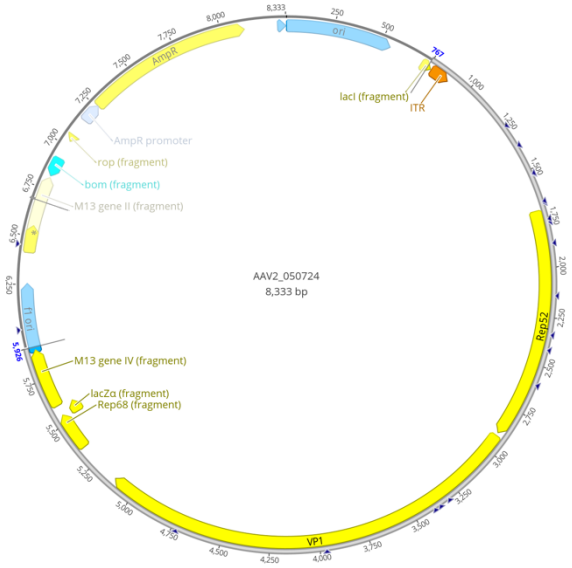
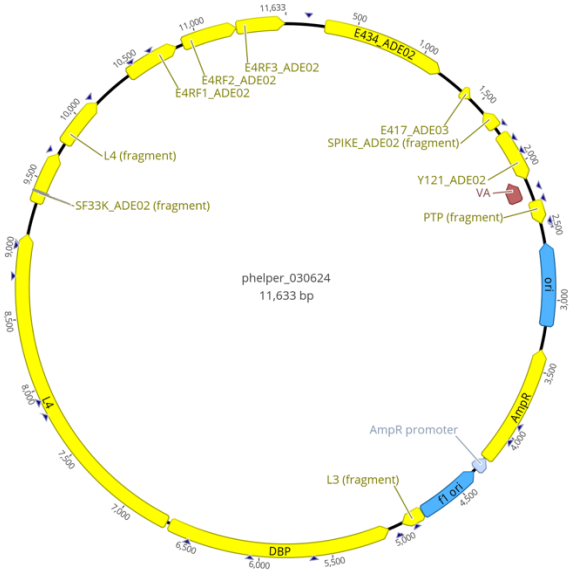
**AAV Batch #: AAV2-04172024-scCAG-mgL**
Serotype: AAV2
AAV construct: pAAV-scCAG-mGreenLantern

**Plasmid map:**

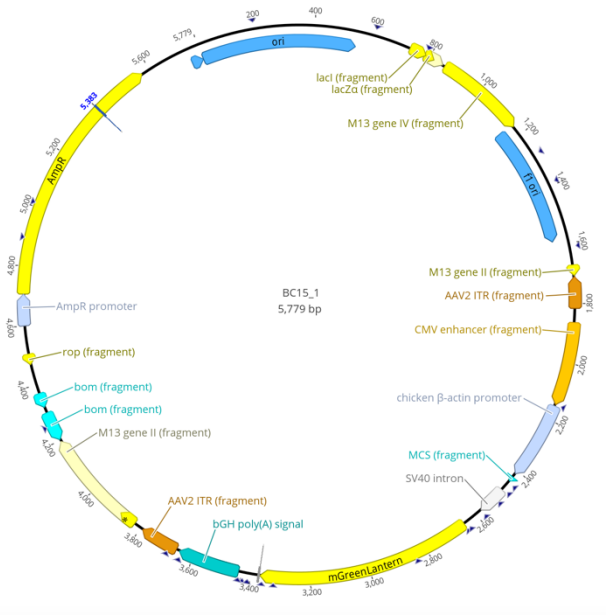
AAV2 (rep/cap):



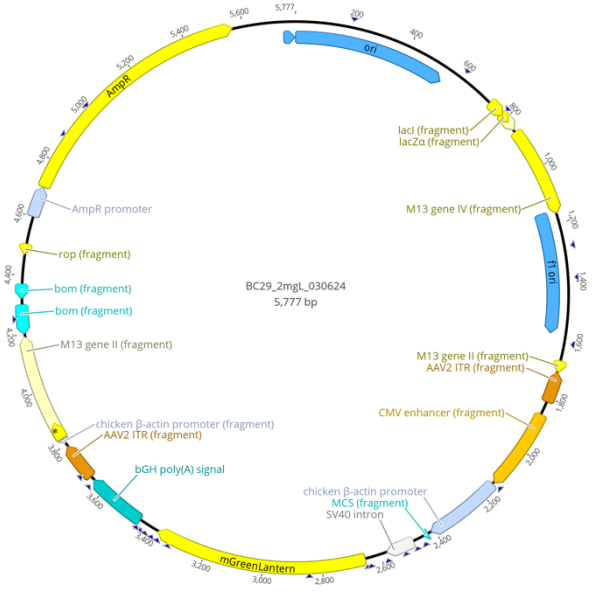

pHelper:



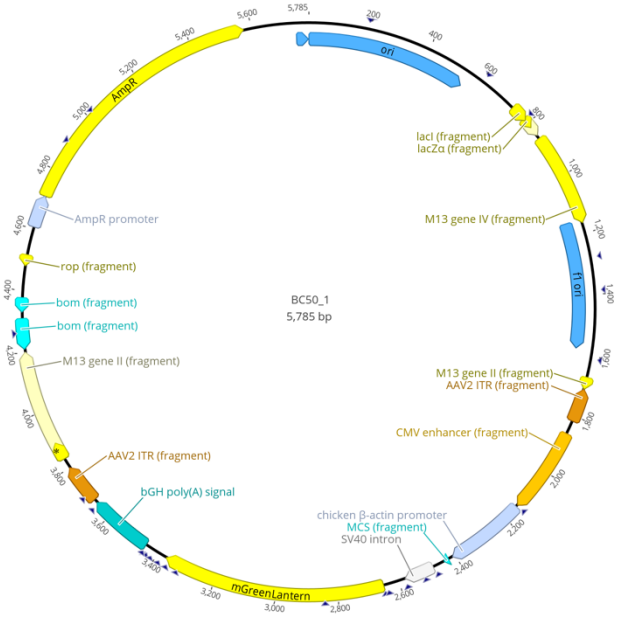
scCAG-mGreenLantern-BC15:



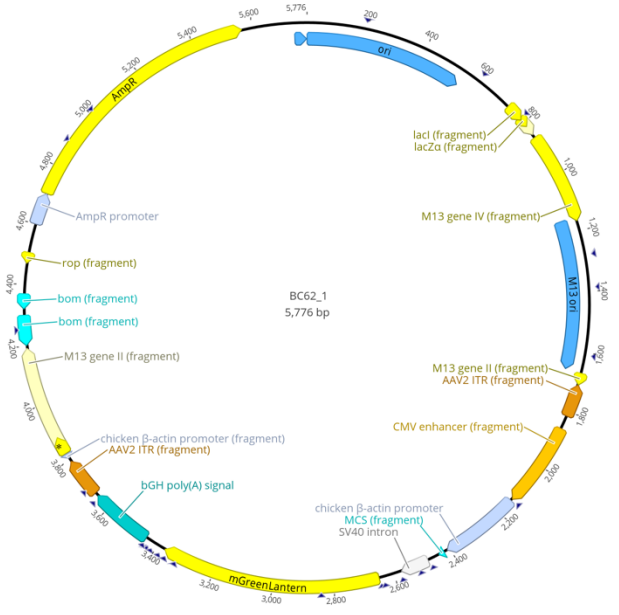

scCAG-mGreenLantern-BC29:



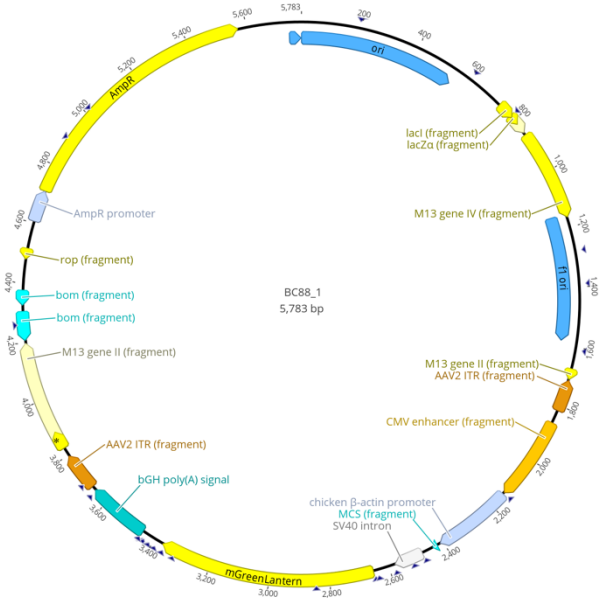
scCAG-mGreenLantern-BC50:




scCAG-mGreenLantern-BC62:



scCAG-mGreenLantern-BC88:

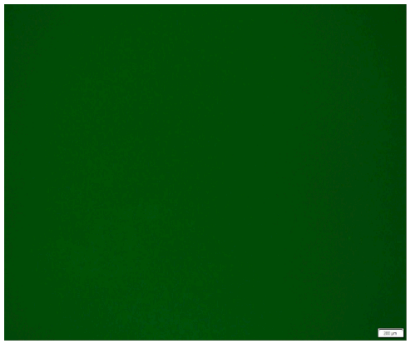



**Packaging protocol:**

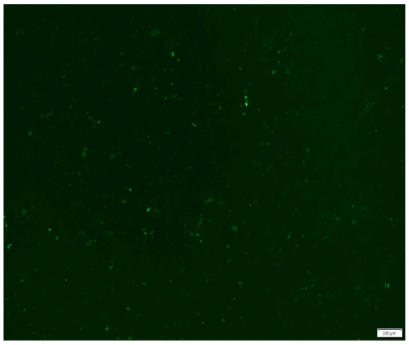
Barcoded AAVs were packaged using a triple transfection method in adherent cells. Seed 0.6E+6 to 0.7E+6 cells/mL into a 125 mL shaker flask containing 30 mL of Expi293 medium (Thermo Fisher – A1435101) the day before transfection. On the following day, prepare the transfection mix of pHelper: AAV2: Barcode (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:**

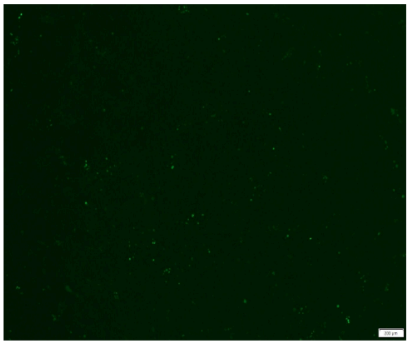
1 uL of packaged virus was used to infect 1 well of a 6-well plate containing HEK293 cells at 70% confluency. The cells were imaged for GFP signal after 48 hrs.



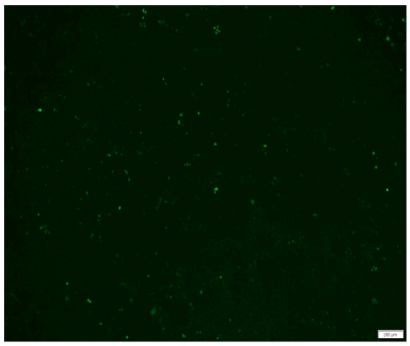
neg



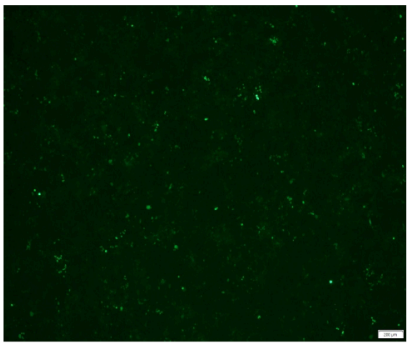
AAV2-BC15
(1.00E+11 Vg/mL)



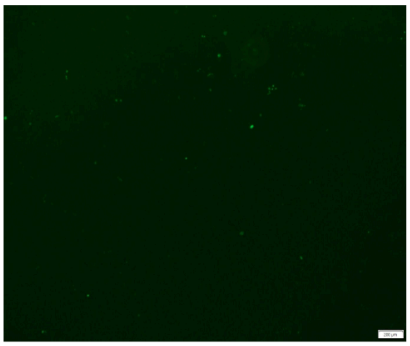
AAV2-BC29
(1.00E+12 Vg/mL)



AAV2-BC50
(2.13E+11 Vg/mL)



AAV2-BC62
(2.34E+12 Vg/mL)



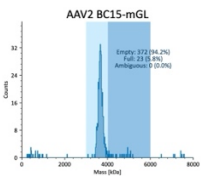
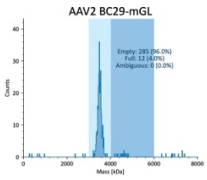
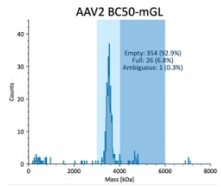
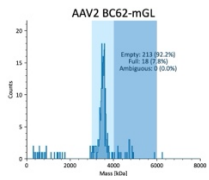
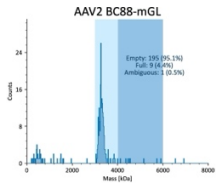
AAV2-BC88
(1.39E+11 Vg/mL)

**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:**

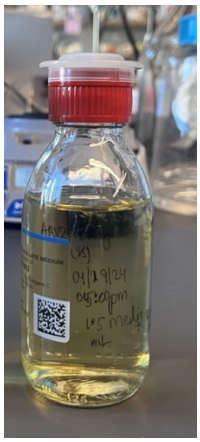
Mass photometry was performed on a SamuxMP (Refyn).

| | 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 |

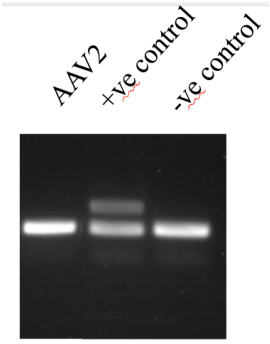
**Fungal and bacterial contamination:**
2 µL of purified AAV was tested for bacterial and fungal contamination using qPCR-based Femto Bacterial DNA Quantification Kit (catalog # E2006) and Femto Fungal DNA Quantification Kit (catalog # E20067), following manufacturer's instructions. A $C_t$ > 30 was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

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

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 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.

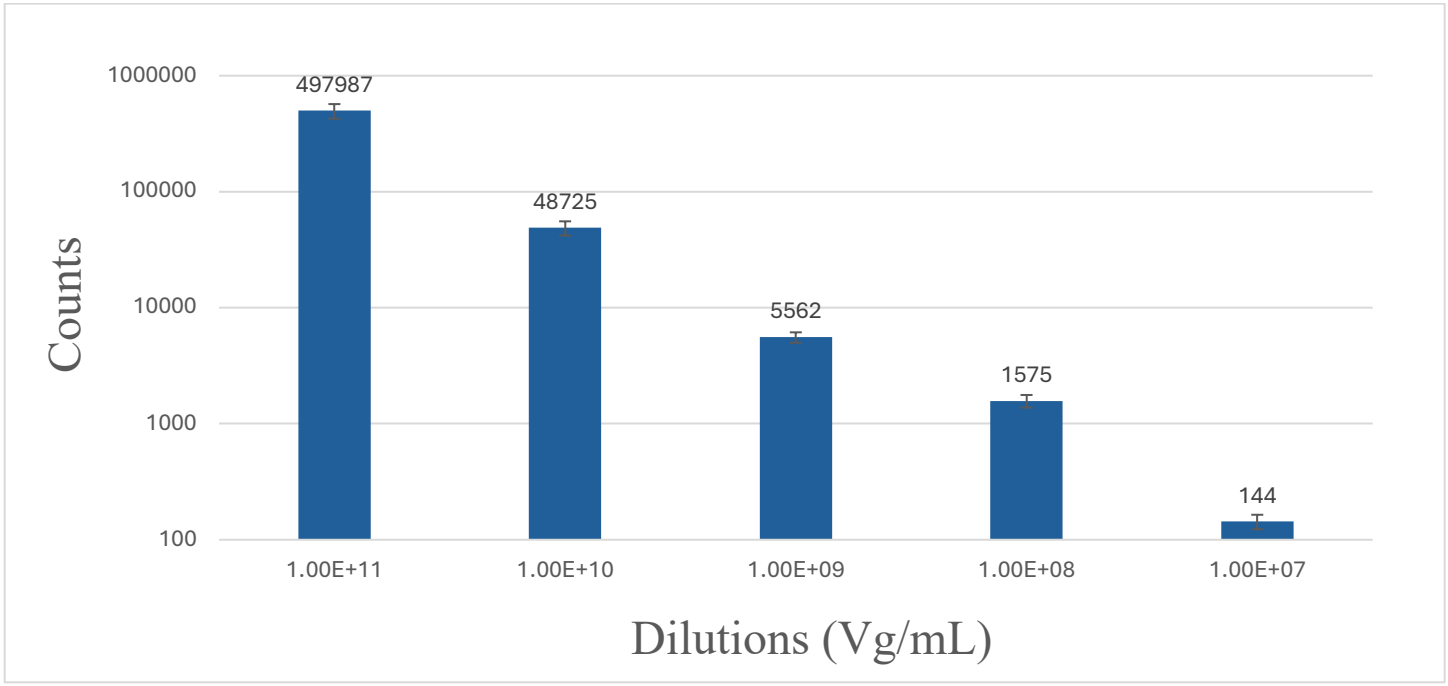
**SAMPLE RESULTS:**

| | | |
|---|---|---|
| **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:** 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 | 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 |


1000000
100000
10000
1000
100
Counts
497987
48725
5562
1575
144
1.00E+11
1.00E+10
1.00E+09
1.00E+08
1.00E+07
Dilutions (Vg/mL)