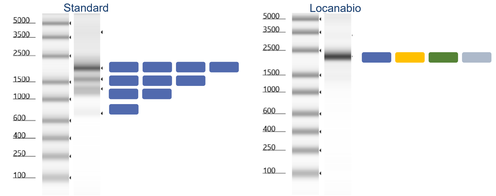


Effective scAAV packaging of 4 snRNAs with mixed promoter and terminator elements

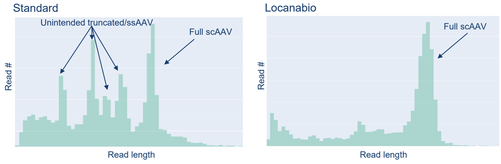
Standard 4x design

Locanabio 4x design

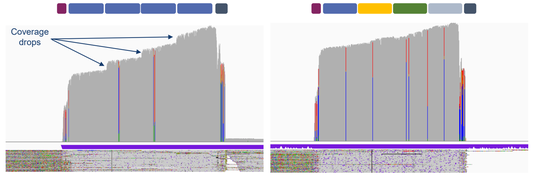
Tapestation of extracted AAV genomes reveals multiple species from standard 4x compared to single band from the Locanabio design



Nanopore sequencing read length analysis confirms Locanabio design efficiently packaged 4x scAAV with very few truncated or ssAAV genomes



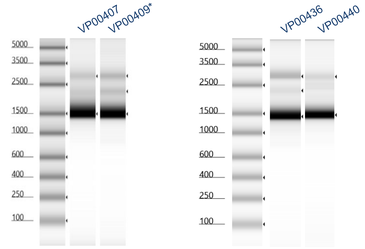
Loss of snRNA cassettes in the standard 4x design confirmed by sharp drops in the nanopore sequencing coverage



Elimination of batch-to-batch variability in the genome integrity of snRNA scAAVs

2x Packaging across various AAV preparations

Locanabio 2x design



*VP00409 was produced in suspension and purified by 2-step chromatography, which is a scalable process

4x Packaging across various AAV preparations

Locanabio 4x design