

Supplementary Data

Supplementary Table S1. Results of 37825 agarose gel extracted adeno-associated viral genomic DNA

| <i>Band</i> | <i>Total Reads</i> | <i>Reads Aligned to Reference</i> | <i>No. of Unmapped Reads</i> |
|-------------|--------------------|-----------------------------------|------------------------------|
| Low MW | 1478 | 1129 | 349 |
| High MW | 2730 | 2469 | 261 |

MW, molecular weight.

Supplementary Table S2. Next-generation sequencing read data for recombinant adeno-associated viral vector lots

| <i>Cis Plasmid Addgene ID</i> | <i>Serotype</i> | <i>Total Trimmed Reads</i> |
|-------------------------------|-----------------|----------------------------|
| 112005 | AAV5 | 48,200 |
| 50459 | AAV5 | 49,042 |
| 105553 | AAVrg | 70,066 |
| 20298 | AAV1 | 57,892 |
| 20298 | AAV5 | 50,976 |
| 44332 | AAV5 | 44,848 |
| 50477 | AAV8 | 53,618 |
| 44361 | AAV2 | 41,782 |
| 111067 | AAV5 | 78,678 |
| 50477 | AAV8 | 92,986 |
| 100844 | AAV1 | 66,916 |
| 105537 | AAV1 | 41,912 |
| 105537 | AAV8 | 87,822 |
| 51502 | AAV1 | 59,492 |
| 100851 | AAV1 | 87,756 |
| 45185 | AAV9 | 65,084 |
| 105677 | AAV1 | 74,394 |
| 100838 | AAV1 | 46,976 |
| 51502 | AAV9 | 41,138 |
| 112164 | AAV1 | 49,460 |
| 108912 | AAV9 | 24,254 |
| 100850 | AAV1 | 71,480 |
| 114472 | AAV2 | 60,428 |
| 50459 | AAV8 | 28,364 |
| 65417 | AAV8 | 22,422 |
| 100852 | AAV1 | 20,808 |
| 105539 | AAV1 | 15,958 |
| 105545 | AAV2 | 40,246 |
| 60227 | AAV1 | 50,932 |
| 26971 | AAV1 | 51,824 |
| 59170 | AAV1 | 46,026 |
| 105545 | AAV1 | 32,410 |
| 107787 | AAV8 | 29,602 |
| 100843 | AAV9 | 48,840 |
| 44362 | AAV8 | 64,430 |
| 99130 | AAV1 | 70,408 |
| 104493 | AAV1 | 51,404 |
| 106183 | AAV1 | 65,246 |
| 106185 | AAV1 | 48,656 |
| 112163 | AAV1 | 67,346 |

(continued)

Supplementary Table S2. (Continued)

| <i>Cis Plasmid Addgene ID</i> | <i>Serotype</i> | <i>Total Trimmed Reads</i> |
|-------------------------------|-----------------|----------------------------|
| 18917 | AAV1 | 29,812 |
| 18917 | AAV9 | 34,628 |
| 121539 | AAV1 | 42,234 |
| 121538 | AAV1 | 31,440 |
| 44361 | AAV8 | 38,452 |
| 27056 | AAV9 | 45,554 |
| 26966 | AAV9 | 33,366 |
| 50475 | AAV2 | 36,030 |
| 37120 | AAVrg | 6,948 |
| 104488 | AAV1 | 33,048 |
| 28306 | PHPeB | 39,940 |
| 44361 | AAV2 | 29,304 |
| 44361 | AAV2 | 14,962 |
| 111568 | AAV5 | 20,724 |
| 44362 | AAV5 | 33,528 |
| 37120 | AAVrg | 4,470 |
| 44362 | AAV2 | 6,252 |
| 58909 | AAV5 | 21,044 |
| 112164 | AAV1 | 44,314 |
| 26973 | AAV9 | 20,360 |
| 115893 | AAV8 | 49,162 |
| 105540 | AAV1 | 10,168 |
| 115892 | AAV8 | 19,072 |
| 20298 | AAV9 | 25,126 |
| 100833 | AAV9 | 25,898 |
| 50475 | AAV8 | 27,064 |
| 104492 | AAV9 | 18,414 |
| 104061 | PHPeB | 17,048 |
| 107787 | AAV8 | 12,176 |
| 44361 | AAV5 | 13,340 |
| 84445 | AAV5 | 13,020 |
| 112005 | AAV5 | 12,466 |
| 62726 | AAV8 | 39,178 |
| 28306 | AAV1 | 31,184 |
| 105679 | AAV5 | 38,332 |
| 37825 | AAVrg | 3,480 |
| 50457 | AAVrg | 6,706 |
| 121538 | AAV5 | 26,036 |
| 105545 | AAV8 | 9,870 |
| 49124 | AAV1 | 9,140 |
| 106186 | AAV2 | 5,460 |
| 119036 | AAV1 | 1,238 |
| 121539 | AAV5 | 26,780 |
| 114472 | AAV8 | 3,608 |
| 26969 | AAV5 | 4,538 |
| 55637 | AAVrg | 35,472 |
| 50457 | AAVrg | 62,060 |
| 111067 | AAV5 | 10,222 |
| 44362 | AAV5 | 42,634 |
| 104492 | AAV1 | 41,816 |
| 100837 | AAV1 | 50,572 |
| 105530 | AAV1 | 40,104 |
| 99121 | AAV1 | 77,618 |

(continued)

Supplementary Table S2. (Continued)

| Cis Plasmid Addgene ID | Serotype | Total Trimmed Reads |
|------------------------|----------|---------------------|
| 114472 | AAV5 | 58,988 |
| 59462 | AAVrg | 27,778 |
| 37825 | AAV5 | 38,240 |
| 26975 | AAV5 | 32,488 |
| 99120 | AAV1 | 28,892 |
| 44361 | AAV2 | 16,180 |
| 100834 | AAV1 | 25,702 |
| 44362 | AAV8 | 20,236 |
| 37120 | AAVrg | 24,108 |
| 75033 | AAV1 | 25,178 |
| 112010 | AAV9 | 36,540 |
| 104488 | AAV9 | 37,048 |
| 50459 | AAV8 | 36,444 |
| 105540 | AAVrg | 15,834 |
| 50459 | AAVrg | 12,600 |
| 87306 | AAV1 | 68,464 |
| 49124 | AAV1 | 33,168 |
| 106186 | AAV2 | 67,858 |
| 119036 | AAV1 | 29,548 |
| 114472 | AAV8 | 64,348 |
| 26969 | AAV5 | 42,106 |
| 104496 | AAV1 | 66,780 |
| 105553 | AAVrg | 65,072 |
| 50942 | AAV1 | 118,108 |
| 44361 | AAV8 | 84,768 |
| 104492 | AAV1 | 93,336 |
| 119741 | AAV5 | 39,924 |

AAV, adeno-associated virus.

Supplementary Table S3. pAAV-CAG-GFP-specific percentage of unmapped reads across serotypes

| Serotype | Total Reads | Unused Reads | Percentage of Unused Reads | Unused Reads Mapping to Cap | Percentage of Unmapped Reads Hitting Cap |
|----------|-------------|--------------|----------------------------|-----------------------------|--|
| AAV1 | 87,326 | 1,169 | 1.34 | 104 | 8.90 |
| AAV2 | 40,108 | 1,078 | 2.69 | 463 | 42.95 |
| AAV5 | 38,222 | 999 | 2.61 | 145 | 14.51 |
| AAV8 | 59,932 | 1,076 | 1.80 | 109 | 10.13 |
| AAV9 | 57,076 | 1,415 | 2.48 | 132 | 9.33 |
| AAVrg | 32,578 | 768 | 2.36 | 173 | 22.53 |
| Php.EB | 41,607 | 687 | 1.65 | 64 | 9.32 |

AAV, adeno-associated virus.

Supplementary Table S4. pAAV-hSyn-EGFP-specific percentage of unmapped reads across serotypes

| Serotype | Total Reads | Unused Reads | Percentage of Unused Reads | Unused Reads Mapping to Cap | Percentage of Unmapped Reads Hitting Cap |
|----------|-------------|--------------|----------------------------|-----------------------------|--|
| AAV1 | 49,324 | 930 | 1.89 | 47 | 5.05 |
| AAV2 | 36,188 | 503 | 1.39 | 160 | 31.81 |
| AAV5 | 68,222 | 1,245 | 1.82 | 224 | 17.99 |
| AAV8 | 57,888 | 834 | 1.44 | 40 | 4.80 |
| AAV9 | 74,948 | 837 | 1.12 | 98 | 11.71 |
| AAVrg | 36,188 | 503 | 1.39 | 155 | 30.82 |

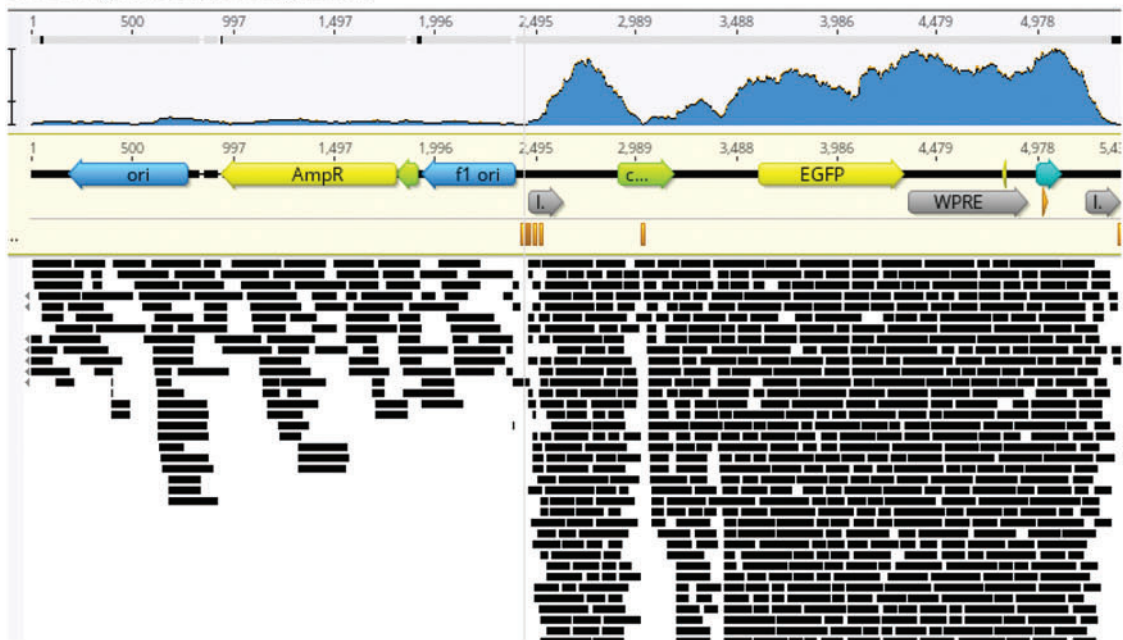
AAV, adeno-associated virus.

Supplementary Table S5. Key resources

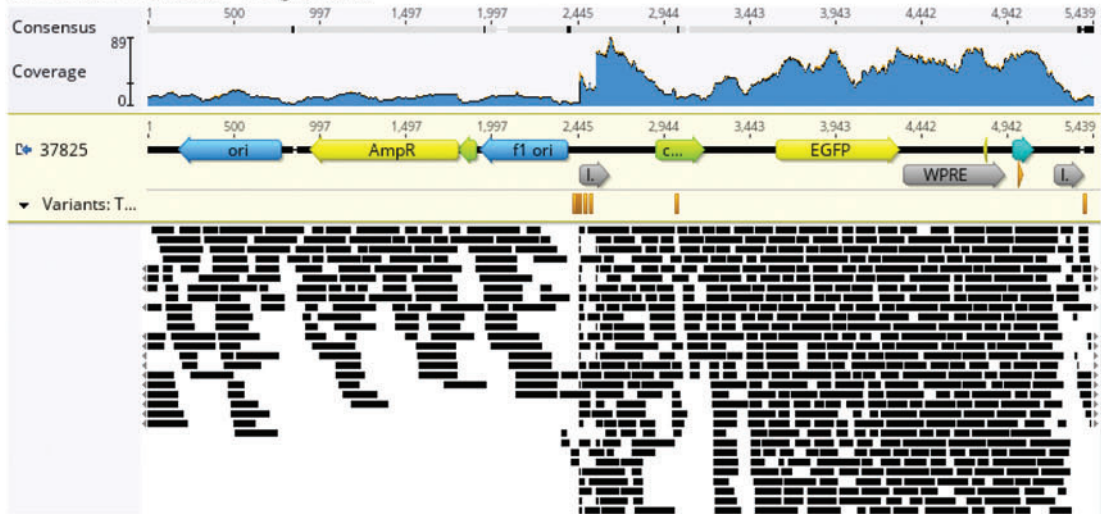
| Reagent or Resource | Source | Identifier |
|--|----------------------------------|---|
| Chemicals, peptides, and recombinant proteins | | |
| Polyethylenimine, linear, MW 25,000 | Alfa Aesar | 43896-03 |
| OptiMEM | Thermo Fisher | 31985-070 |
| TrypLE Express Enzyme (1 ×), phenol red | Thermo Fisher | 12605010 |
| Fetal bovine serum | Seradigm | 1500-500 |
| DMEM | Corning | 10-013-CV |
| EMEM | Corning | 10-009-CV |
| PBS (1 ×) | Corning | 21-040-CV |
| Pluronic F-68 | Thermo Fisher | 24040032 |
| Qiagen's endotoxin-free HiSpeed Gigaprep kit | Qiagen | 1054575 |
| ΦX174 DNA | New England Biolabs | N3023S |
| <i>SacI</i> | New England Biolabs | R0157S |
| <i>HaeIII</i> | New England Biolabs | R0108S |
| 1 kb DNA ladder | New England Biolabs | N3232S |
| Trypan blue | Thermo Fisher | T10282 |
| Benzonase nuclease | Millipore | 71205-3 |
| PureLink Viral RNA/DNA purification Kit | Thermo Fisher | 12280050 |
| Iodixanol (OptiPrep) | Sigma | D1556-250 |
| pHrodo dextran | Thermo Fisher | P35368 |
| Amcon Ultra-15 Centrifugal Filter Unit | Millipore | UFC910024 |
| QIAquick PCR Purification Kit | Qiagen | 28104 |
| PowerUp SYBR Green Master Mix | Thermo Fisher | A25741 |
| Experimental models: cell lines | | |
| AAVpro 293T | Clontech | 632273 |
| Neuro2a | American Type Culture Collection | ATCC CCL-13 |
| Recombinant DNA | | |
| pAAV-CAG-GFP | Addgene | 37825 |
| pAAV-hSyn-EGFP | Addgene | 50465 |
| pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA | Addgene | 20298 |
| AAV-Cre-GFP | Addgene | 68544 |
| AAV-EF1a-DIO-GCaMP6s-P2A-nls-dTomato | Addgene | 51082 |
| AAV-FLEX- <i>rev</i> -ChR2(H134R)-mCherry | Addgene | 18916 |
| rAAV2-retro helper | Addgene | 81070 |
| Software | | |
| Geneious Prime version 2019.1.1 (1.0) | Biomatters, Ltd. | |
| Addgene/open-bio | Addgene | https://github.com/addgene/openbio |

AAV, adeno-associated virus; DMEM, Dulbecco's Modified Eagle Medium; EMEM, Eagle's Minimum Essential Medium; PBS, Phosphate buffered saline.

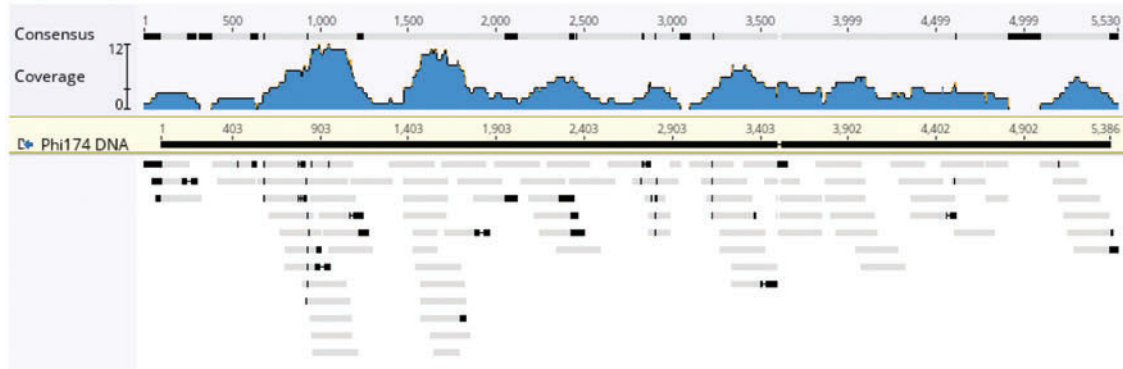
37825 High Molecular Weight Band



37825 Low Molecular Weight Band



ΦX174 DNA



Supplementary Figure S1. Analysis of VGS data from agarose gel extracted DNA. (Top and middle panels) High- and low-molecular-weight bands were extracted and submitted for VGS. Reads were aligned to the reference *cis* plasmid. (Bottom panel) ΦX174 DNA was submitted for VGS, and reads were aligned to the reference sequence. VGS, viral genome sequencing.