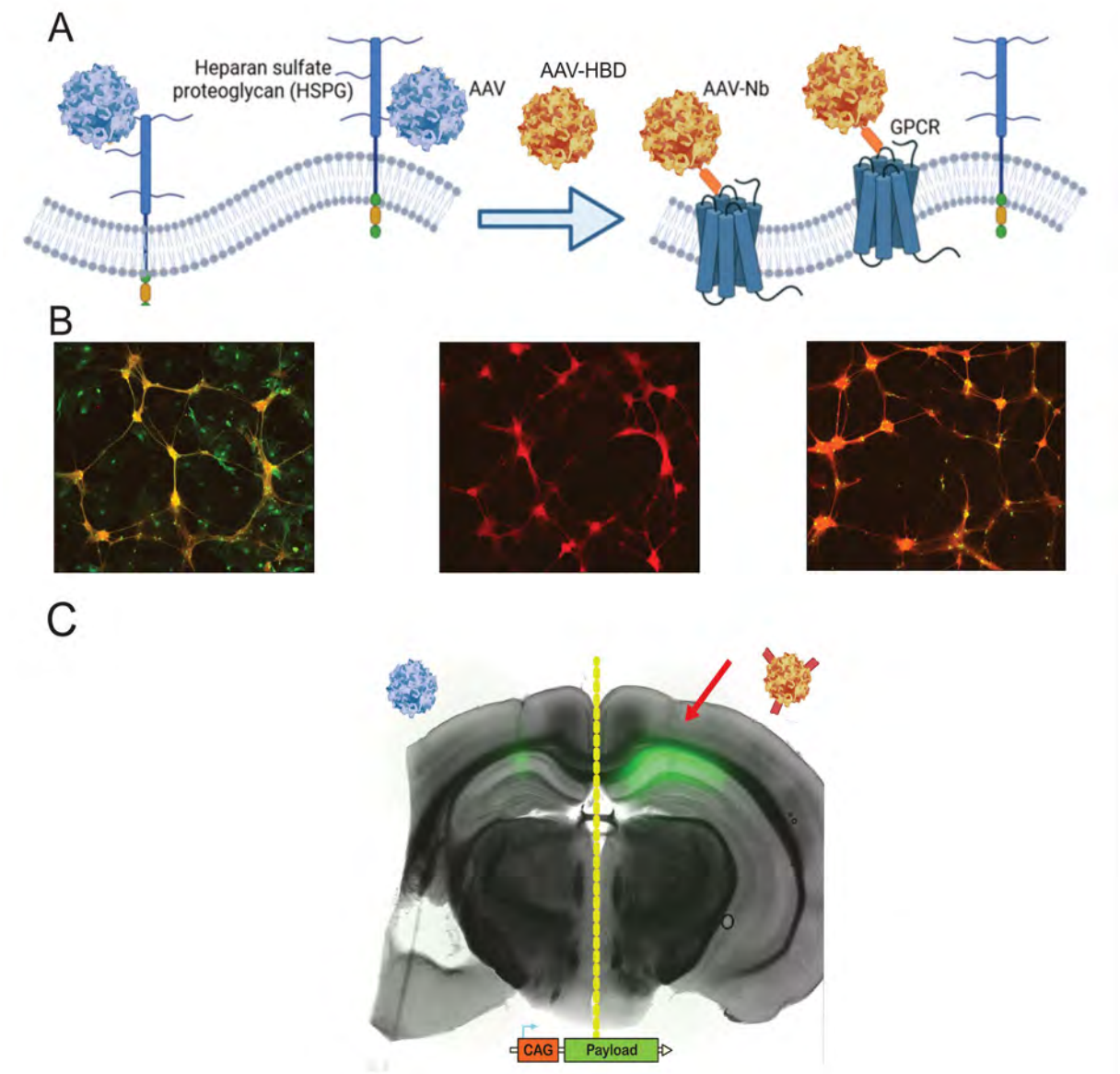
ANNUAL MEETING

BOSTON, MA • May 11-15, 2026

ASGCT 2026 ANNUAL MEETING ABSTRACTS

ASGCT does not permit the use of any robot, spider, scraper, data mining tool, or other automated or manual means to access, copy, harvest, or extract ASGCT Annual Meeting or any other event or research abstracts. Any reproduction, distribution, or external use of the abstracts is strictly forbidden without explicit, prior written approval from ASGCT.

Copyright © 2026 ASGCT. All rights reserved, except where otherwise required by law.



A. Schematic of AAV-Nb infecting cells via redirected tropism, replacing Heparan interaction with mGluR1/5 interaction; B. In vitro validation of AAV-Nb: Primary neuron culture 10 days post infection with virus delivering a GFP payload, counter stained with anti-mGluR5 Antibody (Red). Left: Wild Type AAV-DJ virus, Center: AAV-DJ with Heparan binding domain mutated, Right: AAV-Nb; C. In vivo validation of AAV-Nb: Coronal slice of mouse brain 2 weeks post injection showing virus mediated GFP expression in hippocampus, Left hemisphere: Wild type AAV-DJ, Right hemisphere: AAV-Nb

1025 Identification of Novel Tissue-tropic AAV Capsids Using an In Vivo-Calibrated Barcode Library

ANNUAL MEETING
BOSTON, MA • May 11-15, 2026

Ruixue Chen[1], Yangyang Zhong[1], Qingyu Deng[1], Wenbo Fu[1], Yan Jiao[1], Ying Bai[1], Gang Wang[1]

[1]*Byongen Therapeutics, Beijing, China*

Introduction The discovery of adeno-associated virus (AAV) capsids has been significantly accelerated by barcoded capsid library screening. In this approach, unique barcodes (BCs) are embedded in the 3' UTR and decoded by next-generation sequencing (NGS), enabling parallel assessment of tissue distribution and transgene expression for multiple capsid variants in a single animal. Conventional workflows require individual packaging, purification, and titration of each variant before pooling at a theoretically equimolar ratio. However, the resulting libraries frequently show substantial compositional bias, where viral titers vary several-fold among variants. This problem intensifies with greater library complexity. Consequently, comparisons of variant performance at equivalent doses are difficult, preventing accurate ranking. To address these limitations, we developed CALIBRATOR (Calibrated AAV Libraries with Internal Barcode-Referenced Analytes for True Output Ranking). CALIBRATOR establishes an in vivo calibration system by co-delivering a gradient of internal reference standards. This system compensates for preparation bias, enables normalized comparison of variants, and provides an unbiased functional ranking.

Methods The platform workflow is shown in Figure 1. Barcode sequences were inserted into the 3' UTR of the transgene cassette. The library was designed with two key components, including a six-dose AAV9 internal reference for calibration and dual barcodes (4:1 ratio) assigned to each capsid variant for quantitative tracking. Each virus was individually packaged, pooled prior to purification, and processed using a consistent purification protocol to generate a homogeneous calibrated AAV barcode library. To establish a dose benchmark, the pre-injection library was analyzed by NGS to determine its actual composition. Following tissue collection, DNA and RNA were extracted, followed by barcode amplification and NGS. The abundance of AAV9 barcodes was used to generate tissue-specific standard curves. Each variant's performance was quantified relative to these curves, yielding dose-calibrated fold changes in expression compared with AAV9.

Results We selected a panel of 41 dual-barcoded, tissue-tropic capsid variants, including known controls and in-house designs, together with an AAV9 reference gradient for library production. The resulting library exhibited a precise AAV9 dose gradient ($R^2 = 0.97$) and accurate variant barcode ratios (mean 4.06 vs. 4.0 theoretical), with >98% of variant measurements falling within the reference dose range. Following intravenous administration, AAV9 dose–response standard curves were established across multiple mouse tissues. These curves showed highly linear correlations between AAV9 barcode dose and mRNA expression ($R^2 > 0.9$ in all tissues), providing a robust internal reference for calibration. Based on these curves, calibrated relative expression fold changes were calculated for each capsid variant versus AAV9. In contrast to conventional single-reference normalization, CALIBRATOR achieved near-perfect concordance between the paired barcodes of each variant ($R^2 = 0.99$). Furthermore, the measurements were highly consistent across animals, demonstrating strong reproducibility ($R = 0.87$). Based on the screening results, lead variants targeting multiple tissues were individually validated, achieving ten- to hundred-fold higher mRNA expression than AAV9 in their respective target tissues,

supporting the robustness and reliability of the platform. Notably, the cardiac-tropic capsid BY103 showed superior efficiency and specificity in the heart across multiple doses and species, supporting its potential for cardiac gene therapy.

Conclusion We established CALIBRATOR, an in vivo–calibrated AAV barcode library screening platform that corrects preparation bias and enables reliable capsid ranking. Using this platform, we identified AAV variants with distinct tissue tropism, including a promising candidate with cardiac specificity.

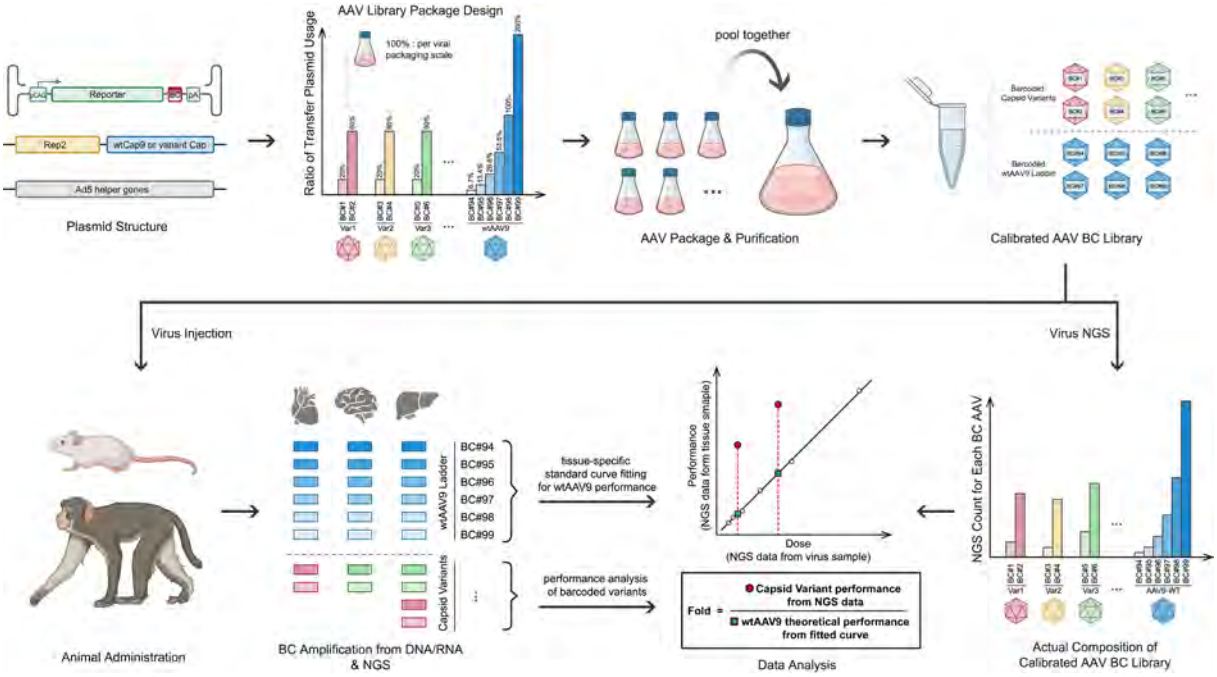


Figure 1. Workflow for the CALIBRATOR platform. This workflow outlines inserting barcodes into the 3' UTR to build the plasmid structure; designing the AAV barcode library package, with each capsid variant carrying two barcodes at a 4:1 ratio and a six-dose series of wild type AAV9 (wtAAV9 ladder) as an internal reference; preparing and verifying the calibrated BC library; conducting animal administration; and using NGS-based analysis to generate standard curves for calibrated evaluation of variant performance.

1026 Reprogramming AAV performance through a rational platform targeting non-receptor-binding capsid regions

Yiwen Li[1 2], Giulia Massaro[2], Ahad A. Rahim[2], Paul Dalby[1]

[1]University College London, Department of Biochemical Engineering, London, United Kingdom, [2]University College London, School of Pharmacy, London, United Kingdom