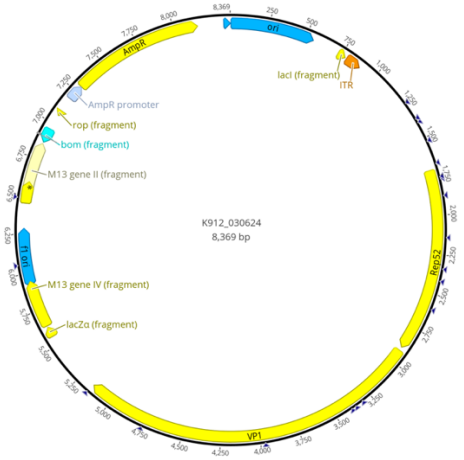
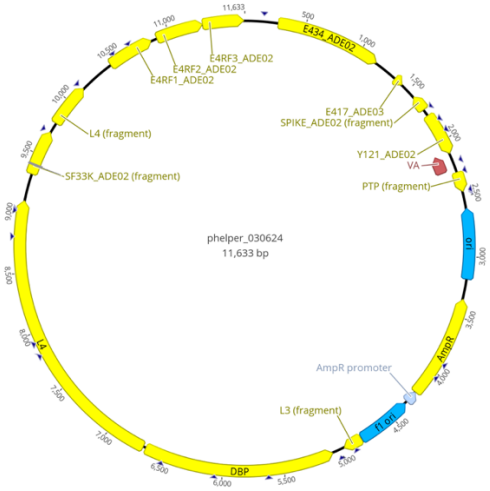
AAV Batch #: K912-04172024-scCAG-mgL
Serotype: K912
AAV construct: pAAV-scCAG-mGreenLantern

Plasmid maps of constructs used:

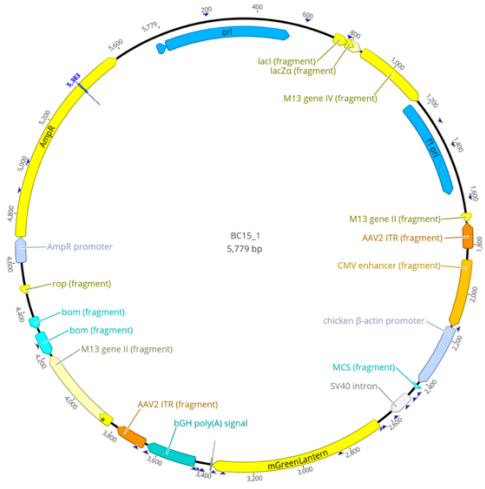
K912 (rep/cap):



pHelper:

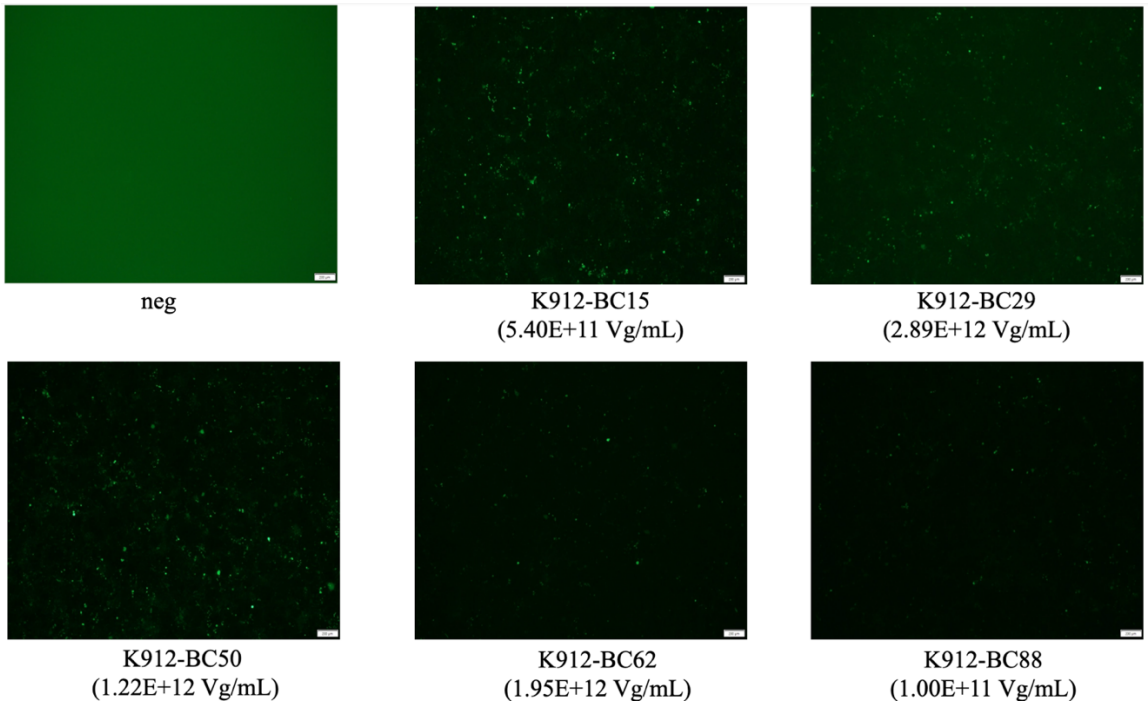


scCAG-mGreenLantern-BC:



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.

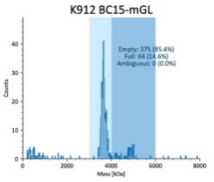
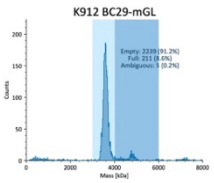
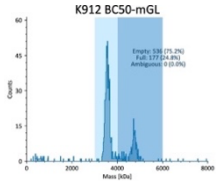
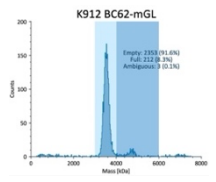
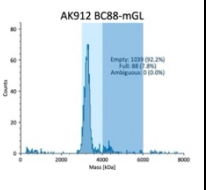


Titering:
AAV titers were measured using a qPCR AAV titer kit (ABM, catalog# G931), following the manufacturer's protocol.

AAV construct	Titer (Vg/mL)
K912-CAG-BC15_1 mgL	5.40E+11
K912-CAG-BC29_2 mgL	2.89E+12
K912-CAG-BC50_1 mgL	1.22E+12
K912-CAG-BC62_ mgL	1.95E+12
K912-CAG-BC88_1 mgL	1.00E+11

Mass photometry:

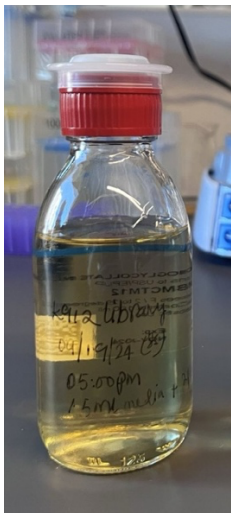
Mass photometry was performed on a SamuxMP (Refyn).

	K912-BC15	K912-BC29	K912-BC50	K912-BC62	K912-BC88
					
% full	14.6	8.6	24.8	8.3	7.8
% empty	85.4	91.2	75.2	91.6	92.2
% ambiguous	0.0	0.2	0.0	0.1	0.0

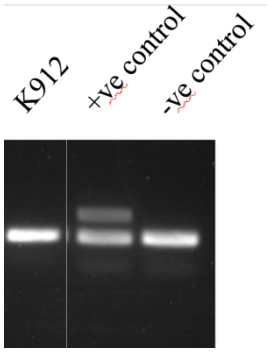
Fungal and bacterial contamination:
2 µL of purified AAV was tested for bacterial and fungal contamination using qPCR-based Femto Bacterial DNA Quantification Kit (Zymo, catalog # E2006) and Femto Fungal DNA Quantification Kit (Zymo, catalog # E20067), following manufacturer's instructions. A C_t > 30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

Test	K912 library mix
Fungus (C_t)	39.354
Bacteria (C_t)	32.359

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:
The final library pool was tested for mycoplasma contamination, using Myco-Sniff-Valid Mycoplasma PCR Detection kit (MP Biomedicals, Catalog# 093050301), following the manufacturer's protocol. No mycoplasma contamination was noted:



Endotoxin:
The final library pool was tested for endotoxin contamination. The samples were submitted for testing to Charles River Laboratories and analyzed using PTS kinetic LAL analysis.

SAMPLE RESULTS:

Serial Number/Bay: MCS 19211062/2	**Start/End Temp (°C):** 37.0:37.0	
Sample Name: K912 Library		
Sample ID: N/A	**Sample Lot:** N/A	**Dil./Conc.:** 1:500 mL/mL
Dilution: N/A		
Sample Comments: N/A		
Cartridge Lot Number: 4516155	**Cartridge Range:** 0.5-0.005 EU/mL	
Calibration Code: 418156542390	**Archived Spike Concentration:** 0.043 EU/mL	
Range: 181-965 seconds	**Y-Intercept:** +2.148	**Slope:** -0.363
Endotoxin Value: 111 EU/mL	**Sample CV Limit:** <25%	**Status:** VALID
Endotoxin Limit: No Limit **Status:** N/A	**Spike CV Limit:** <25%	**Status:** VALID
Alert Limit: N/A	**Spike Recovery Range:** 50-200%	**Status:** VALID
Cartridge Type: LAL Cartridge	**Performed With:** N/A	

SAMPLE DATA				SPIKE DATA				
Channel	Reaction Time	CV%	Sample Value	Channel	Reaction Time	CV%	Spike Value	Spike Recovery %
1	242	0.6%	111 EU/mL	2	230	1.2%	0.0426 EU/mL	99%
3	244			4	226			

Dilution curve analysis:
Final titer of the pooled dilution curve was 2.25E+11 vg/mL. Barcodes from the pooled library were amplified by PCR in triplicate 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	**BC62**	1.00E+11	1325010	1252307	1456315	1344544	103397
2	**BC29**	1.00E+10	58997	56517	61319	58944	2401
3	**BC50**	1.00E+09	14405	13180	13966	13850	621
4	**BC15**	1.00E+08	1342	1288	1322	1317	27
5	**BC88**	1.00E+07	1095	1133	1123	1117	20