

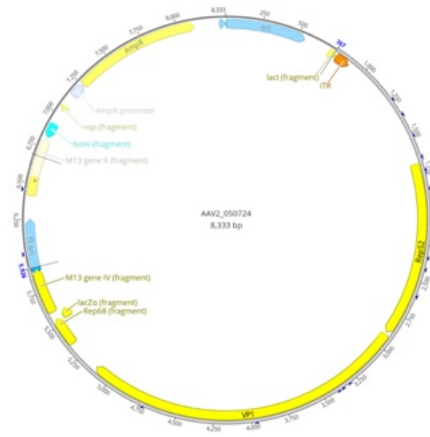
**AAV Batch #: AAV2-04172024-scCAG-mgL**

Serotype: AAV2

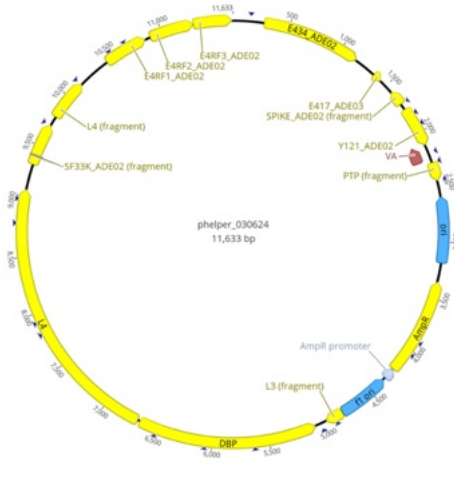
AAV construct: pAAV-scCAG-mGreenLantern

**Plasmid maps:**

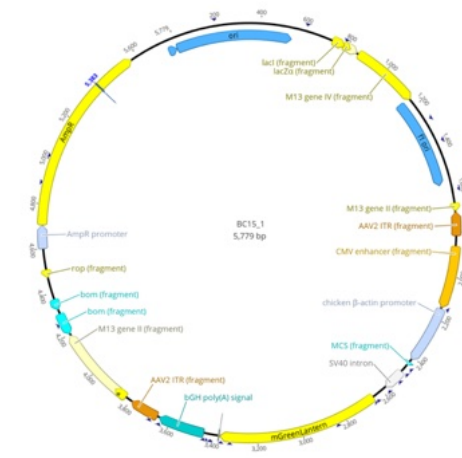
AAV2 (rep/cap):



pHelper:



scCAG-mGreenLantern-BC:

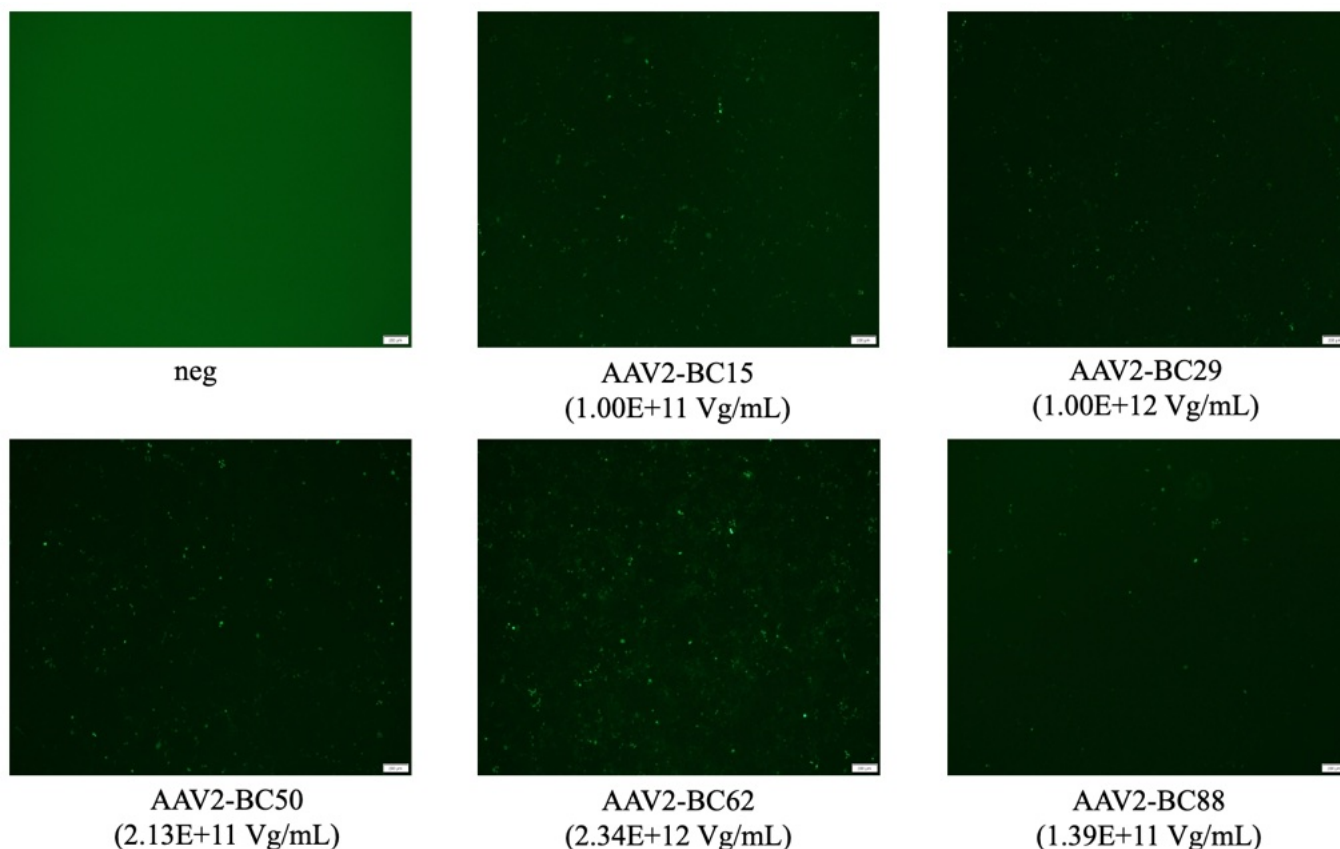


**Packaging protocol:**

Barcoded AAVs were packaged using a triple transfection method in adherent cells. Seed  $0.6 \times 10^6$  to  $0.7 \times 10^6$  cells/mL into a 125 mL shaker flask containing 30 mL of Expi293 medium (Thermo Fisher – A1435101) the day before transfection. On the following day, prepare the transfection mix of pHelper: AAV2: Barcode (BC)=1.67:1:1. Then, add the FectoVIR reagent to the mix and gently invert the solution (do not vortex) to prevent disintegration of the Fecto/DNA complex. Incubate the mixture for 15 minutes at room temperature. After incubation, add the required volume of the transfection mix to each flask. Finally, incubate the cells 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  $\mu$ L 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:**

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

AAV construct	Titer (Vg/mL)
AAV2-CAG-BC15_1 mgL	1.00E+11
AAV2-CAG-BC29_2 mgL	1.00E+12
AAV2-CAG-BC50_1 mgL	2.13E+11
AAV2-CAG-BC62_ mgL	2.34E+12
AAV2-CAG-BC88_1 mgL	1.39E+11

## Mass photometry:

Mass photometry was performed on a SamuxMP (Refyn).

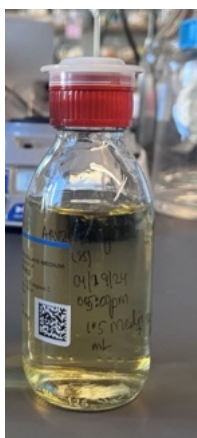
	AAV2-BC15	AAV2-BC29	AAV2-BC50	AAV2-BC62 4	AAV2-BC88
% full	5.8	4	6.8	7.8	4.4
% empty	94.2	96	92.9	92.2	95.1
% ambiguous	0.0	0.2	0.3	0.1	0.5

## Fungal and bacterial contamination:

2  $\mu$ L 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.

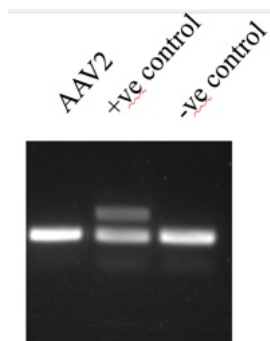
Test	AAV2 library mix
Fungus ( $C_t$ )	39.335
Bacteria ( $C_t$ )	34.197

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



### Mycoplasma:

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



### 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 19211061/1				Start/End Temp (°C): 37.0/37.0				
Sample Name: AAV2 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: 78.3 EU/mL				Sample CV Limit: <25%		Status: <span>VALID</span>		
Endotoxin Limit: No Limit		Status: N/A		Spike CV Limit: <25%		Status: <span>VALID</span>		
Alert Limit: N/A				Spike Recovery Range: 50-200%		Status: <span>VALID</span>		
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	266	5.1%	78.3 EU/mL	2	246	2.3%	0.0490 EU/mL	114%
3	286			4	254			

### Dilution curve analysis:

Final titer of the pooled dilution curve was 2.48E+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	BC62	1.00E+11	576735	437692	479533	497987	71335
2	BC50	1.00E+10	43890	53560	59722	48725	6838
3	BC29	1.00E+09	6218	5153	5316	5562	574
4	BC15	1.00E+08	1798	1437	1489	1575	195
5	BC88	1.00E+07	120	154	157	144	21

