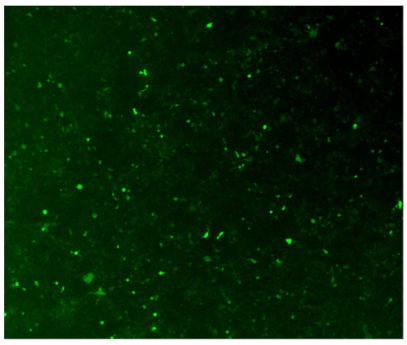
AAV Batch #: AAV8-04172024-scCAG-mgL
Serotype: AAV8
GOI: pAAV-scCAG-mgL

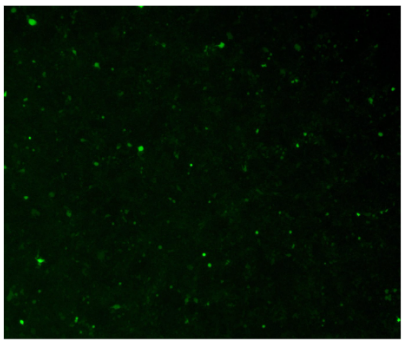
Plasmid map:

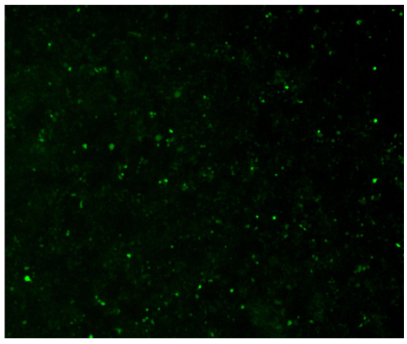
Packaging protocol:
Barcoded AAVs were packaged using a triple transfection method in adherent cells.

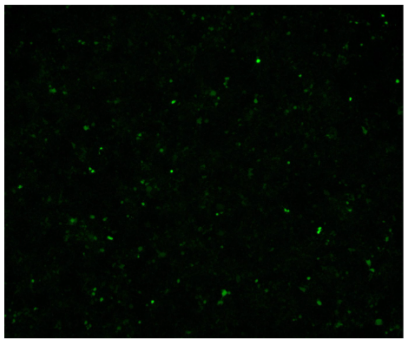
In vitro infectivity test:
1 uL of packaged virus was used to infect 1 well of a 6-well plate containing HEK293 cells at 70% confluency.

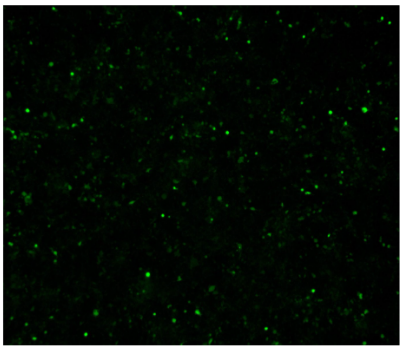

neg


AAV8-BC8


AAV8-BC11


AAV8-BC12

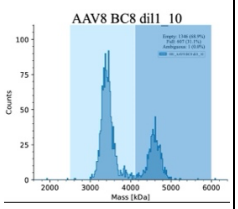
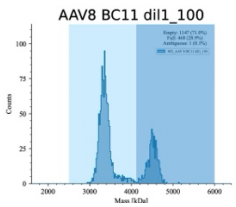
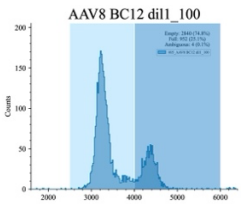
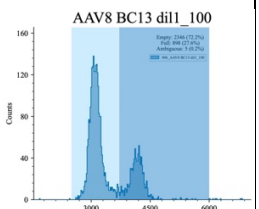
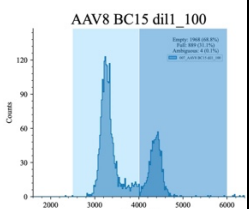

AAV8-BC13


AAV8-BC15

Tittering:
The titers were measure using qPCR AAV titer kit (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:

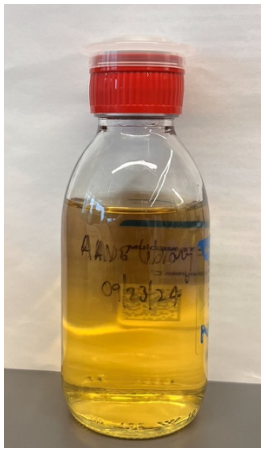
	Construct 1	Construct 2	Construct 3	Construct 4	Construct 5
					
% full	68.9	71	74.8	72.2	68.8
% empty	31.1	28.9	25.1	27.6	31.1
% ambiguous	0.0	0.1	0.1	0.2	0.1

Fungal and bacterial contamination:

2 µL of purified AAV was tested for bacterial and fungal contamination using qPCR-based Femto Bacterial DNA Quantification Kit (catalog # E2006) and Femto Fungal DNA Quantification Kit (catalog # E20067), following manufacturer's instructions. A C_t > 30 was considered no contamination. (UD – undetermined)

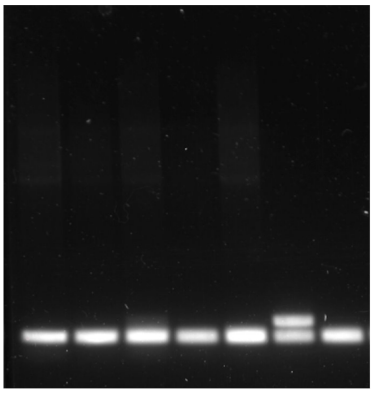
Test	AAV8 library mix	BC8	BC11	BC12	BC13	BC15
Fungus (C_t)	39.335	UD	UD	UD	UD	UD
Bacteria (C_t)	34.197	33.575	31.51	32.341	33.491	32.326

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 (Catalog# 093050301), following the manufacturer's protocol, before making the library pool.



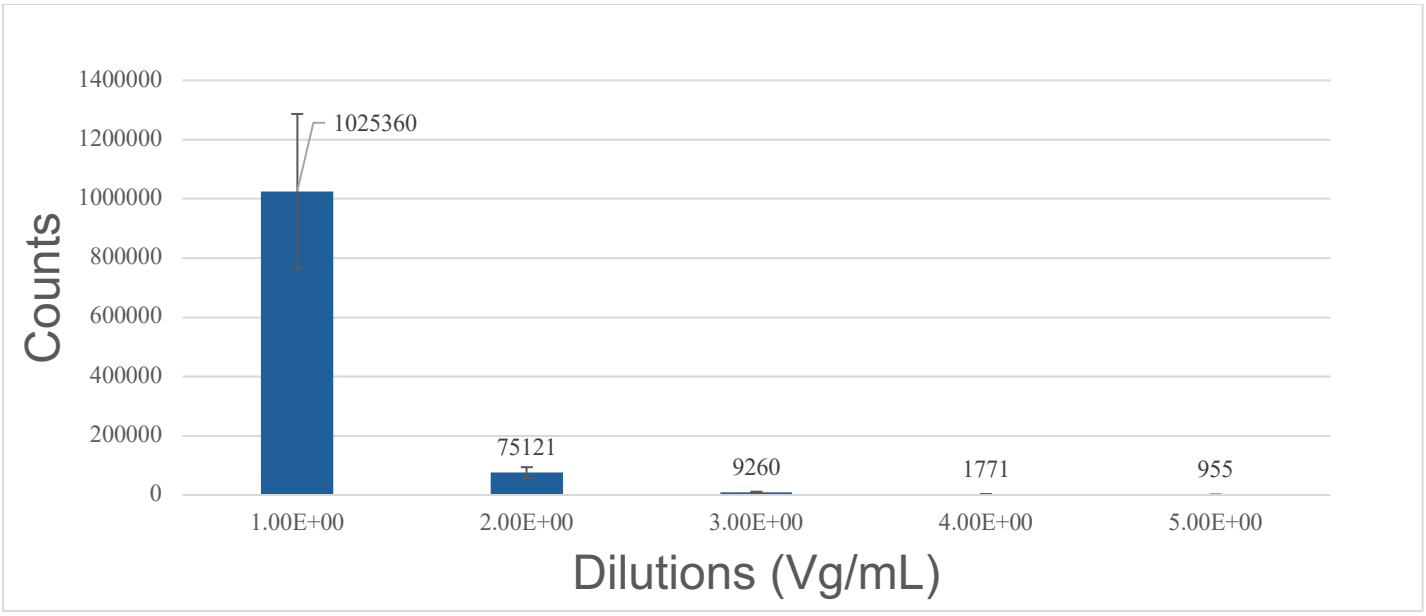
Lane1: AAV8 BC8,
Lane2: AAV8 BC11,
Lane3: AAV8 BC12,
Lane4: AAV8 BC13,
Lane5: AAV8 BC15,
Lane6: +ve control,
Lane7: -ve control

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:
Final titer of the pooled dilution curve 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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1000000
800000
600000
400000
200000
0
Counts
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75121
9260
1771
955
1.00E+00
2.00E+00
3.00E+00
4.00E+00
5.00E+00
Dilutions (Vg/mL)