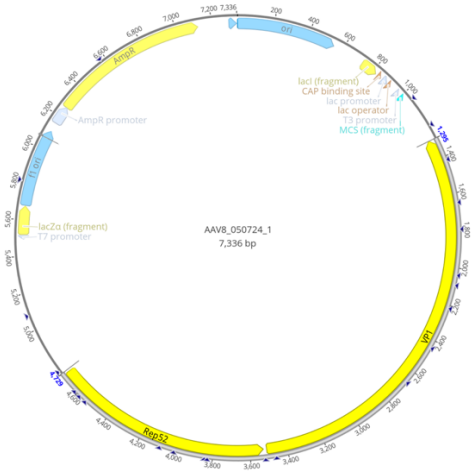
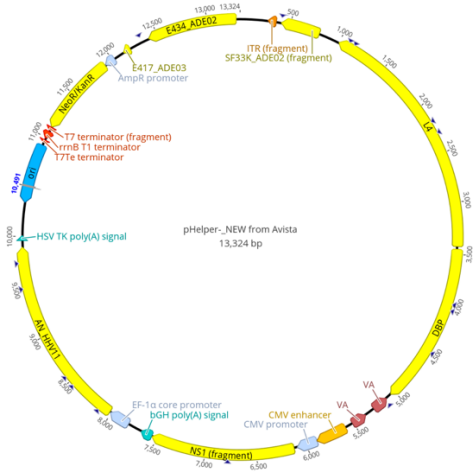
AAV Batch #: AAV8-04172024-scCAG-mgL
Serotype: AAV8
AAV construct: pAAV-scCAG-mGreenLantern

Plasmid map:

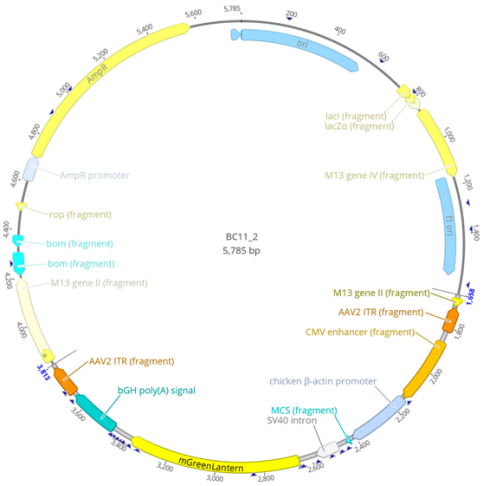
AAV8 (rep/cap):



pHelper:



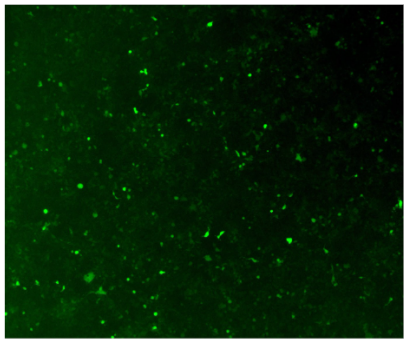
scCAG-mGreenLantern-BC:

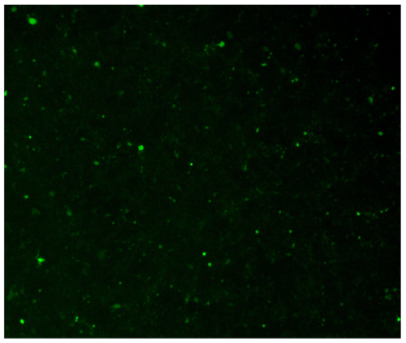


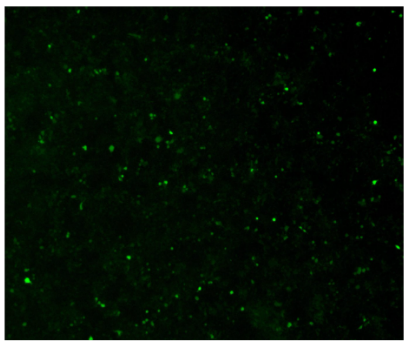
Packaging protocol:
Barcoded AAVs were packaged using a triple transfection method in adherent cells. 0.6E+6 to 0.7E+6 cells/mL were seeded into a 125 mL shaker flask containing 30 mL of Expi293 medium (Thermo Fisher – A1435101) the day before transfection. On the following day, the transfection mix of pHelper: K912: Barcode (BC)=1.67:1:1 was prepared. Then, the FectoVIR reagent was added to the mix and the solution was gently inverted (avoiding vortexing) to prevent disintegration of the Fecto/DNA complex. The mixture was incubated for 15 minutes at room temperature. After incubation, the required volume of the transfection mix was added to each flask. Finally, the cells were incubated at 37°C for 72 hours post-transfection. After three days of culturing, cell lysates were subjected to three freeze-thaw cycles. Cell debris was removed by centrifugation and the supernatants were purified through AAV2-based affinity column. The final viral elute was carried on for titrations.

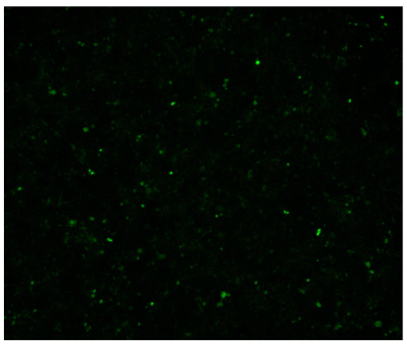
In vitro infectivity test:
1 uL of packaged virus was used to infect 1 well of a 6-well plate containing HEK293 cells at 70% confluency. The cells were imaged for GFP signal after 48 hrs.

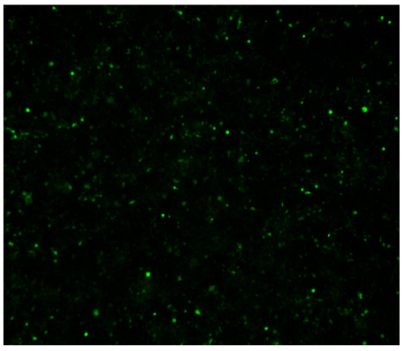

neg


AAV8-BC8


AAV8-BC11


AAV8-BC12


AAV8-BC13

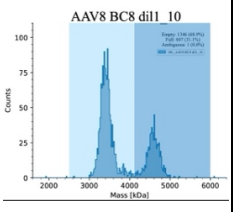
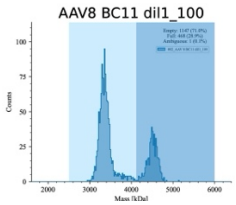
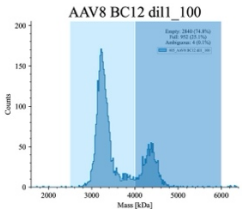
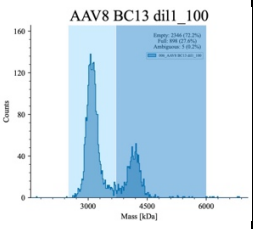
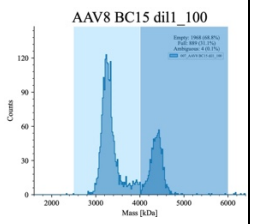

AAV8-BC15

Titering:
The titers were measured using qPCR AAV titer kit (ABM, catalog# G931), following the manufacturer's protocol.

AAV construct	Titer (Vg/mL)
AAV8-CAG-BC8 mgL	6.03e+12
AAV8-CAG-BC11 mgL	2.54e+12
AAV8-CAG-BC12 mgL	4.99e+12
AAV8-CAG-BC13 mgL	1.81e+12
AAV8-CAG-BC15 mgL	5.49e+12

Mass photometry:

Mass photometry was performed on a SamuxMP (Refyn).

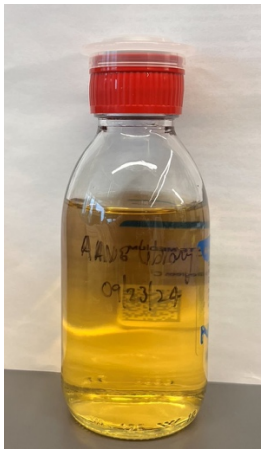
	AAV8-BC8	AAV8-BC11	AAV8-BC12	AAV8-BC13	AAV8-BC15
					
% full	68.9	71	74.8	72.2	68.8
% empty	31.1	28.9	25.1	27.6	31.1
% ambiguous	0.0	0.1	0.1	0.2	0.1

Fungal and bacterial contamination:

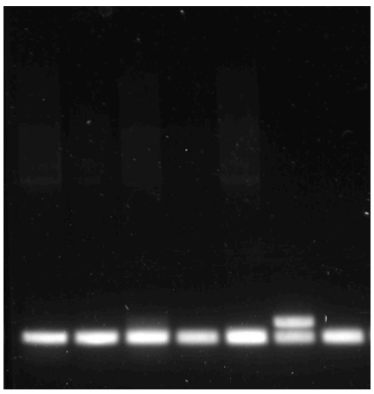
2 µL of purified AAV was tested for bacterial and fungal contamination using qPCR-based Femto Bacterial DNA Quantification Kit (Zymo, catalog # E2006) and Femto Fungal DNA Quantification Kit (Zymo, catalog # E20067), following manufacturer's instructions. A C_t > 30 was considered to be free of contamination. (UD – undetermined)

Test	AAV8 library mix	BC8	BC11	BC12	BC13	BC15
Fungus (C_t)	39.335	UD	UD	UD	UD	UD
Bacteria (C_t)	34.197	33.575	31.51	32.341	33.491	32.326

1 µL of purified AAV was also tested in 100 mL of FTM media, culturing at 37 ^{0}C for 14 days. Media was clear and free of contamination.



Mycoplasma:
Each of the AAV dilution was investigated for mycoplasma contamination, using Myco-Sniff-Valid Mycoplasma PCR Detection kit (MP Biomedicals, Catalog# 093050301), following the manufacturer's protocol, before making the library pool. No mycoplasma was detected.



Lane1: AAV8 BC8,
Lane2: AAV8 BC11,
Lane3: AAV8 BC12,
Lane4: AAV8 BC13,
Lane5: AAV8 BC15,
Lane6: +ve control,
Lane7: -ve control

Endotoxin:

Each of the AAV dilutions and the final library pool was investigated for endotoxin contamination. The samples were analyzed using Nextgen PTS instrument.

	Endotoxin (Eu/mL)
BC8	<50
BC11	<50
BC12	<5
BC13	7.95
BC15	48.9
AAV8 library	<5

Dilution curve analysis:

The final titer of the pooled dilution curve library was 4.24E+11 vg/mL. Barcodes from the pooled library were amplified by PCR and then subjected to sequencing on an Illumina iSeq100. Quantification of barcodes revealed the following library balance:

X-axis label		**dilution (Vg/mL)**	**counts_1**	**counts_2**	**counts_3**	**Avg counts**	**Std dev**
1	**BC8**	1.00E+11	830990	922945	1322145	1025360	261103
2	**BC12**	1.00E+10	59983	69158	96221	75121	18840
3	**BC11**	1.00E+09	7712	8478	11589	9260	2053
4	**BC13**	1.00E+08	1411	1557	2344	1771	502
5	**BC15**	1.00E+07	594	935	1335	955	371