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(54) **METHODS FOR USING
TRANSCRIPTION-DEPENDENT DIRECTED
EVOLUTION OF AAV CAPSIDS**

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Publication Classification

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C12N 7/00 (2006.01)
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CPC **C12N 15/11** (2013.01); **C12N 7/00**
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2310/11 (2013.01); **C12N 2320/33** (2013.01);
C12N 2750/14123 (2013.01); **C12N 2830/008**
(2013.01)

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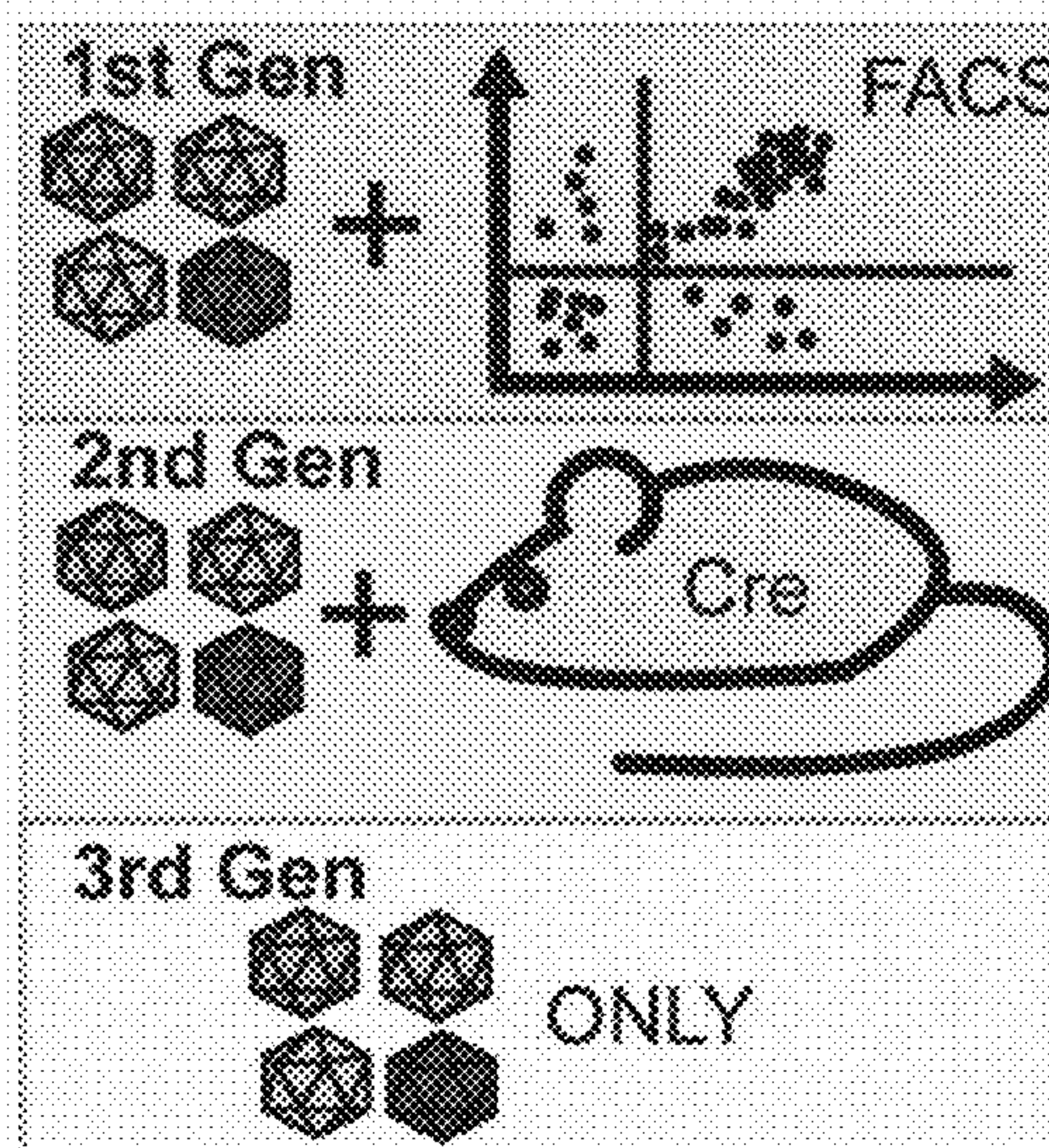
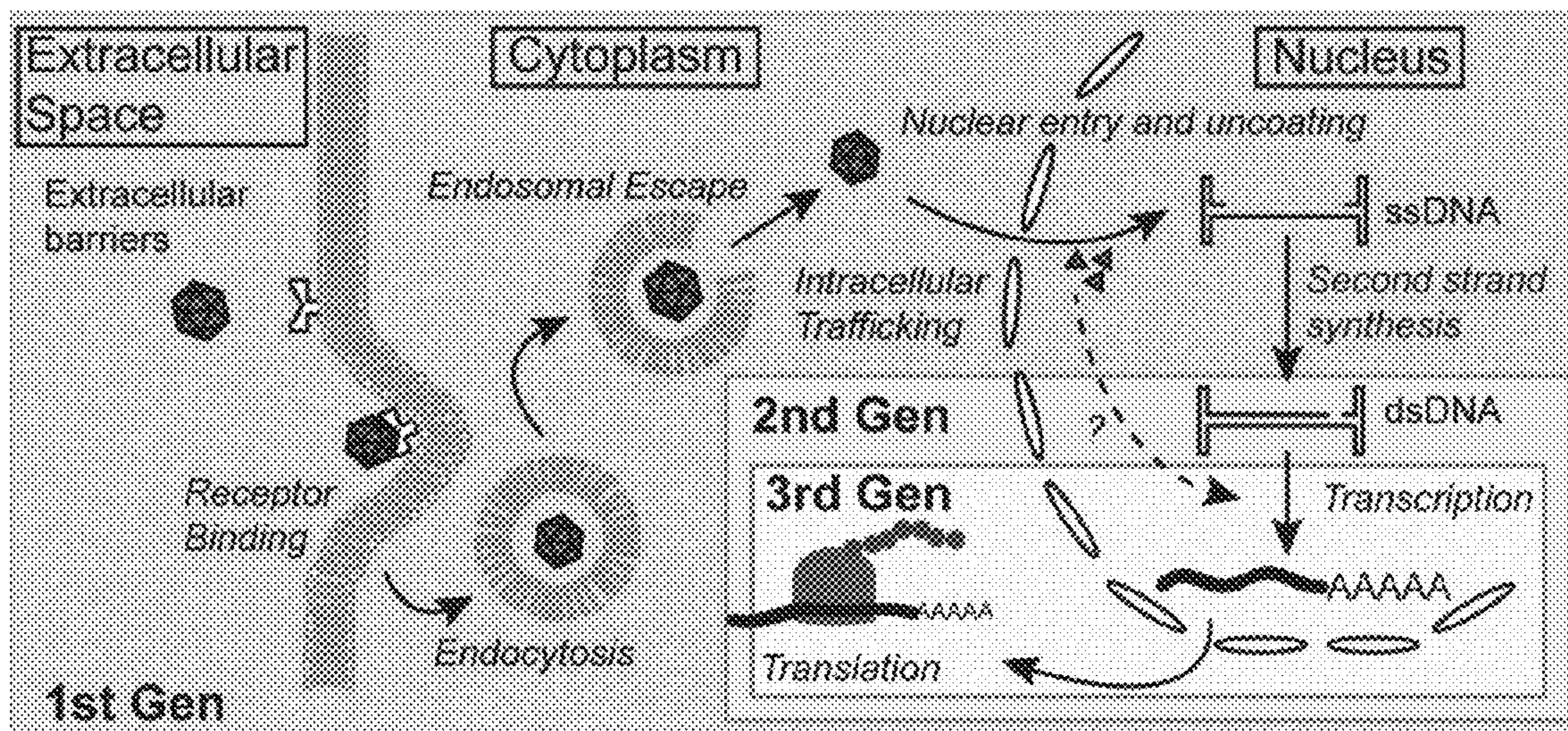
Related U.S. Application Data

(63) Continuation of application No. 17/337,341, filed on Jun. 2, 2021, now Pat. No. 11,459,558, which is a continuation of application No. PCT/US2020/016273, filed on Jan. 31, 2020.

(57) **ABSTRACT**

Disclosed are methods for performing transcription-dependent directed evolution (TRADE) and novel AAV capsids selected using such methods.

Specification includes a Sequence Listing.



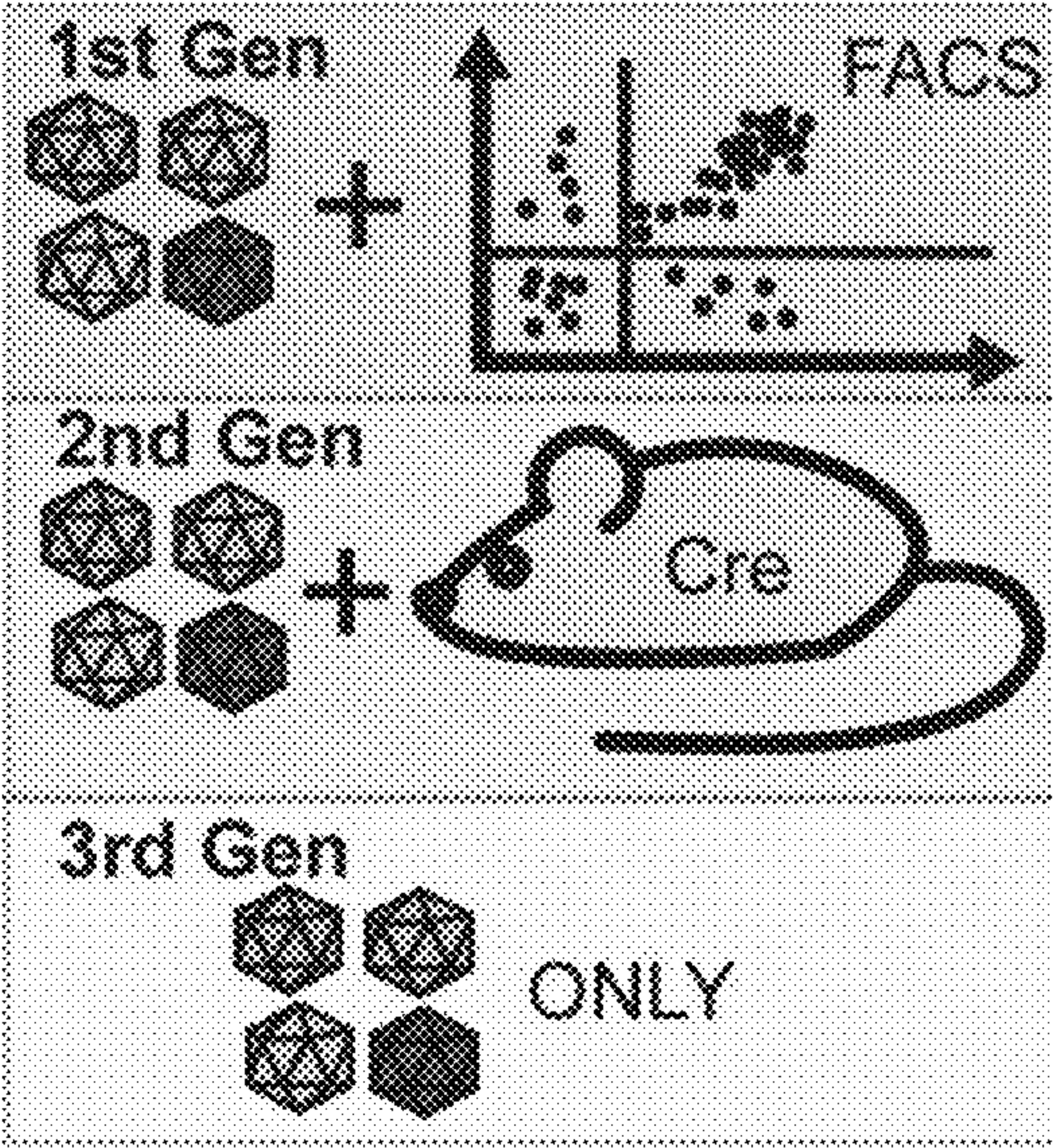
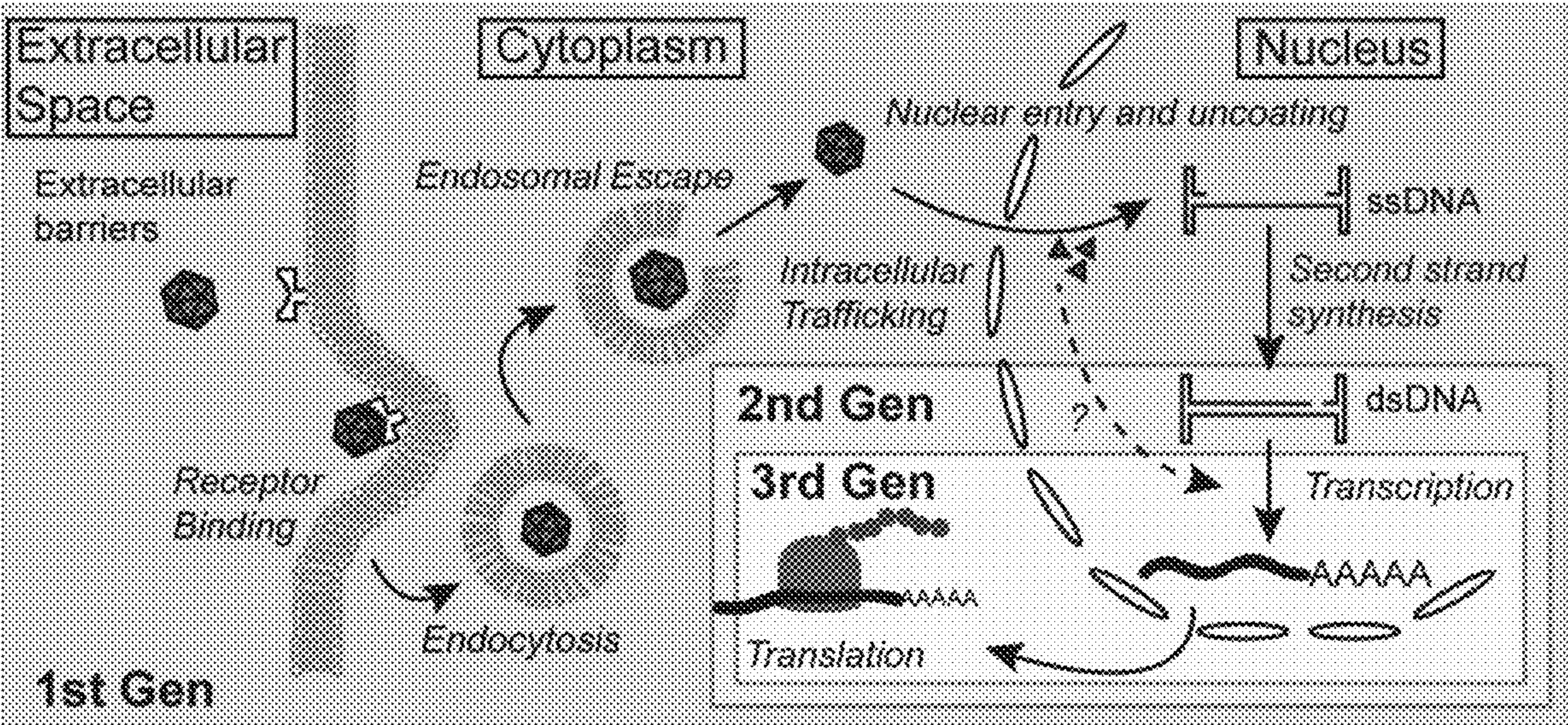


FIG. 1

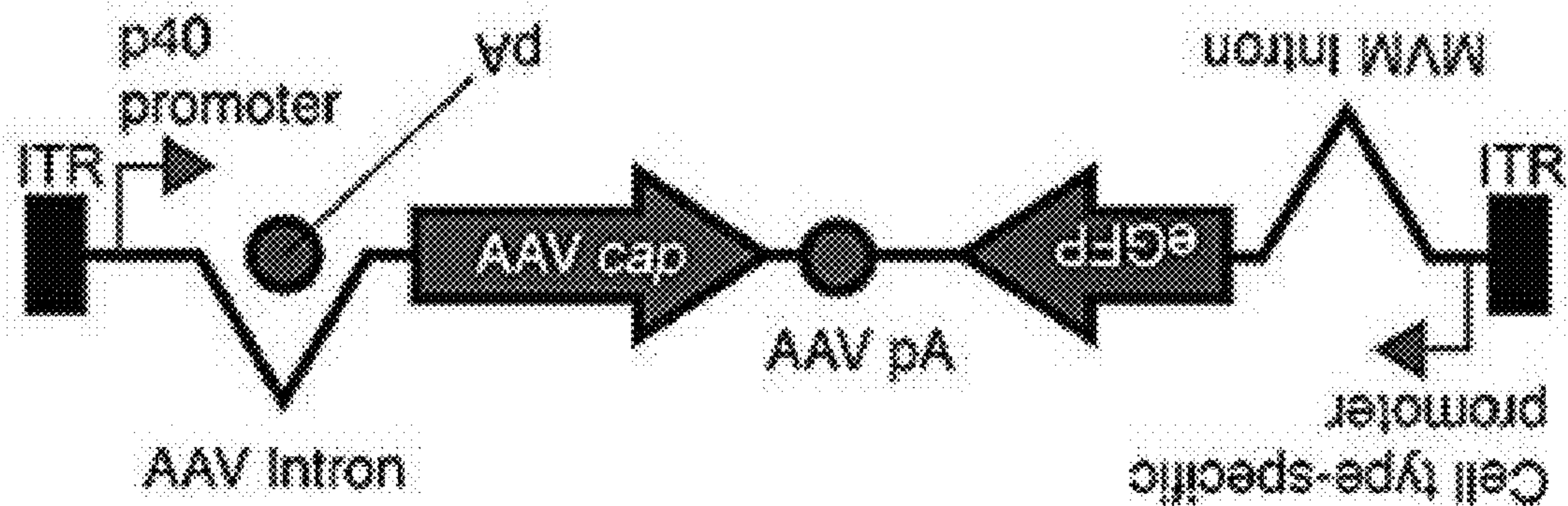


FIG. 2A

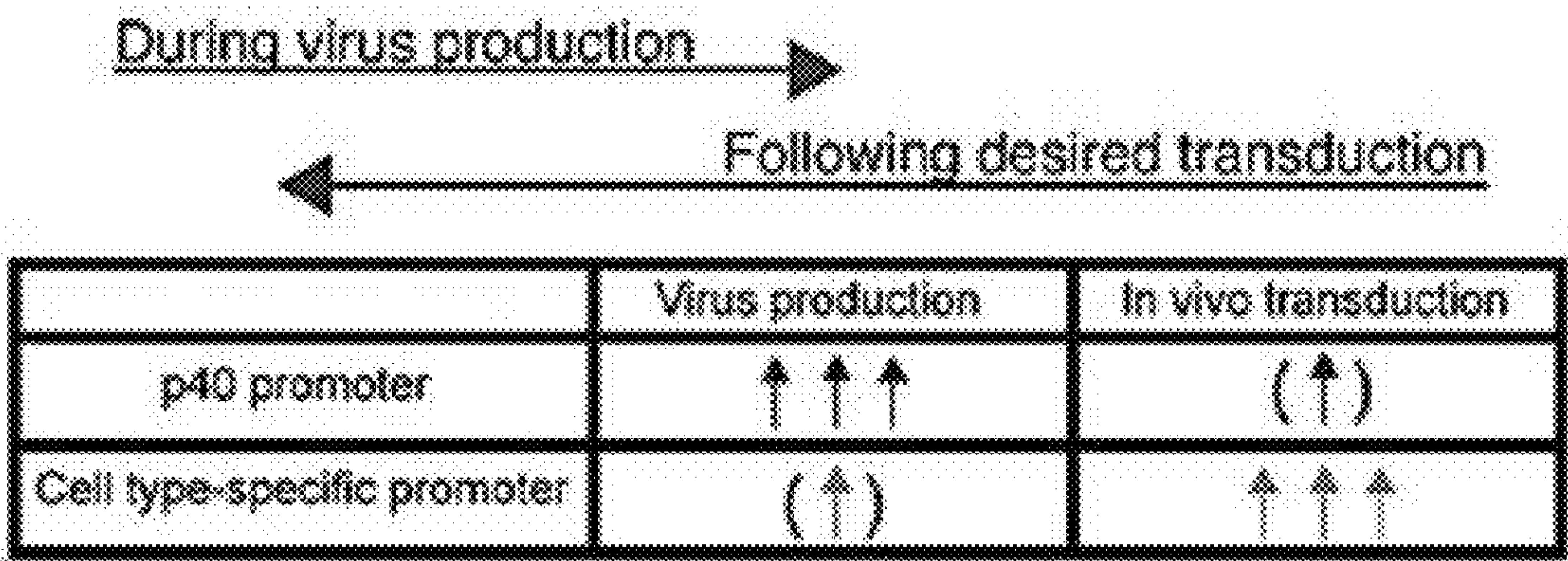


FIG. 2B

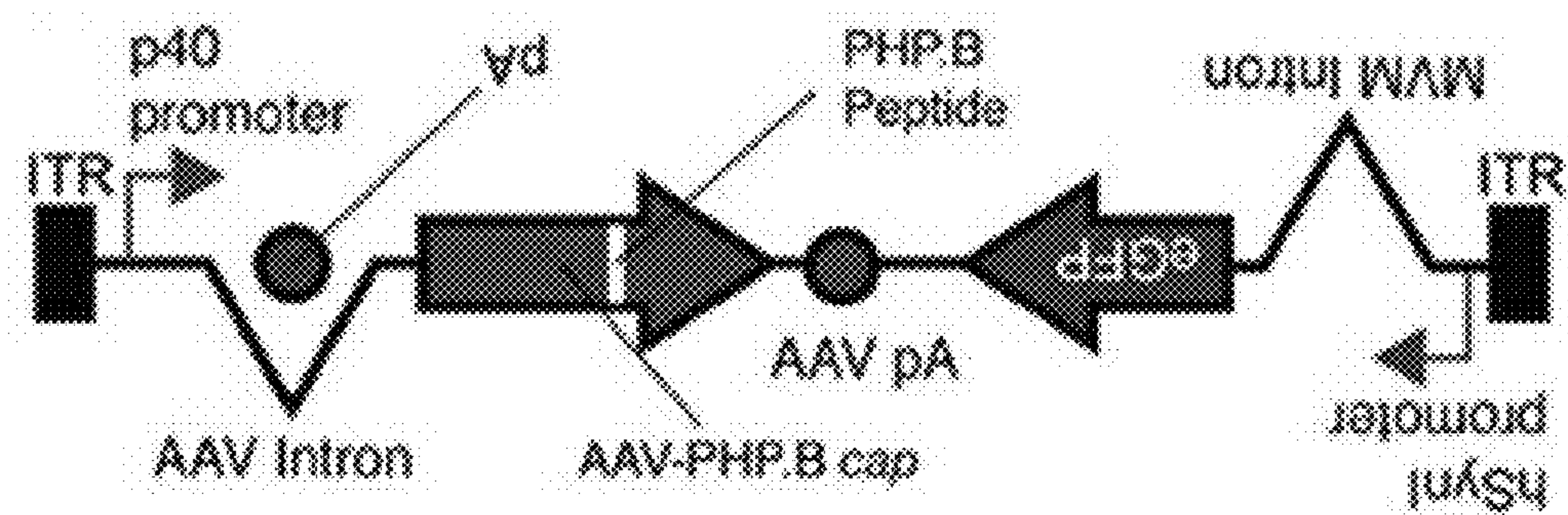


FIG. 3A

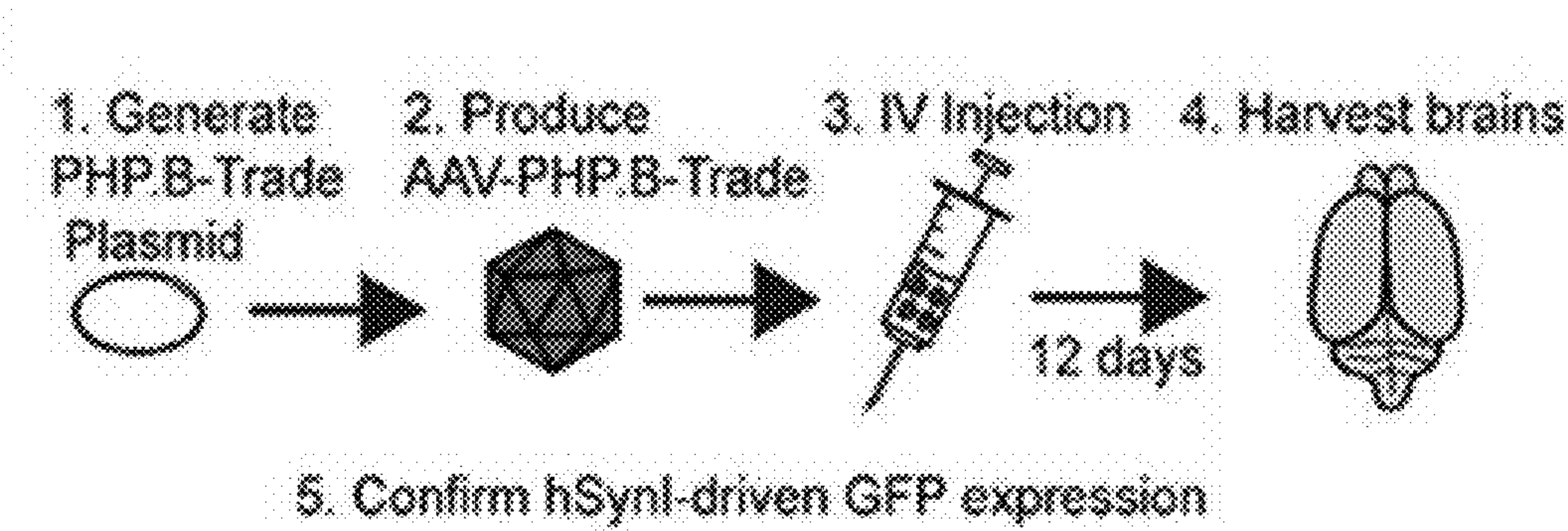
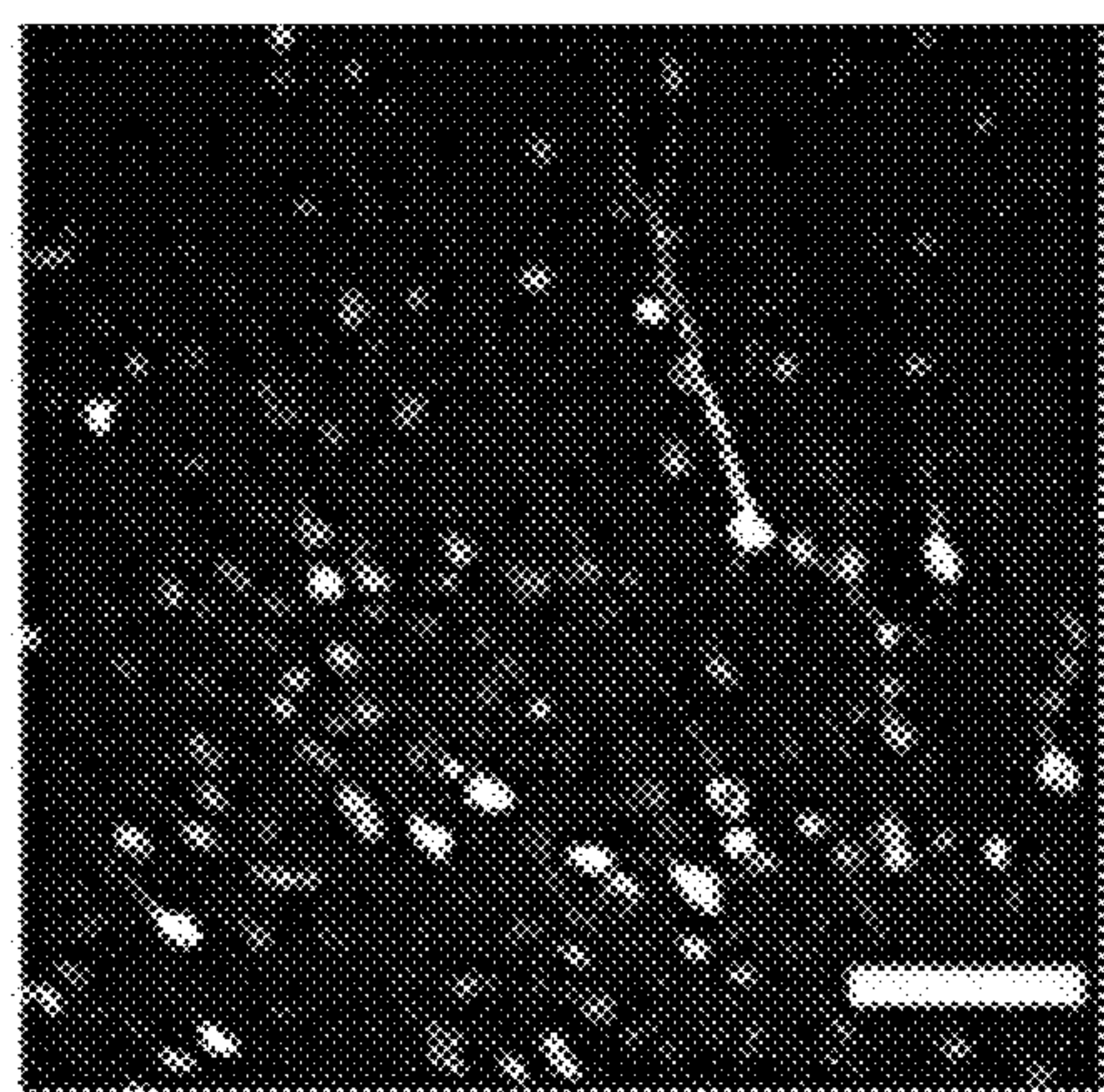
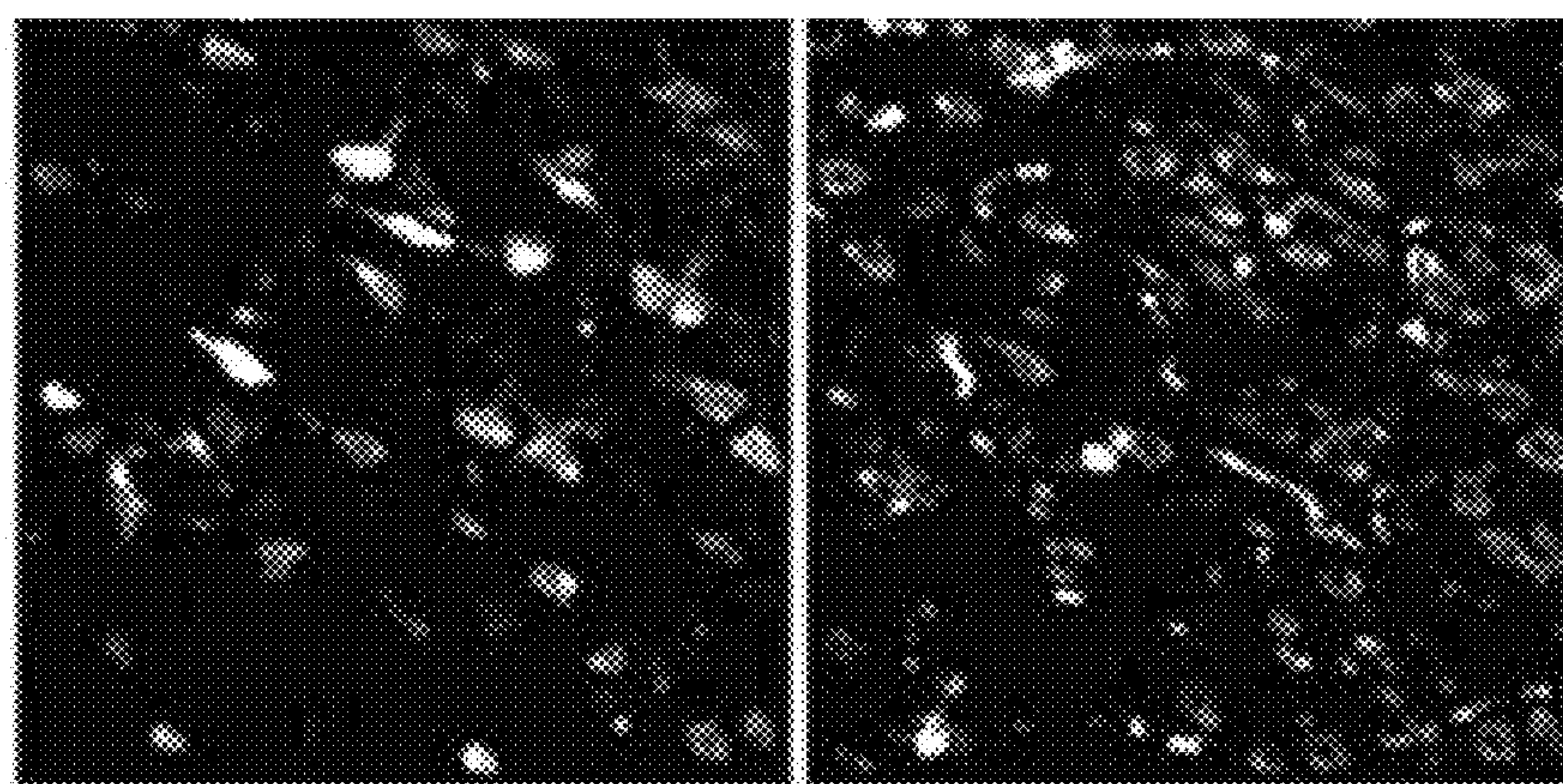


FIG. 3B



Anti-GFP

FIG. 3C



GFP

Anti-HuC/D

6. Recover and sequence hSynl-driven transcripts

FIG. 3D

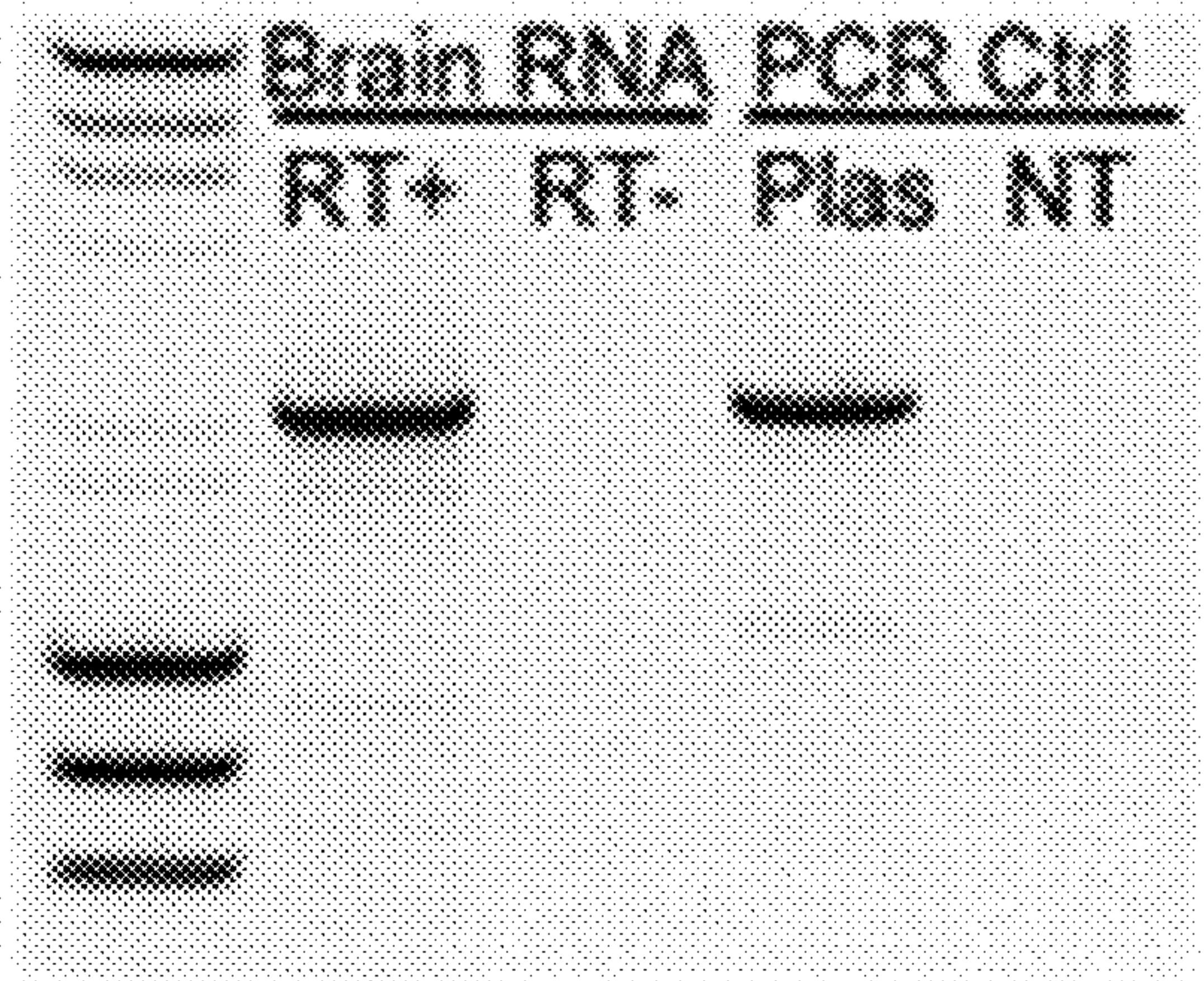


FIG. 3E

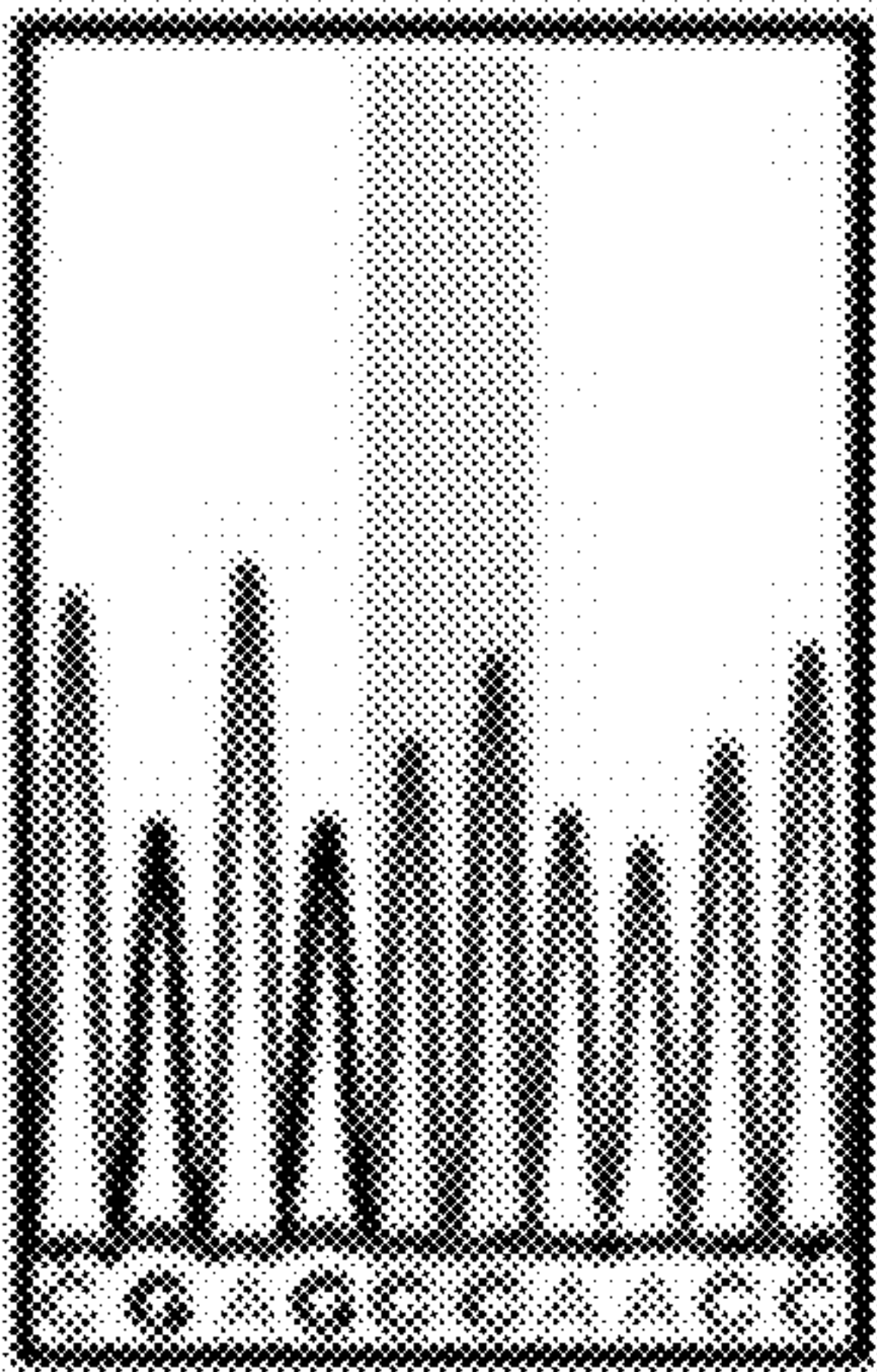


FIG. 3F

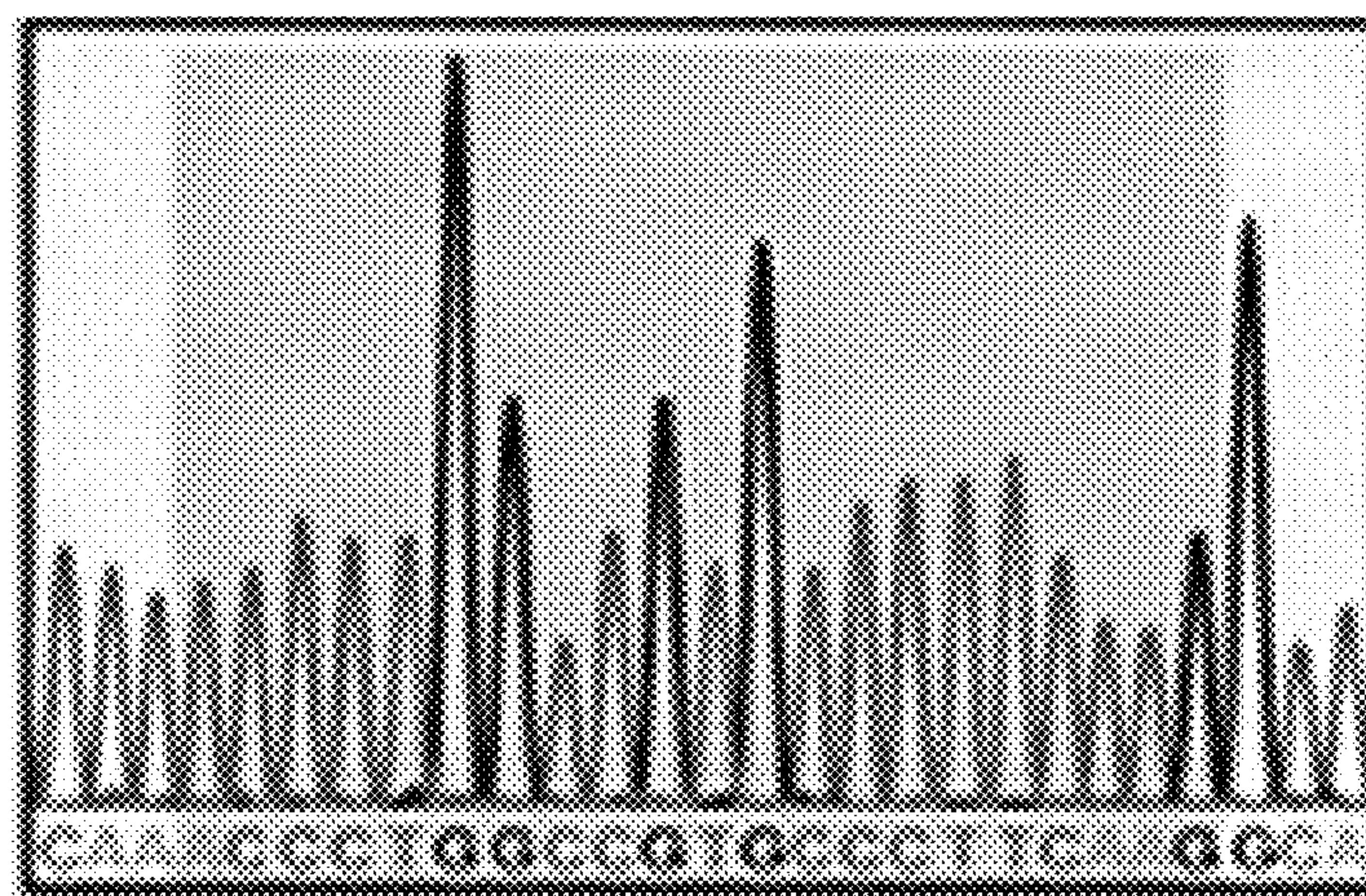


FIG. 3G

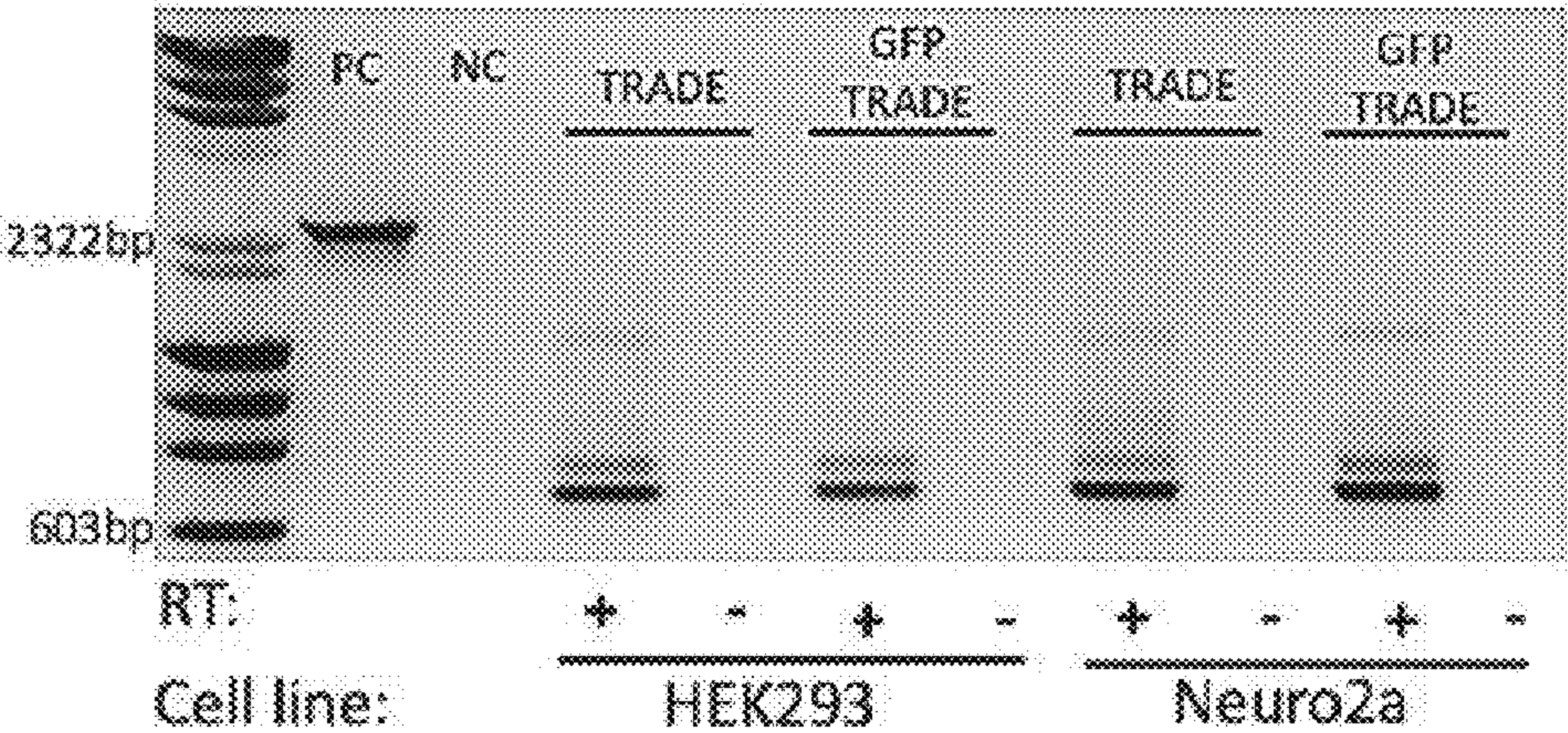


FIG. 4

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGG
AATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCCAAGGCAAATC
ACAACATCAAGACAACGCTCGAGGTCTTGTGCTTCCGGGTTACAAATACCTTG
GACCCGGCAACGGAAGCTCGACAAGGGGGAGCCGGTCAACGCAGCAGACGCGG
CGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAA
CCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTCCAGGAGCGGCTCAAAG
AAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAG
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cccagtatctactggccaagtgcagcgtggagatcgagtgaggagctgcagaaggaaaaacagcaagcgcctggaac
ccggagatccagtacacttccaactattacaagcttaataatgttgaatttgcgttgaatactgaagggtgtatagtgaac
cccccccatgggcaccagatacCTGACTCGTAATCTGTAA

FIG. 5

Splice acceptor

rh64	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh53	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh50	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh52	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
hu.39	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh51	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh57	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh61	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh49	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh58	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAACTTT	522
AAV7	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
cy.2	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh62	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAACTTT	522
rh48	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAACTTT	522
rh55	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh54	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAACTTT	522
rh60	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAATTTC	522
rh38	GCAAG--AAAGGCCAGCCG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.41	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.66	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.49	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.67	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.42	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.37	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
rh40	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
AAV10	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
rh.2	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AGAAAGAGACTCAACTTT	522
rh25	GCAAG--ACAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.6	GCAAG--ACAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
hu.17	GCAAG--ACAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
rh.16	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	507
rh.17	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	507
rh.18	GCAAG--ACAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
rh.12	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	507
rh.14	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	507
rh.24	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	507
bb.1	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
bb.2	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
rh.19	GCAAG--AAAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAACTTT	522
pi.3	GCAAG--TCAGGCCAGCAG-----CCCCTC-----AAAAAGAGACTCAATTTC	504

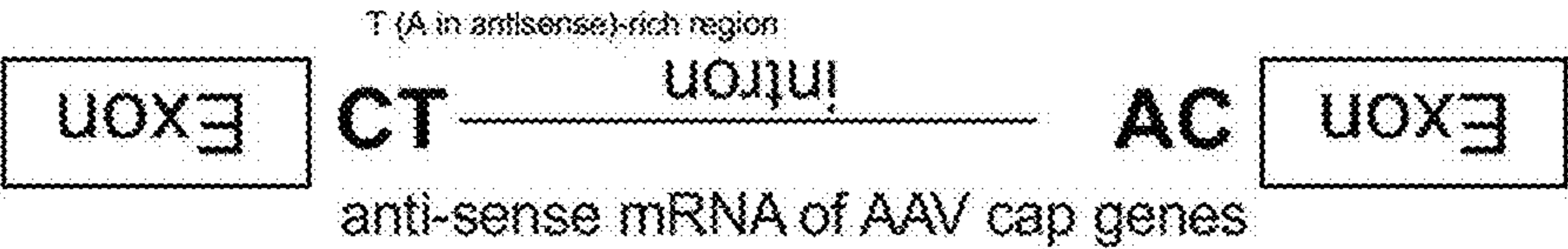


FIG. 6A

Splice acceptor

pi.1	GCAAG---TCAGGCCAGCAG-----CCCCTG-----	AAAAAGAGACTCAATTTTT	504
pi.2	GCAAG---TCAGGCCAGCAG-----CCCCTG-----	AAAAAGAGACTCAATTTTT	504
AAV8	GCAAG---AAAGGCCAACCAG-----CCCCTC-----	AAAAAGAGACTCAATTTTT	522
rh.43	GCAAG---AAAGGCCAACCAG-----CCCCTC-----	AAAAAGAGACTCAATTTTT	519
rh.1	GCAAG---AAAGGCCAACCAG-----CCCCTC-----	AAAAAGAGACTCAATTTTT	522
rh.8	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
cy.5	GCAAG---AAAGGCCAGCCG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
rh.13	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
cy.3	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
cy.4	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
cy.6	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
rh.22	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	507
rh.19	GCAAG---ACAGGCCAGCAG-----CCCCTG-----	AAAAAGAGACTCAACTTTT	522
rh.23	GCAAG---ACAGGCCAGCAG-----CCCCTG-----	AAAAAGAGACTCAACTTTT	522
rh.36	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	507
rh.37	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	507
rh.35	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	507
AAV1	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
hu.48	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
hu.43	GCAAG---AAAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAACTTTT	522
hu.46	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
hu.44	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
AAV6	GCAAG---ACAGGCCAGCAG-----CCCCTT-----	AAAAAGAGACTCAATTTTT	519
hu.34	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.35	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
AAV2	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.52	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.51	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.47	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.45	GAAAG---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.56	GAAAA---GCGGGCAACCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.57	GAAAA---GCGGGCAACCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	516
hu.63	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.64	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.58	GAAAA---GCGGGCAACCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.49	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.13	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.29	GAAAG---TCGGGCAACCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.28	GAAAG---TCGGGCAACCAG-----CTTCCA-----	AGAAAAAGATTCAATTTC	519
hu.24	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519
hu.21	GAAAA---GCGGGCCAGCAG-----CTTCCA-----	AGAAAAAGATTCAATTTTT	519

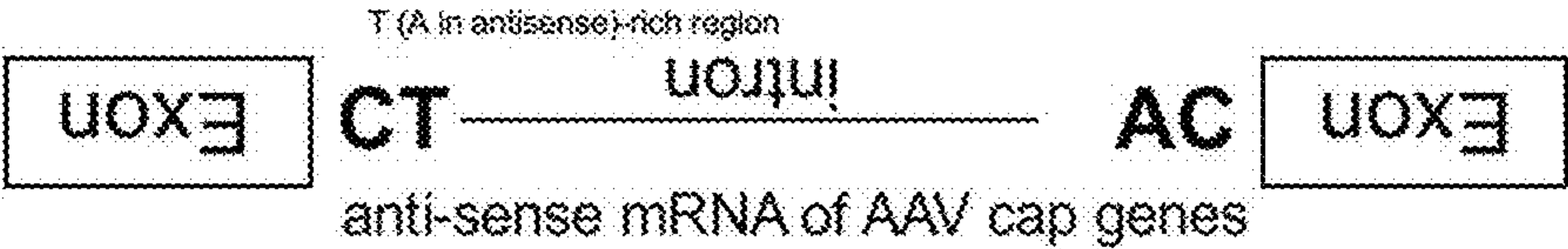


FIG. 6B

Splice acceptor

hu.22	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.20	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.19	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.27	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.23	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.15	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
hu.16	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
hu.18	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
hu.7	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
hu.60	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
hu.25	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.4	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTAAATTTT	519
hu.1	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTAAATTTT	519
hu.2	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTAAATTTT	519
hu.3	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTAAATTTT	522
hu.9	GAAAA--GCGGGCCATCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.11	GAAAA--GCGGGCCATCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.10	GAAAG--GCGGGCCATCAG--CTTCA	AGAAACAGATTCAATTTT	519
hu.55	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGACTCAATTTT	519
hu.53	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGACTCAATTTT	519
hu.54	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGACTCAATTTT	519
hu.61	GAAAA--GCGGGCCAGCAG--CTTCA	AGAAAAAGATTCAATTTT	519
AAV3A	GCAAA--TCGGGCCAAACAG--CTTCC	AGAAAAAGACTCAATTTT	519
AAV3B	GCAAA--TCGGGCCAAACAG--CTTCC	AGAAAAAGACTCAATTTT	519
AAV13	GCAAA--TCAGGCCAGCAG--CCCCT	AGAAAAAGACTCAATTTT	516
ch.5	GCAAA--TCAGGCCAGCAG--CCCCT	AGAAAAAGACTCAATTTT	519
AAV9	GCAAA--TCGGGTTCACAG--CCCCT	AAAAAGAGACTCAATTTT	519
AAV9hu.14	GCAAA--TCGGGTTCACAG--CCCCT	AAAAAGAGACTCAATTTT	519
hu.31	GCAAA--TCGGGTTCACAG--CCCCT	AAAAAGAAACTCAATTTT	519
hu.32	GCAAA--TCGGGTTCACAG--CCCCT	AAAAAGAAACTCAATTTT	519
rh.32	GCAAA--AAAGGCCAAACA--ACCAACC	AAAAAGAGACTCAACTTT	516
rh.33	GCAAA--AAAGGCCAAACA--ACCAACC	AAAAAGAGACTCAACTTT	516
rh.34	GCAAA--AAAGGCCAAACA--ACCAACC	AAAAAGAGACTCAACTTT	516
AAV11	GCAAA--AAAGGCCAAACA--ACCAACC	AGAAAGAGGCTCAACTTT	516
AAV12	GCCAAAGAAAAAGCAAAAGACGGCGAACCACCGACTCTGCTAGAAAGGACACTCGACTTT		546
AAV4	GCAAA--AAAGGCCAGCAG--CCCCT	AAAAAGAGGCTCGTTTTC	516
BAAV	GCAAG--AAAGGCCAAACAG--CTTCC	AGAAAGAGACTCAACTTT	519
Avian	CTAAG--AAAAACAAGAG--CTTCC	CAAGGAAAGACCTTCC	543
AAV5	AAGAG--GACTCCAG--CTTCC	ACCTCGT	496
Go.1	AAGAG--GACTCCAG--CTTCC	ACCTCGT	496
Goos	TTAAG--AAGCCTAAAC--CTTCC	AGGAAGTCAGTCCG	522

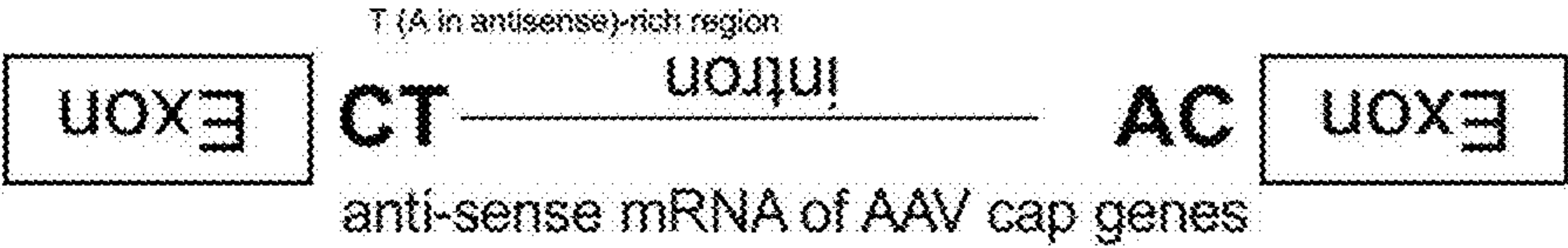


FIG. 6C

Splice donor

rh64	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2212
rh53	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh50	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh52	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.39	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh51	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh57	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh61	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh49	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh58	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
AAV7	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2214
cy.2	TGTTTACTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2214
rh62	TGTTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2214
rh48	TGTTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2214
rh55	TGTTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2214
rh54	TGTTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2214
rh60	TGTTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2208
rh38	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.41	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.66	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.40	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.67	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.42	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.37	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh40	TACATATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
AAV10	AACTTATTCTGAGCCTCGCCCCCAATTGGTACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh.2	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh25	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.6	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
hu.17	TGTGTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh.16	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2196
rh.17	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2196
rh.18	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh.12	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2202
rh.14	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2202
rh.24	TACTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2202
bb.1	CACCTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
bb.2	CACCTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
rh.10	CACCTTATTCTGAGCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2217
pl.3	TGTATATAGCGAACCCTCGCCCCCAATTGGCACTCGTTAATTCAGCCGTAATCTGTAA--	2196

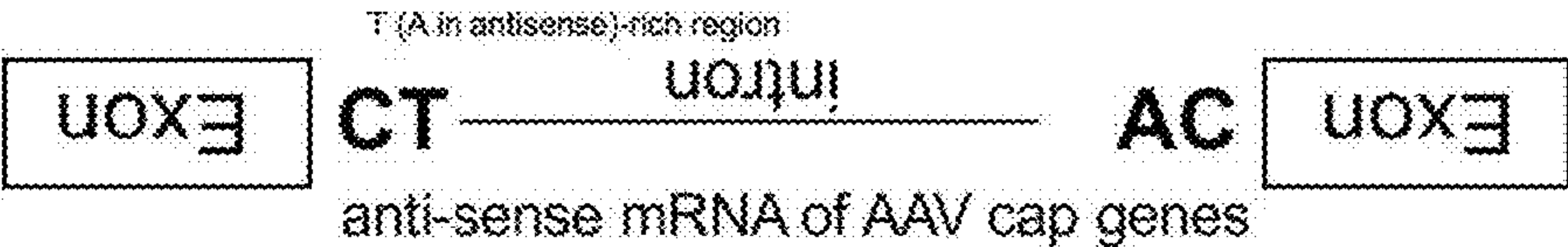


FIG. 6D

Splice donor

pi.1	TGTATATAGTGAACCCCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2196
pi.2	TGTATATAGTGAACCCCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2196
AAV8	CGTGTACTCTGAACCCCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2217
rh43	CGTGTACTCTGAACCCCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
rh1	TGTTTACTCTGAACCCCGCCCCCAATTGGTACTCGTTACCTCAGCCGTAATCTGTAA~	2214
rh.8	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
cy.5	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
rh.13	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
cy.3	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
cy.4	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
cy.6	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
rh.22	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2187
rh.19	GCTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
rh.23	TACTTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2058
rh.36	TGTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2190
rh.37	TGTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2190
rh.35	TGTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2190
AAV1	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
hu.48	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
hu.43	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2214
hu.46	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
hu.44	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
AAV6	ACTTTATAGCGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2211
hu.34	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.35	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
AAV2	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.52	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.51	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.47	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.45	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.56	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.57	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2205
hu.63	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.64	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.58	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.49	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.13	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.29	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.28	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.24	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208
hu.21	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACTCGTTACCTCAGCCGTAATCTGTAA~	2208

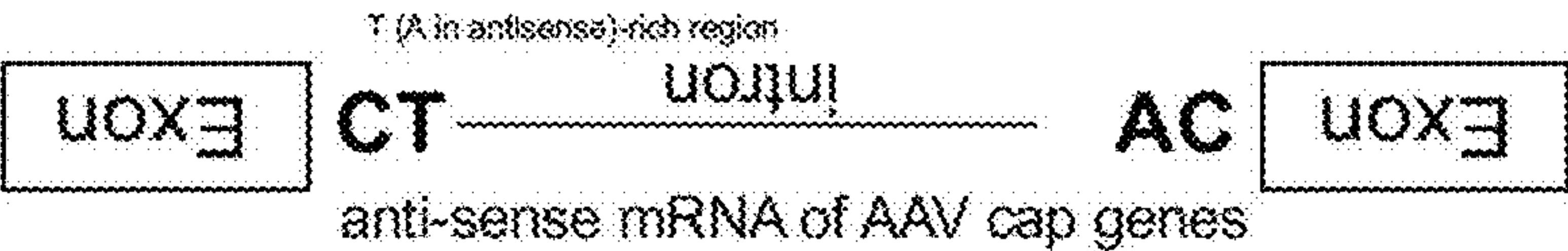


FIG. 6E

Splice donor

hu.22	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.20	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.19	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.27	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.23	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.15	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.16	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.18	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.7	CGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.60	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.25	CGTGTACTTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.4	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.1	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.2	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2209
hu.3	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2211
hu.9	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.11	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.10	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2208
hu.55	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2205
hu.53	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2205
hu.54	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2205
hu.61	-----	
AAV3A	TGTTTATAGTGAACCTCGCCCCCTATTGGAAACCCGGTATCTCAACCGAAACTTGTGA--	2211
AAV3B	TGTTTATAGTGAACCTCGCCCCCTATTGGAAACCCGGTATCTCAACCGAAACTTGTAA--	2211
AAV13	TGTGTATTCAGAGCCCTCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2202
ch.5	TGTTTATTCCTGAACCCCGCCCCCTATTGGCACTCGTTATCTTAAACCGAAACTTGTAA--	2208
AAV9	TGTATATAGTGAACCCCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2211
AAV9hu.14	TGTATATAGTGAACCCCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2211
hu.31	TGTATATAGTGAACCCCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2211
hu.32	TGTATATAGTGAACCCCGCCCCCAATTGGCACCAGATACCTGAACTCGTAATCTGTAA--	2211
rh.32	GAAGTATACAGAGCCCGCGGGTTATTGGCTCTCGTTATCTGAACTAATCATTTTGTAA--	2202
rh.33	GAAGTATACAGAGCCCGCGGGTTATTGGCTCTCGTTATCTGAACTAATCATTTTGTAA--	2202
rh.34	GAAGTATACAGAGCCCGCGGGTTATTGGCTCTCGTTATCTGAACTAATCATTTTGTAA--	2202
AAV11	GAAGTATACAGAGCCCGCGGGTTATTGGCTCTCGTTATCTGAACTAATCATTTTGTAA--	2202
AAV12	CAACTACACGAACCTCCGGGCTATTGGGCTCCCGTTTCTCAACCCACCACCTTGTAA--	2229
AAV4	GAAATACACTGAGCCTAGGGCTATCGGTAACCGGCTACCTCACCCACCACCTGTAA--	2205
BAAV	AGCCTACAAAGAGCCCGAGGGCCATTGGATCCCGATACCTCAACCAACCACCTCTAG--	2211
Avian	ATCCTATTCCGAACCTCCGTCCCATCGGTAACCGGTTACTTACCAAACTCTGTAA--	2232
AAV5	GGAATACAGAAACCACCAGACCTATCGGGAACCCGATACCTTAACCGACCCCTTTAA--	2175
Go.1	GGAATACAGAAACCACCAGACCTATCGGGAACCCGATACCTCAACCGACCCCTTTAA--	2181
Goose	TGGATATGTAGAAGATAGATTGATTGGAAACCAGATACCTAACTCAAAAATCTGTAA--	2199

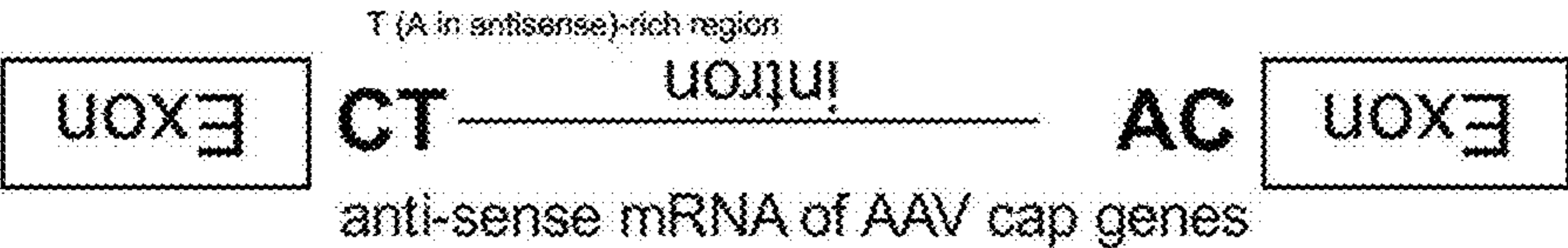


FIG. 6F

ATGGCTGCTGACGGTTATCTTCCAGATTGGCTCGAGGACAACCTTTCTGAAGGC
 ATTCGTGAGTGGTGGGCTCTGAAACCTGGAGTCCCTCAACCCAAAGCGAACCA
 ACAACACCAGGACAACCGTCGGGGTCTTGTGCTTCCGGGTACAAATACCTCG
 GACCCGGTAACGGACTCGACAAAGGAGAGCCGGTCAACGAGGCGGACGCGGC
 AGCCCTCGAACACGACAAAGCTTACGACCAGCAGCTCAAGGCCGGTGACAACC
 CGTACCTCAAGTACAACCACGCCGACGCCGAGTTTCAGGAGCGTCTTCAAGAA
 GATACGTCTTTTGGGGGCAACCTTGGCAGAGCAGTCTTCCAGGCCAAAAAGAG
 GATCCTTGAGCCTCTTGGTCTGGTTGAGGAAGCAGCTAAAACGGCTCctggaaaga
 agaggccctgtagatcagtcctcagggaaccggactcatcatctgggtgttggcaaatcgggcaaacagccctgccaga
 aaaagactaaatttcggtcagactggcgactcagagtcagtcaccagaccctcaaccctcgggagaaccaccagcag
 cccccacaagtttgggatctaatacaatggcttcaggcgggtggcgccaccaatggcagacaataacgaggggtgccga
 tggagtgggttaattcctcaggaaattggcattggcattcccaatggctggggcgacagagtcattaccaccagcacca
 gaacctgggcccctgccacttacaacaaccatctctacaagcaaatctccagccaatcaggagcttcaaaccgacaa
 ccactactttggctacagcacccttgggggtattttgactttaacagattccactgccacttctaccacgtgactggca
 ggcactcatttaacaacaacttggggattccggcccaagaaactcagcttcaagctcttcaacatccaagttaagagg
 tcacgcagaacgatggcacgacgactattgccaaataacctiaccagcacgggttcaagtggttacggactcggagtaic
 agctcccgtagctgctcgggtcggcgccaccaaggctgtctcccgccgtttccagcggacgtcttcatggtccctcagtat
 ggatacctcaccttgaacaacgggaagtcaagcgggtgggacgctcatctttactgcttggagtacttcccttcgcaga
 tgctaaggactggaaataacttccaattcagctataccttcagaggatgtacctttcacagcagctacgctcacagcca
 gagtttggatcgcttgatgaatcctcttattgatcagtaictgtactacctgaacagaaacgcaagggaacaacctctggaa
 caaccaaccaatcacggctgctttttagccaggctgggcctcagctatgtctttgcaggccagaaattggctacctgg
 gccctgctaccggcaacagagactttcaaagactgctaaccgacaacaacaacagtaactttccttggacagcggcc
 agcaaatatcatctcaatggcccgactcgttggatgaatccaggaccagctatggccagtcacaaggacgatgaag
 aaaaattttccctatgcacggcaatctaataatttggcaagaaggggacaacggcaagtaacgcagaattagataat
 gtaatgattacggatgaagaagagattcgtaccaccaatcctgtggcaacagagcagtatggaactgttggcaata
 acttgcagagctcaaatacagctcccacgactagaactgtcaatgatcagggggcccttacctggcatggtgtggcaa
 gatcgtgacgtgtaccttcaaggacctatctgggcaaaagattcctcacacggatggacactttcatccttctcctctgatg
 ggaggctttggactgaaacatccgcctctcacaatcatgatcaaaaatactccgggtacCGGCAAATCCTCC
 GACGACTTTCAGCCCGGCCAAGTTTGCTTCATTTATCACTCAGTACTCCACTGG
 ACAGGTCAGCGTGGAATTGAGTGGGAGctacagaaagaaaaacagcaaacgttggaaatccag
 agattcagtacacttccaactacaacaagtcgtttaatgtggactttactgtagacactaatgggtgtttatagtgaacctcg
 ccttatggaaacccggatctcacACGAAACTTGTA

FIG. 7A

ATGGCTGCTGACGGTTATCTTCCAGATTGGCTCGAGGACAACCTTTCTGAAGGC
 ATTCGTGAGTGGTGGGCTCTGAAACCTGGAGTCCCTCAACCCAAAGCGAACCA
 ACAACACCAGGACAACCGTCGGGGTCTTGTGCTTCCGGGTACAAATACCTCG
 GACCCGGTAACGGACTCGACAAAGGAGAGCCGGTCAACGAGGCGGACGCGGC
 AGCCCTCGAACACGACAAAGCTTACGACCAGCAGCTCAAGGCCGGTGACAACC
 CGTACCTCAAGTACAACCACGCCGACGCCGAGTTTCAGGAGCGTCTTCAAGAA
 GATACGTCTTTTGGGGGCAACCTTGGCAGAGCAGTCTTCCAGGCCAAAAGAG
 GATCCTTGAGCCTCTTGGTCTGGTTGAGGAAGCAGCTAAAACGGCTCctggaaaga
 agagggcctgtagatcagtcctcaggaaccggactcatcatctgggtgttgcaaatcgggcaaacagcctgccaga
 aaaagactaaatttcggtcagactggcgactcagagtcagtcaccagaccctcaacctctcggaagaaccaccagcag
 cccccacaagtttgggatctaatacaatggcttcaggcggtggcgccaccaatggcagacaataacgagggtgccga
 tggagtggttaattcctcaggaattggcattgcgattcccaatggctggcgacagagtcaccaccagcacca
 gaacctggggccctgccacttacaacaaccatctctacaagcaaatctccagccaatcaggagcttcaaacgacaa
 ccactactttggctacagcacccttgggggtattttgactttaacagattccactgccacttctaccacgtagctggca
 ggcactcattacaacaactggggattccggcccaagaaactcagcttcaagctcttcaacatccaagttaaagagg
 tcacgcagaacgatggcacgacgactattgccaatcacttaccagcacgggttcaagtggttacggactcggaglatc
 agctcccgtagctgctcgggtcggcgccaccaaggctgctcccgccgttccagcgagctcttcatggctccctcaglat
 ggatactcaccctgaacaacgggaagtcagcggtgggacgctcatctttactgacctggaglatctcccttcgcaga
 tgcataaggactggaaataacttccaattcagctatacttcgaggatglatcttccacagcagctacgctcacagcca
 gagtttggatcgcttgatgaatcctcttattgatcaglatctgtactacctgaacagaacggcaaggaacaacctctggaa
 caaccaaccaatcacggctgcttttagccaggctgggcctcagctatgtctttgcaggccagaaattggctacctgg
 gccctgctacgggcaacagagacttcaagagctgctaacgacaacaacaacaglaacttcccttggacagcgcc
 agcaaatatcatcicaatggcccgactccctgggtgaatccaggaccagctatggccagtcacaaggacgatgaag
 aaaaattttccctatgcacggcaatctaataatttggcaagaagggacaacggcaagtaacgcagaattagataat
 gtaatgattacggatgaagaagagattcgtaccaccaatcctgtggcaacagagcaglatggaaactgtggcaata
 acttgcagagctcaaatcagctcccacgactagaactgtcaatgatcagggggcccttacCTGGCATGGTGT
 GGCAAGATCGTGACGTGTACCTTCAAGGACCTATCTGGGCAAAGATTCTCACA
 CGGATGGACACTTTCATCCTTCTCCTCTGATGGGAGGCTTTGGACTGAAACATC
 CGCCTCCTCAAATCATGATCAAAAATACTCCGGTACCGGCAAATCCTCCGACGA
 CTTTCAGCCCGGCCAAGTTTGCTTCATTTATCACTCAGTACTCCACTGGACAGG
 TCAGCGTGGAATTGAGTGGGAGctacagaaagaaaacagcaaacgttgggaatccagagattcag
 tacacttccaactacaacaagtctgttaattgtggactttactgtagacactaatggtgtttatagtgaaacctcgccctattg
 gaacccggtatctcacACGAAACTTGTA

FIG. 7B

Splice acceptor

rh64	CAGCCT--AGATCGTSTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCAGAAAGCCAGAA-AT	2097
rh53	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh50	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh52	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.39	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh51	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh57	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh61	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh49	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh58	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
AAV7	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
cy.2	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
rh62	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
rh48	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
rh55	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
rh54	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2099
rh60	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2093
rh38	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.41	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.66	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.40	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.67	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.42	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.37	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh40	CAGCCTGGAGATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAAAT	2102
AAV10	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh.2	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh25	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.6	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
hu.17	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh.16	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2081
rh.17	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2081
rh.18	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh.12	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2087
rh.14	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2087
rh.24	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2087
bb.1	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
bb.2	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
rh.10	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCAGAGAT	2102
pi.3	CAGCCTGGAGATTGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAATCCCGAGAT	2081
pi.1	CAGCCTGGAGATTGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAATCCCGAGAT	2081
pi.2	CAGCCTGGAGATTGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAATCCCGAGAT	2081
AAV8	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCCGAGAT	2102
rh43	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAAGCCCGAGAT	2096
rh1	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAATCCAGAGAT	2099
rh.8	CAGCCTGGAAATCGAGTGGGAGTTGCAGAAAGGAGAAACAGCAAGGCGCTGGAATCCAGAGAT	2096

T (A in antisense)-rich region



anti-sense mRNA of AAV cap genes

FIG. 8A

Splice acceptor

cy.5	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
rh.13	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
cy.3	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
cy.4	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
cy.6	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
rh.22	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2072
rh.19	CAGCGTGGAGATCGAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAGAT	2093
rh.23			
rh.36	CAGCGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAAAT	2075
rh.37	CAGCGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAAAT	2075
rh.35	CAGCGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCAGAAAT	2075
AAV1	GAGTGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2096
hu.48	GAGTGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2096
hu.43	GAGTGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2099
hu.46	GAGTGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2096
hu.44	GAGTGTGGAAATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2096
AAV6	GAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2096
hu.34	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.35	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
AAV2	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.52	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.51	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.47	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.45	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.56	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.57	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2090
hu.63	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.64	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.58	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.49	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.13	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.29	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.28	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.24	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.21	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.22	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.20	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.19	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.27	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.23	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.15	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.16	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093
hu.18	CAGCGTGGAGATTCAGTGGGAA	TTGCAGAAAGAAAACAGCAAAACGCTGGAAATCCCGAAGT	2093

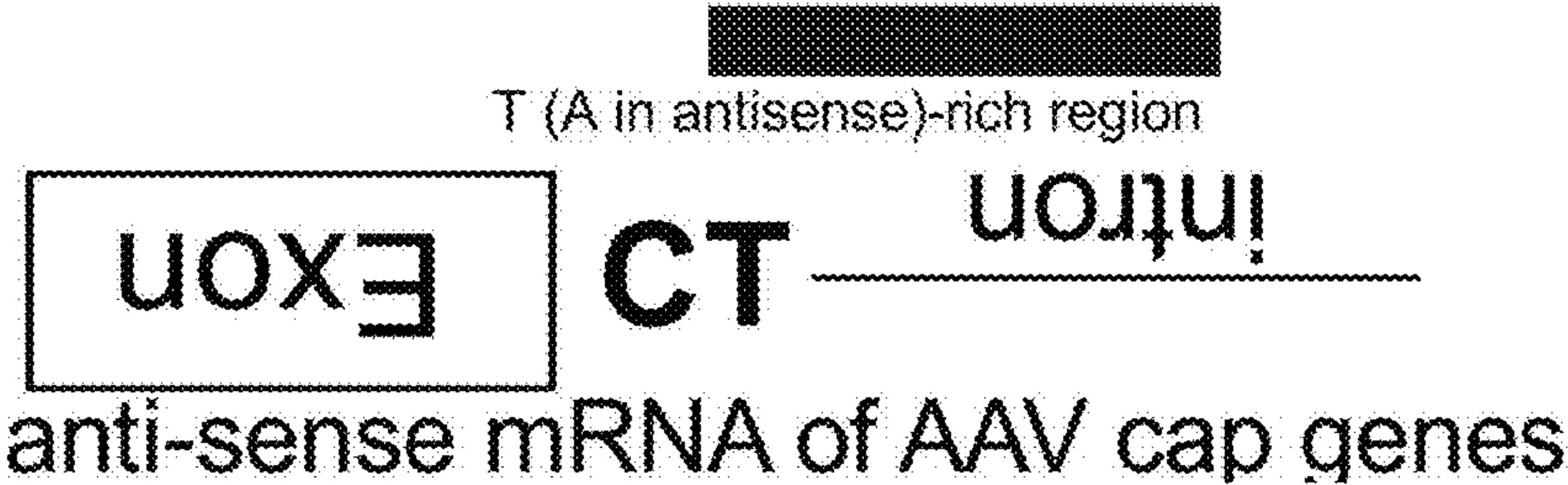
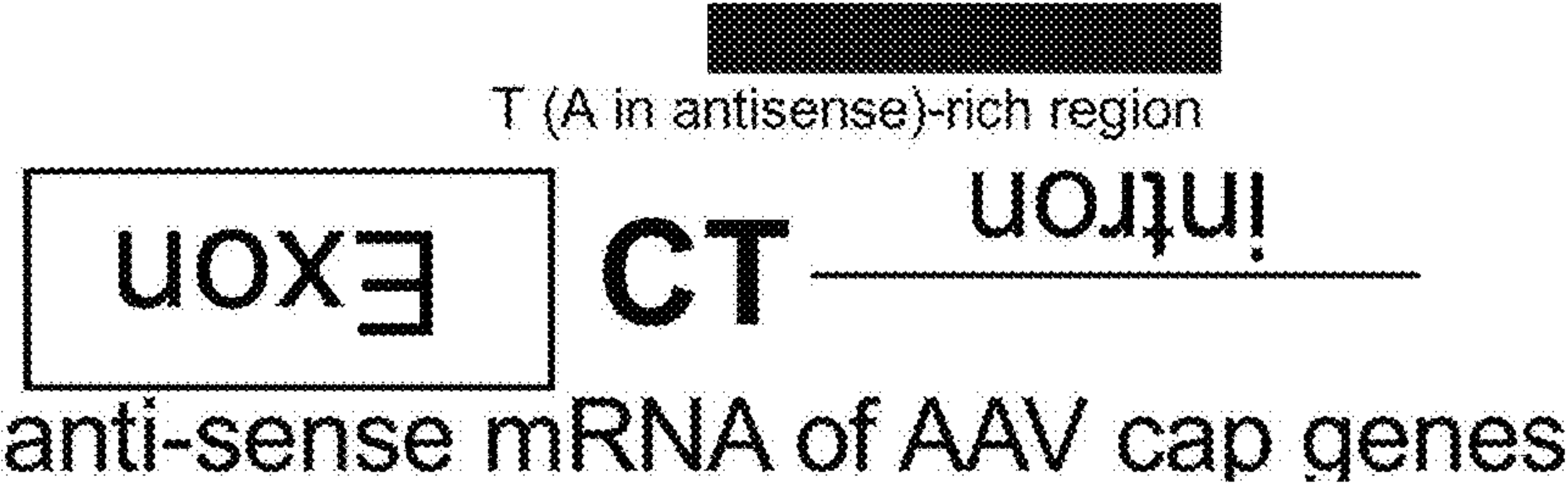


FIG. 8B

Splice acceptor

hu.7	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.60	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.25	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.4	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.1	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.2	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.3	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
hu.9	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.11	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.10	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
hu.55	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2090
hu.53	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2090
hu.54	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2090
hu.61			
AAV3A	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
AAV3B	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
AAV13	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2087
ch.5	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2093
AAV9	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
AAV9hu.14	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
hu.31	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
hu.32	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
rh.32	CGCTGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2087
rh.33	CGCTGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2087
rh.34	CGCTGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2087
AAV11	CGCTGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2087
AAV12	TGCCGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2114
AAV4	GTCCGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2090
BAAV	GGCTGTTTCAGATTGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2096
Avian	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2117
AAV5	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2060
Go.1	CAGCGTGGAGATCGAATGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2066
Goose	TACACTAGACATGCTGTGGGCA	TTGCAGAAAGCAGAACAGCAAAACGCTGGAATCCCGAAAT	2084



anti-sense mRNA of AAV cap genes

FIG. 8C

Splice donor

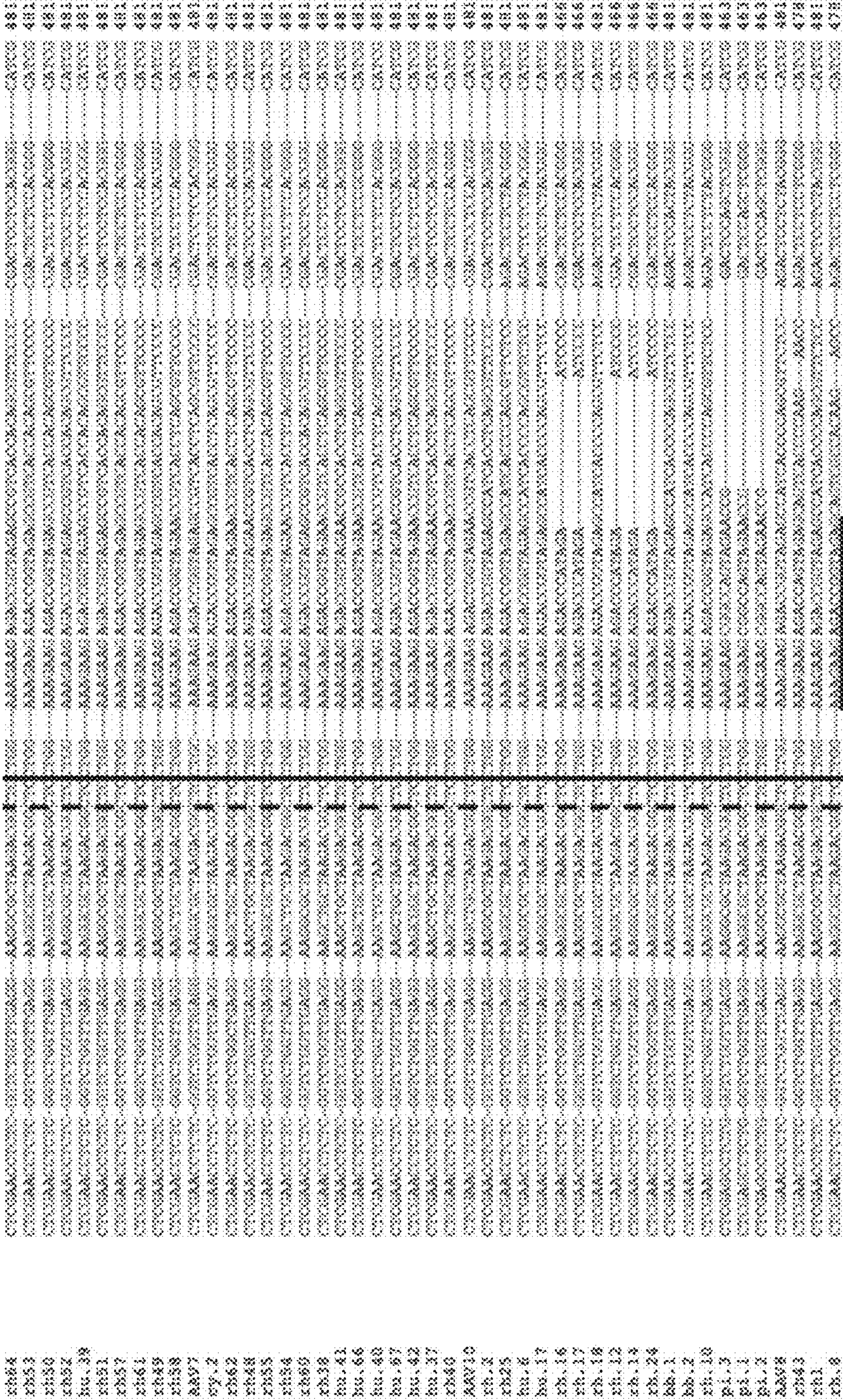


FIG. 8D

Splice donor

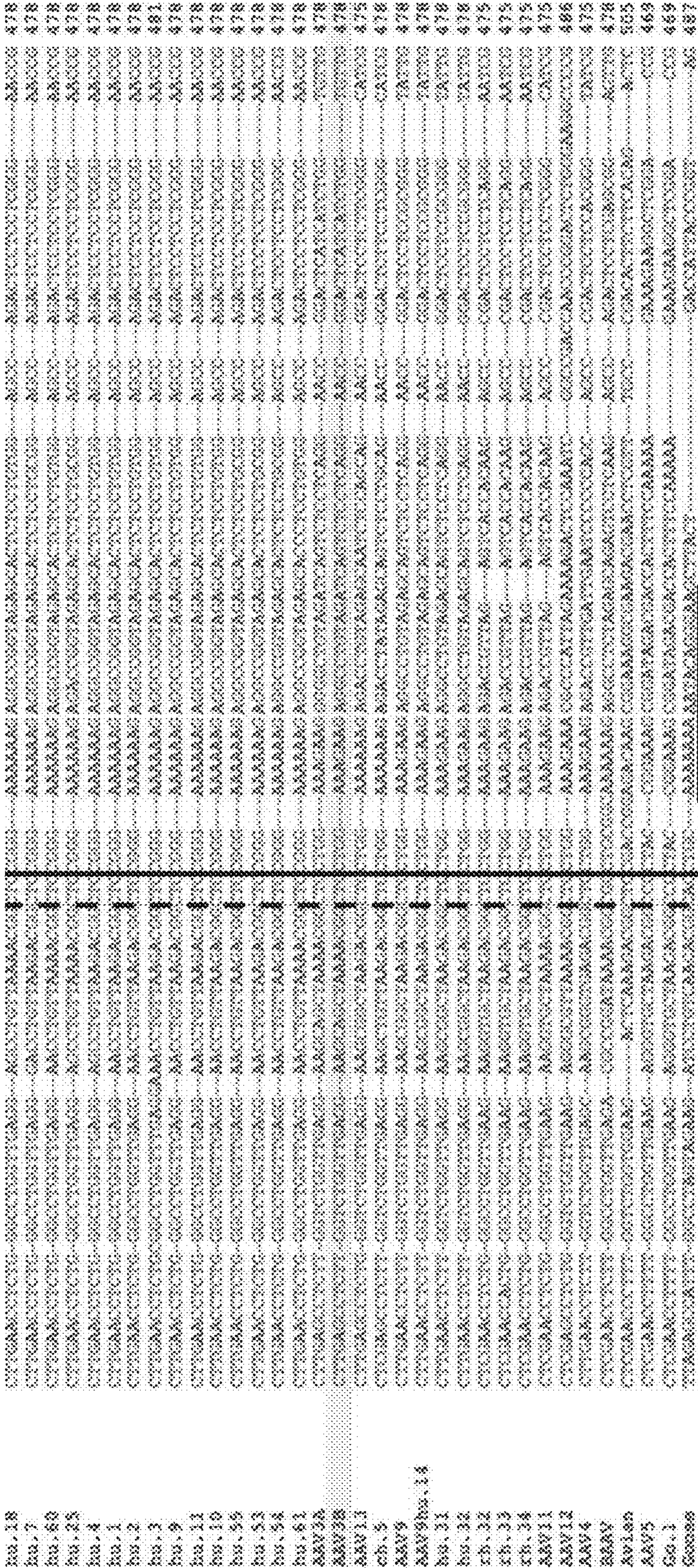


FIG. 8F

Splice donor

rh64	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh53	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh50	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh52	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
hu.39	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh51	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh57	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh61	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh49	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
rh58	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATTTG	1862
AAV7	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
cy.2	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh62	GGAGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh48	GGAGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh55	GGAGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh54	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh60	GGAGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1859
rh38	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.41	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.66	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.40	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.67	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.42	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
hu.37	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
rh40	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
AAV10	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
rh.2	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGGCCCATCTG	1862
rh25	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
hu.6	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
hu.17	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
rh.16	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1841
rh.17	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1841
rh.18	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
rh.12	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1847
rh.14	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1847
rh.24	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1847
bb.1	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
bb.2	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
rh.10	GGAGCCCTTACCTGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCTATCTG	1862
pi.3	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1841
pi.1	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1841
pi.2	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1841
AAV8	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1862
rh43	GGGGCCCTTACCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1856
rh1	GGAGTTATTCCTGGTATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGGCCCTATTTG	1859
rh.8	GGGTTGATTCCTGGCATGGTCTGGCAGAAATACAGACGTTGTACCT-GCAGGGTCCCATCTG	1856



FIG. 9A

Splice donor

cy.5	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
rh.13	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
cy.3	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
cy.4	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
cy.6	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
rh.22	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1832
rh.19	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1853
rh.23	GGGGCTCTGGCCGGCATGGTCTGGCAGAAACCGGACGTTGTACCT-GCAGGGTCCCATCTG	1853
rh.36	GGAGCACTGGCTGGCATGGTCTGGCAGAAACCGGACGTTGTATCT-GCAGGGTCCCATCTG	1835
rh.37	GGAGCACTGGCTGGCATGGTCTGGCAGAAACCGGACGTTGTATCT-GCAGGGTCCCATCTG	1835
rh.35	GGAGCACTGGCTGGCATGGTCTGGCAGAAACCGGACGTTGTATCT-GCAGGGTCCCATCTG	1835
AAV1	GGAGCATTAGCTGGCATGGTCTGGCAAGATAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1856
hu.48	GGAGCATTAGCTGGCATGGTCTGGCAAGATAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1856
hu.43	GGAGCATTAGCTGGCATGGTCTGGCAAGATAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1859
hu.46	GGAGCATTAGCTGGCATGGTCTGGCAAGATAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1856
hu.44	GGAGCATTAGCTGGCATGGTCTGGCAAGATAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1856
AAV6	GGAGCCTTAGCTGGATGGTCTGGCAAGACAGAGACGTTGTACCT-GCAGGGTCCCATTTG	1856
hu.34	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.35	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
AAV2	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.52	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.51	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.47	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.45	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT-TCAGGGGCCCCATCTG	1853
hu.56	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.57	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1850
hu.63	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.64	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.58	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.49	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.13	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.29	GGCCTTCTTTCAGGCATGGTCTGGCAAGACAGAGACGTTGTACCT-GCAGGGGCCCCATTTG	1853
hu.28	GGCCTTCTTTCAGGCATGGTCTGGCAAGACAGAGACGTTGTACCT-GCAGGGGCCCCACTTG	1853
hu.24	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.21	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.22	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.20	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.19	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.27	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.23	GGCCTTCTTTCAGGCATGGTCTGGCAGGACAGAGACGTTGTACCT-GCAGGGGCCCCATCTG	1853
hu.15	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853
hu.16	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853
hu.18	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853
hu.7	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853
hu.60	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853
hu.25	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGATGTGTACCT-TCAGGGACCCATTTG	1853
hu.4	GGAGCGTTAGCTGGTATGGTCTGGCAGGATCGAGACGTTGTACCT-GCAGGGACCCATTTG	1853



FIG. 9B

Splice donor

hu.1	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1853
hu.2	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1853
hu.3	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1856
hu.9	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1853
hu.11	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1853
hu.18	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1853
hu.55	GGAGCATTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1850
hu.53	GGAGCATTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1850
hu.54	GGAGCATTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1850
hu.61	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1850
AAV3A	GGGGCGTTACCTGGCATGGTGTGGCAAGATCGTGACGTGTACCT-TCAAGGACCTATCTG	1856
AAV3B	GGGGCGTTACCTGGCATGGTGTGGCAAGATCGTGACGTGTACCT-TCAAGGACCTATCTG	1856
AAV13	GGAGCGTTACCTGGTATGGTGTGGCAGGATCGAGACGTGTACCT-GCAGGGAGCCCATTTG	1847
ch.5	CGAATCTTACCTGGAAATGGTGTGGCAGGACCGCCATGTCTATCT-TCAAGGTCCCATTTG	1853
AAV9	CGAATACTTTCGGGTATGGTGTGGCAGGACAGAGATGTGTACCT-GCAAGGAGCCCATTTG	1856
AAV9hu.14	CGAATACTTTCGGGTATGGTGTGGCAGGACAGAGATGTGTACCT-GCAAGGAGCCCATTTG	1856
hu.31	CGAATACTTTCGGGTATGGTGTGGCAGGACAGAGATGTGTACCT-GCAAGGAGCCCATTTG	1856
hu.32	CGAATACTTTCGGGTATGGTGTGGCAGGACAGAGATGTGTACCT-GCAAGGAGCCCATTTG	1856
rh.32	CGAGTGCTTTCCTGGCATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGGCCCAATTTG	1847
rh.33	CGAGTGCTTTCCTGGCATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGGCCCAATTTG	1847
rh.34	CGAGTGCTTTCCTGGCATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGGCCCAATTTG	1847
AAV11	CGAGTGCTTTCCTGGCATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGGCCCAATTTG	1847
AAV12	CGAATGTGTTTCGGGAATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGGCCCATTTG	1874
AAV4	GGAGCCGTTTCCTGGAAATGGTGTGGCAAAACAGAGACATTTACTA-CCAAGGTGCCATTTG	1850
BAAV	GGCGTGTTACCTGGGAATGGTGTGGCAGGACAGAGACATTTACTA-CCAAGGGCCCATTTG	1856
Avian	GGGGCGCTTTCCTGGGATGGTGTGGCAAAACAGAGACATTTACCCCTACAGGGAGCCCATTTG	1878
AAV5	GAAATCGTTTCCTGGCAGCGTGTGGATGGAGAGGGACGTGTACCT-CCAAGGAGCCATCTG	1823
Go.1	CGAGTGCTTTCCTGGCAGCGTGTGGATGGAGAGGGACGTGTACCT-CCAAGGAGCCATCTG	1829
Goose	GGAGCTTTACCTAGGAATGGTGTGGCAGAACAGGGCATATATATCT-GCAGGGAGCTATTTG	1844

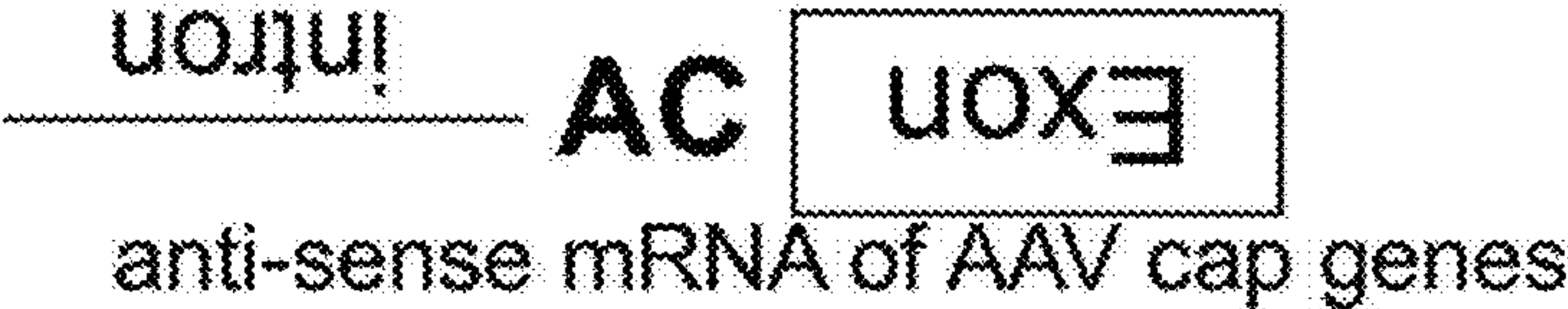


FIG. 9C

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aacaatggcagccaagccgtgggacgttcatcttcttactgcCTggaalatcttccctctcagatgctgagaacgggc
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gctgatgaatcctctcatcgaccaatccgtgtattACCTgaacagaactcaaaatcagtcgggaagtgcccaaac
aaggacttgcgtttagccgtgggtctccagctggcatgtctgttcagcccaaaaactggctACctggaccctgttatcg
gcagcagcgcttCTaaaacaaaaacagacaacaacacagcaatttACctggactgggtgcttcaaaatataa
cctcaatgggcgtgaatccatcatcaacccctggcactgctatggcctcacacaaagacgacgaagacaagttcttcc
catgagcgggtgcatgatttggaaaagagagcgccggagcttcaaaacaCTgcatlggacaatgtcatgattacag
acgaagaggaaatfaaagccactaaccctgtggccaccgaaagattgggaccgtggcagtcatttccagagca
gcagcacagaccctgcgaccggagatgtgcatgctatgggagcattACctggcatgggtgtggcaagatagagacg
tgtACctgcagggtcccatlggggccaaaattccctcacacagatggacactttacccgtctctcttatgggcggctt
ggactcaagaacccgcctctcagatccctcatcaaaaacacgcctgttccgtcgaaatccctccggcgagtttcagct
acaaagtgttgccttcatcaccacaatactccacaggacaagtgagtggtgaaatgaatgggagCTgcagaaag
aaaacagcaagcgctggaatcccgaaagtgagtcacatccaattatgcaaaatctgccaacgttgatttactgtgg
acaacaatggactttatactgagcctcggcccatlggcacccttACcttACccgtccctctgtaa

FIG. 10

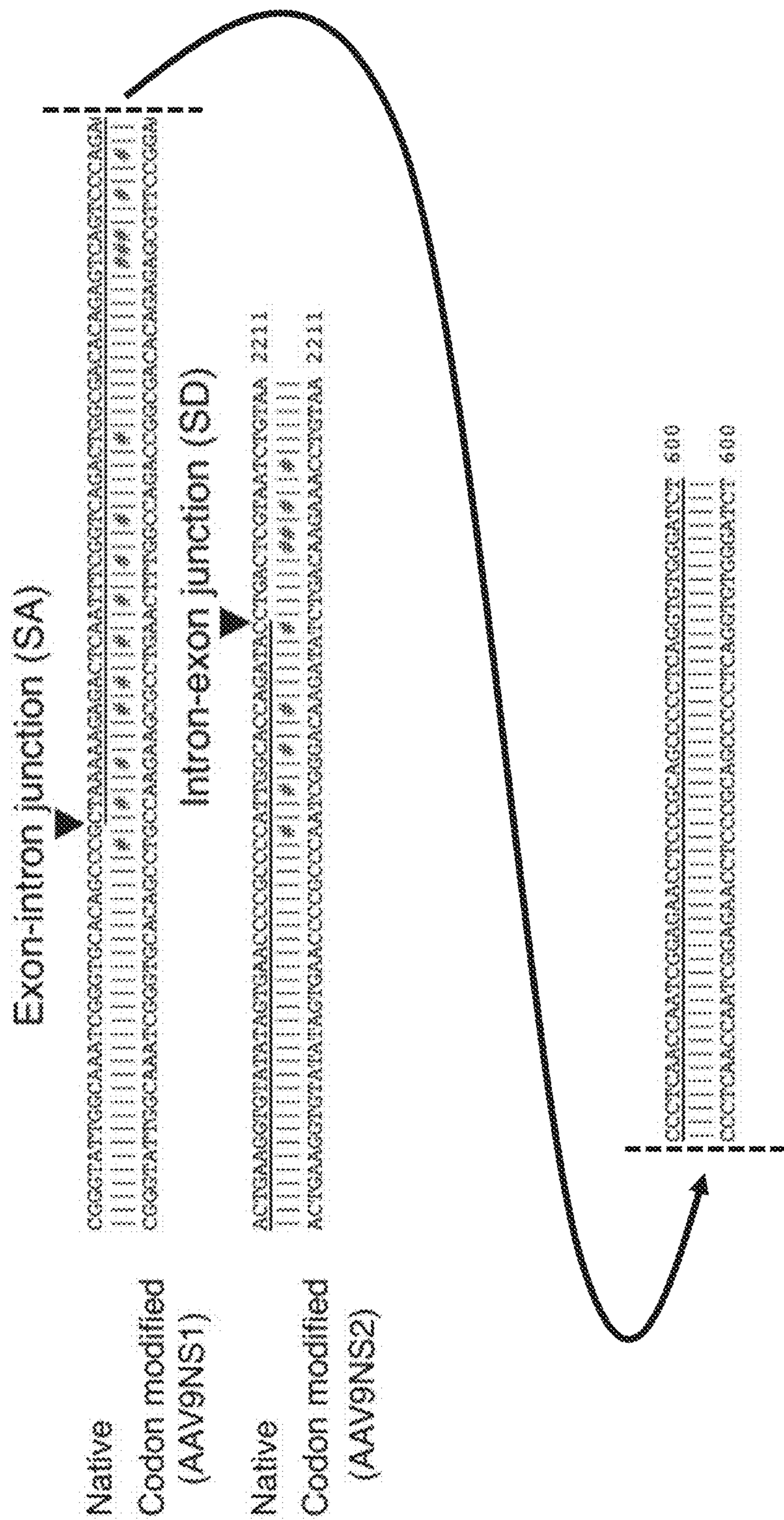


FIG. 11

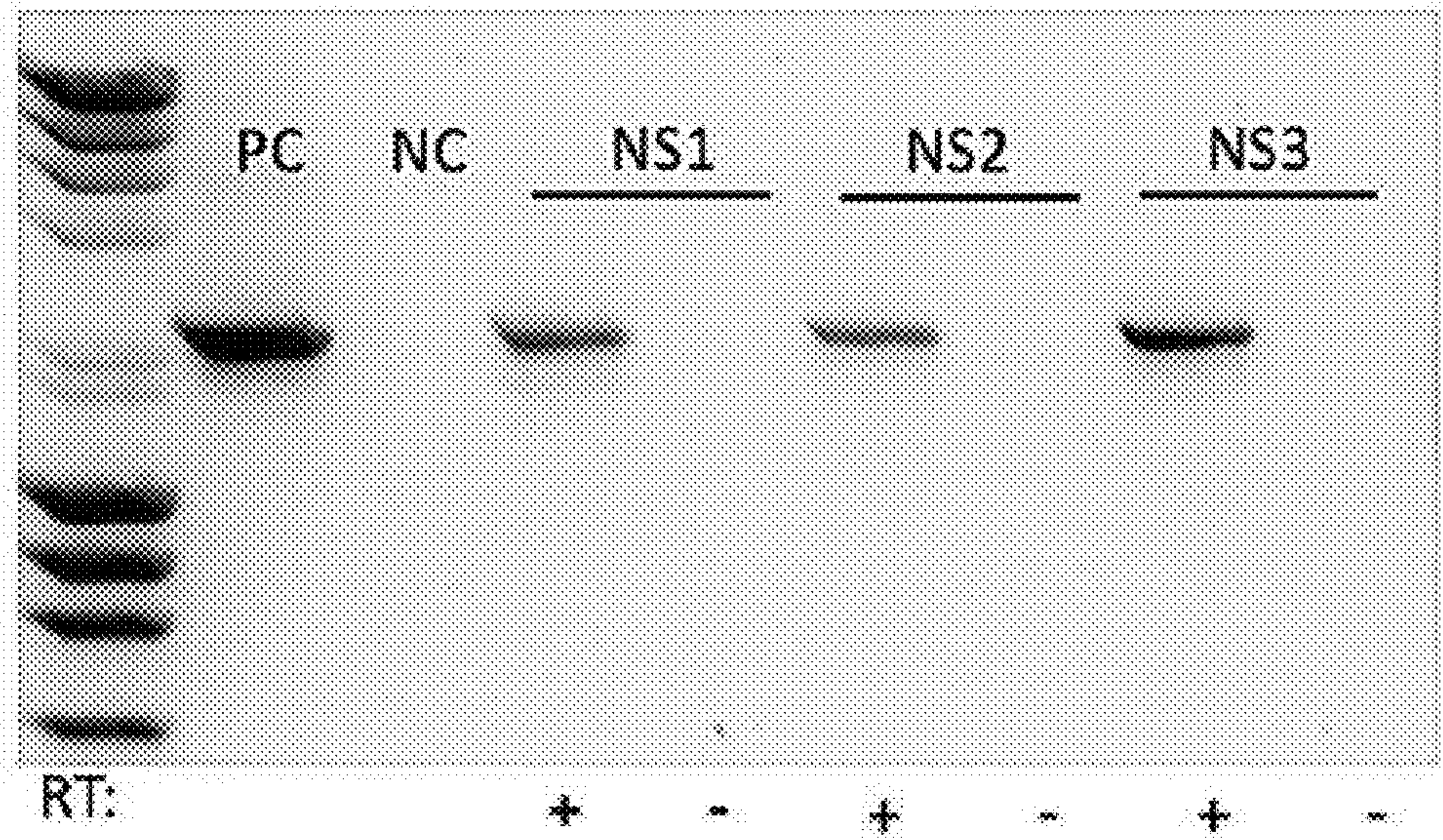


FIG. 12

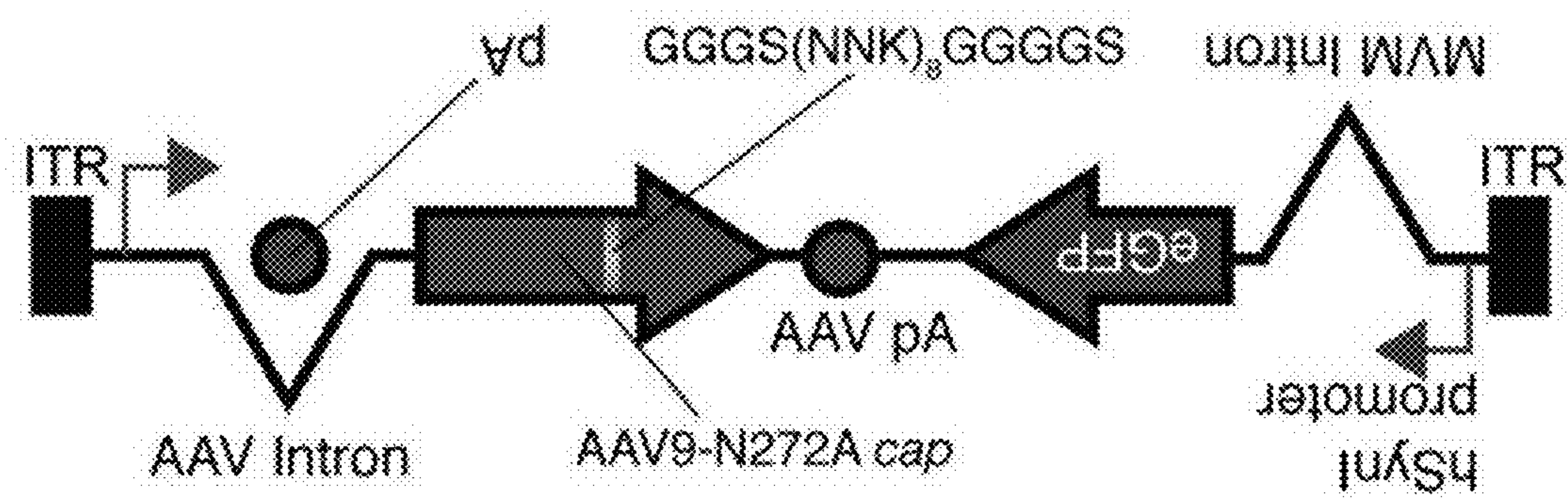


FIG. 13A

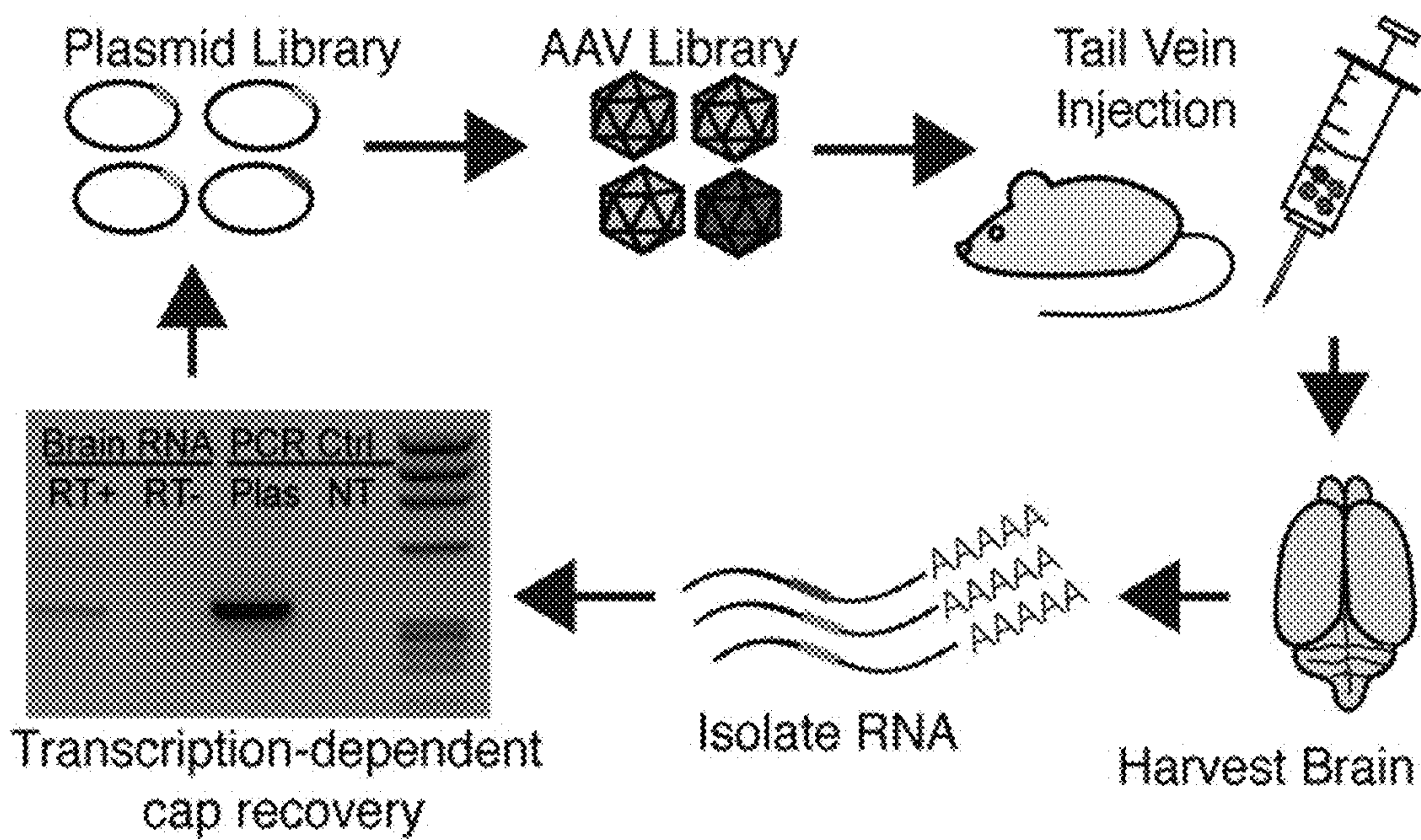
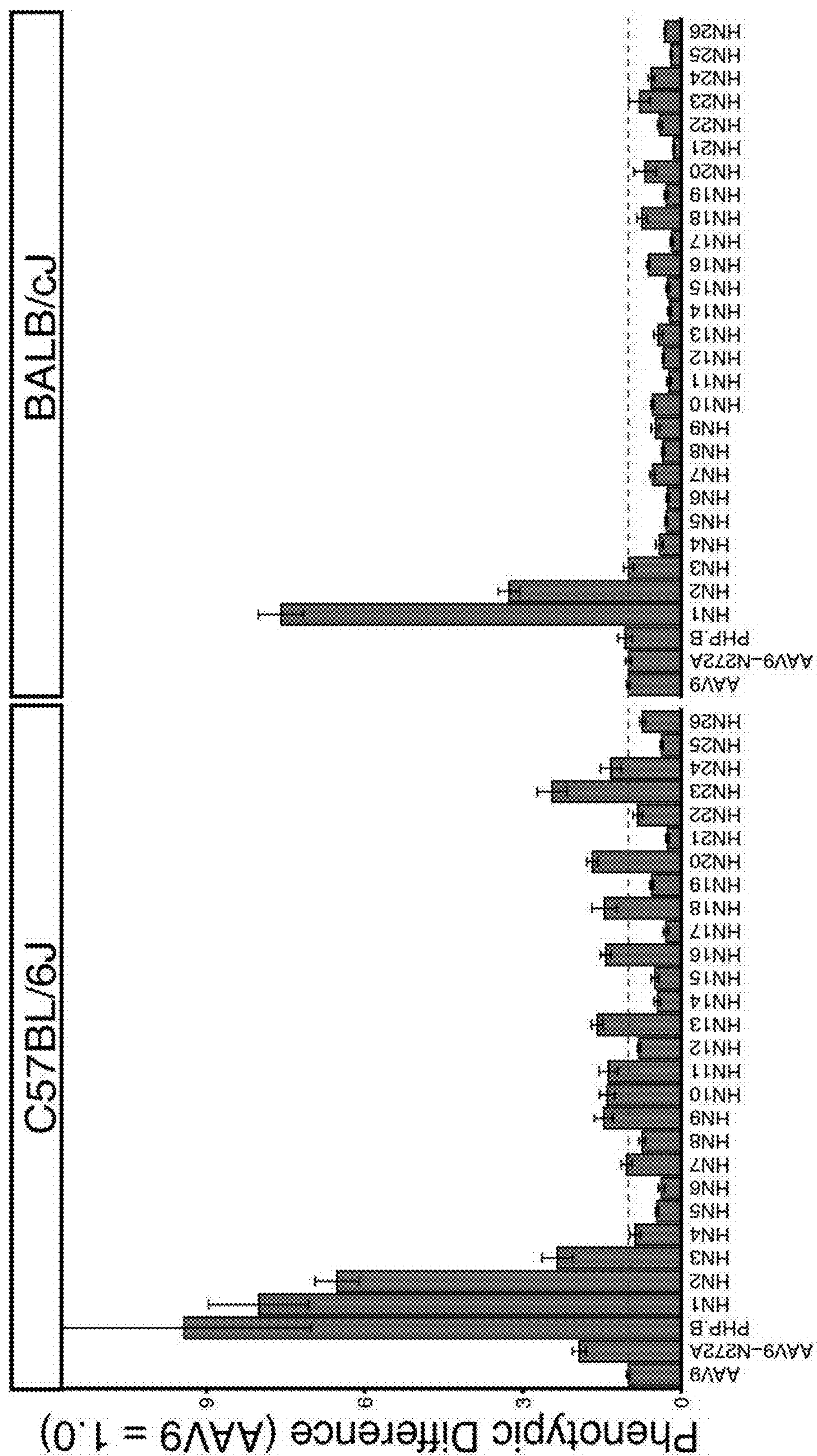


FIG. 13B



FIG. 14A

B
4
7
G
L

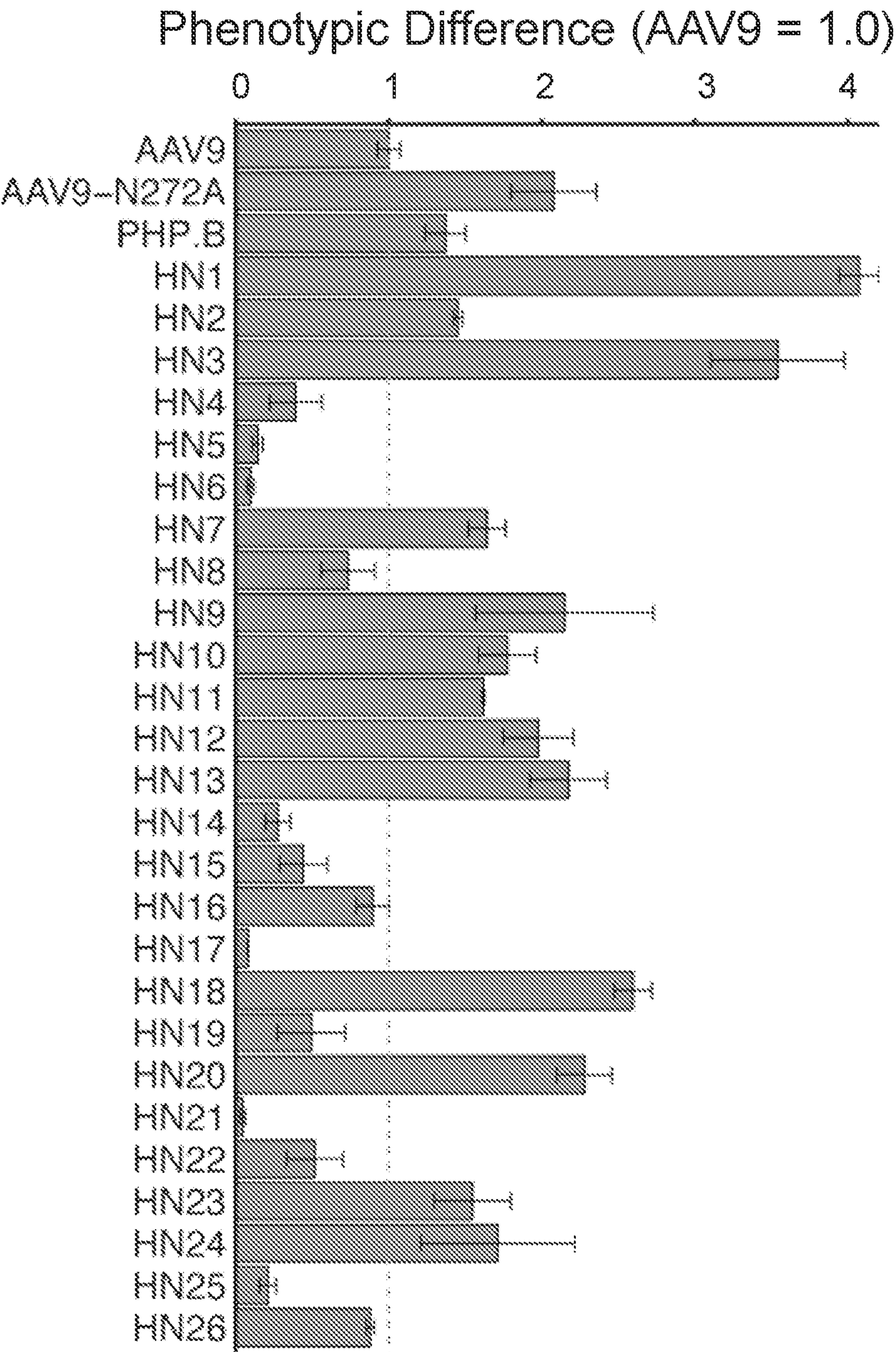


FIG. 14C

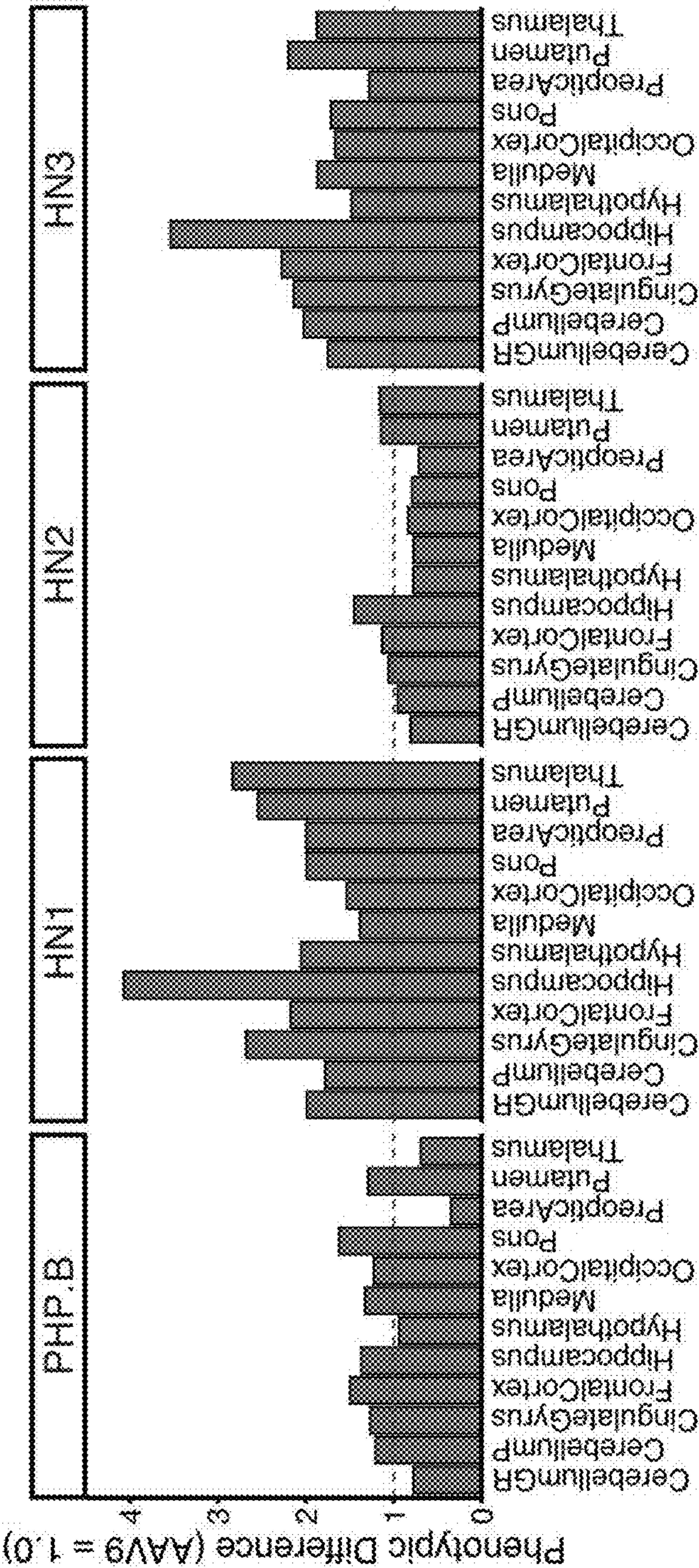


FIG. 14D

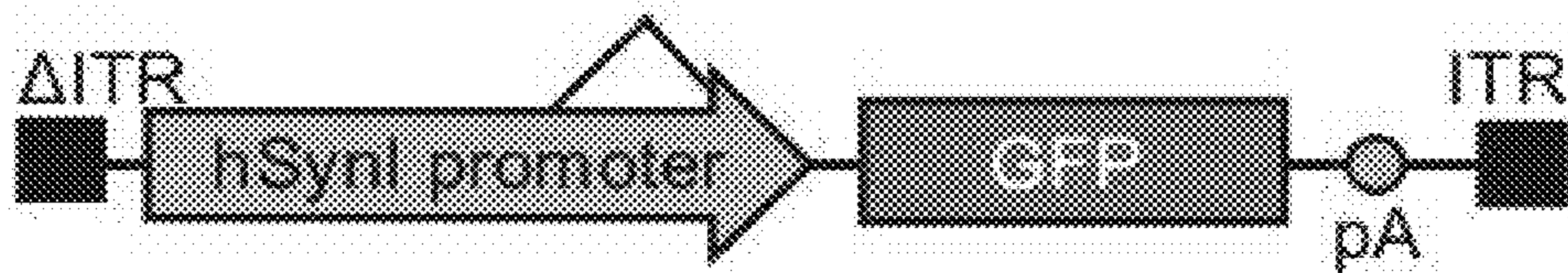


FIG. 15A

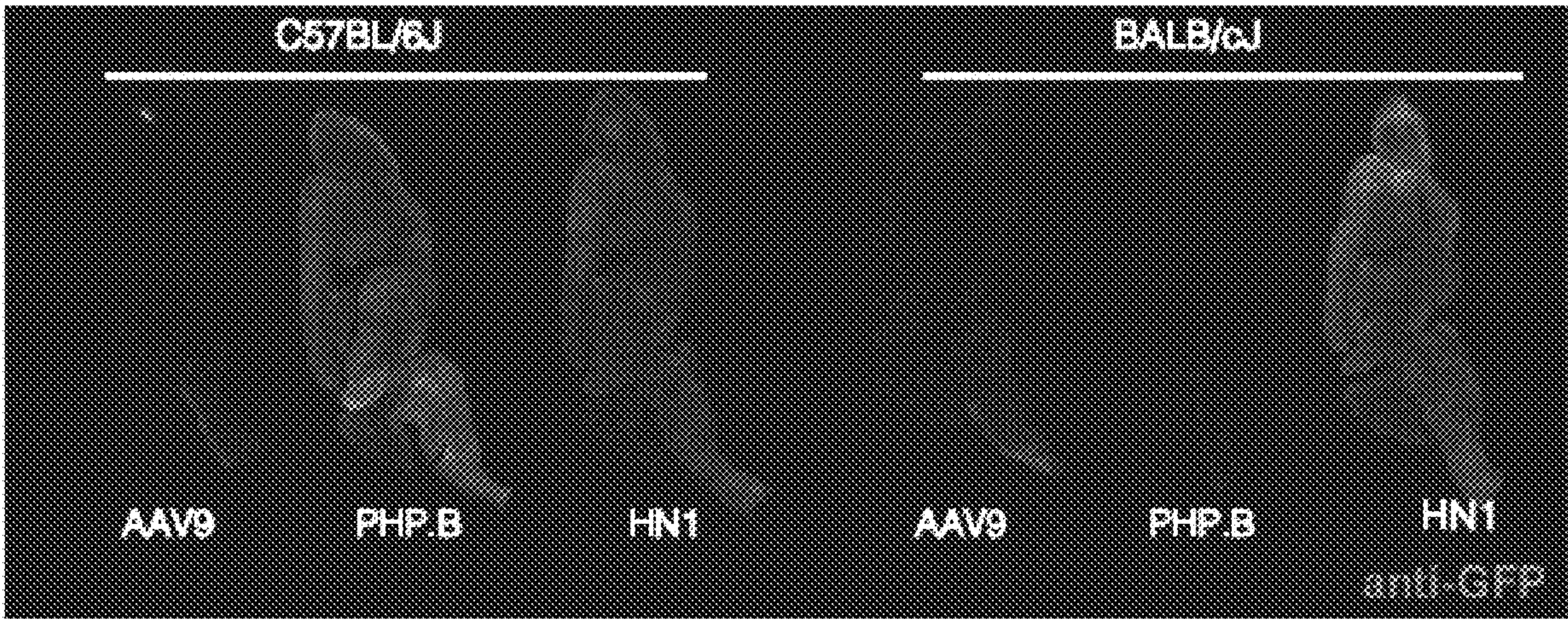


FIG. 15B

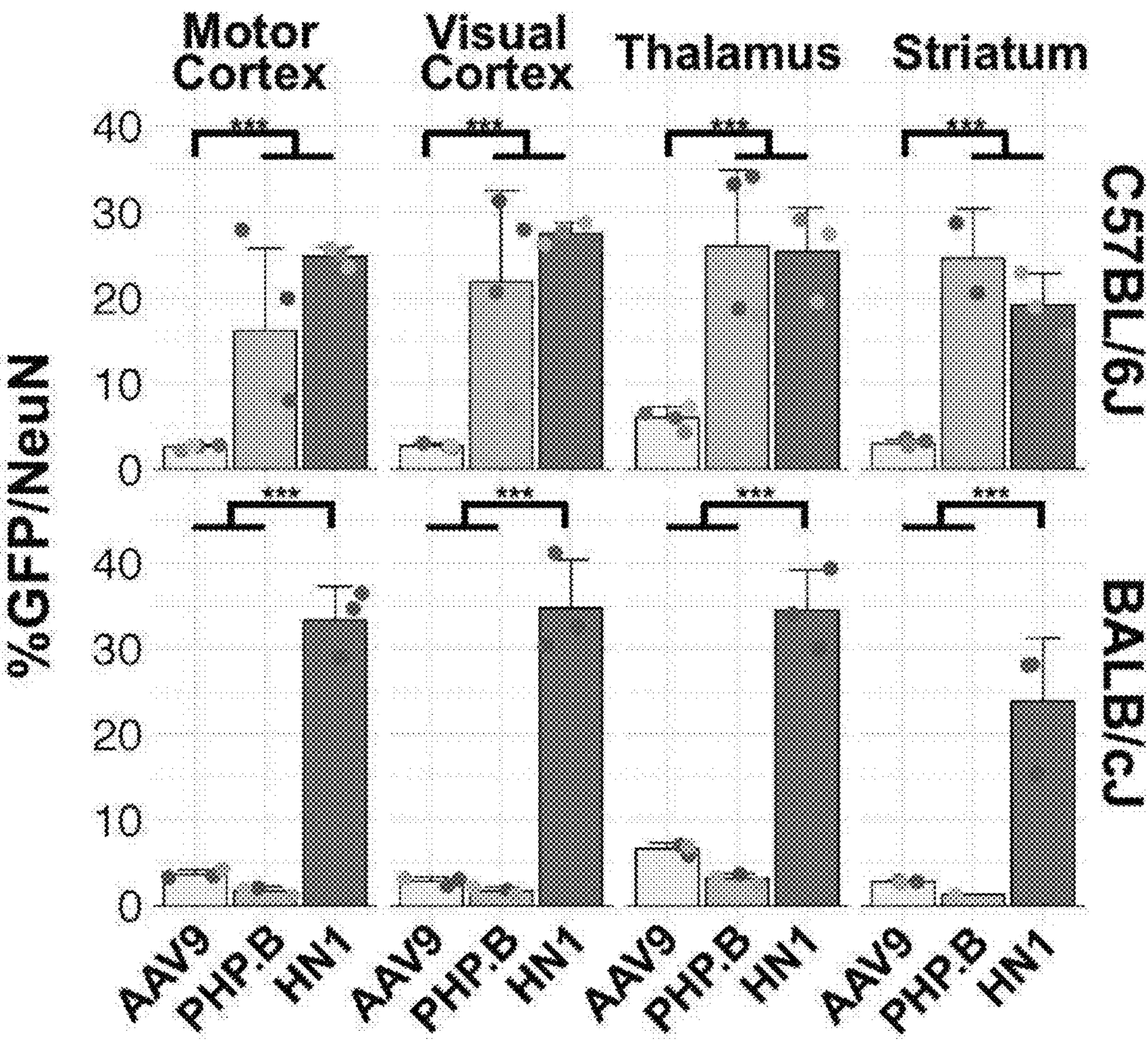


FIG. 15C

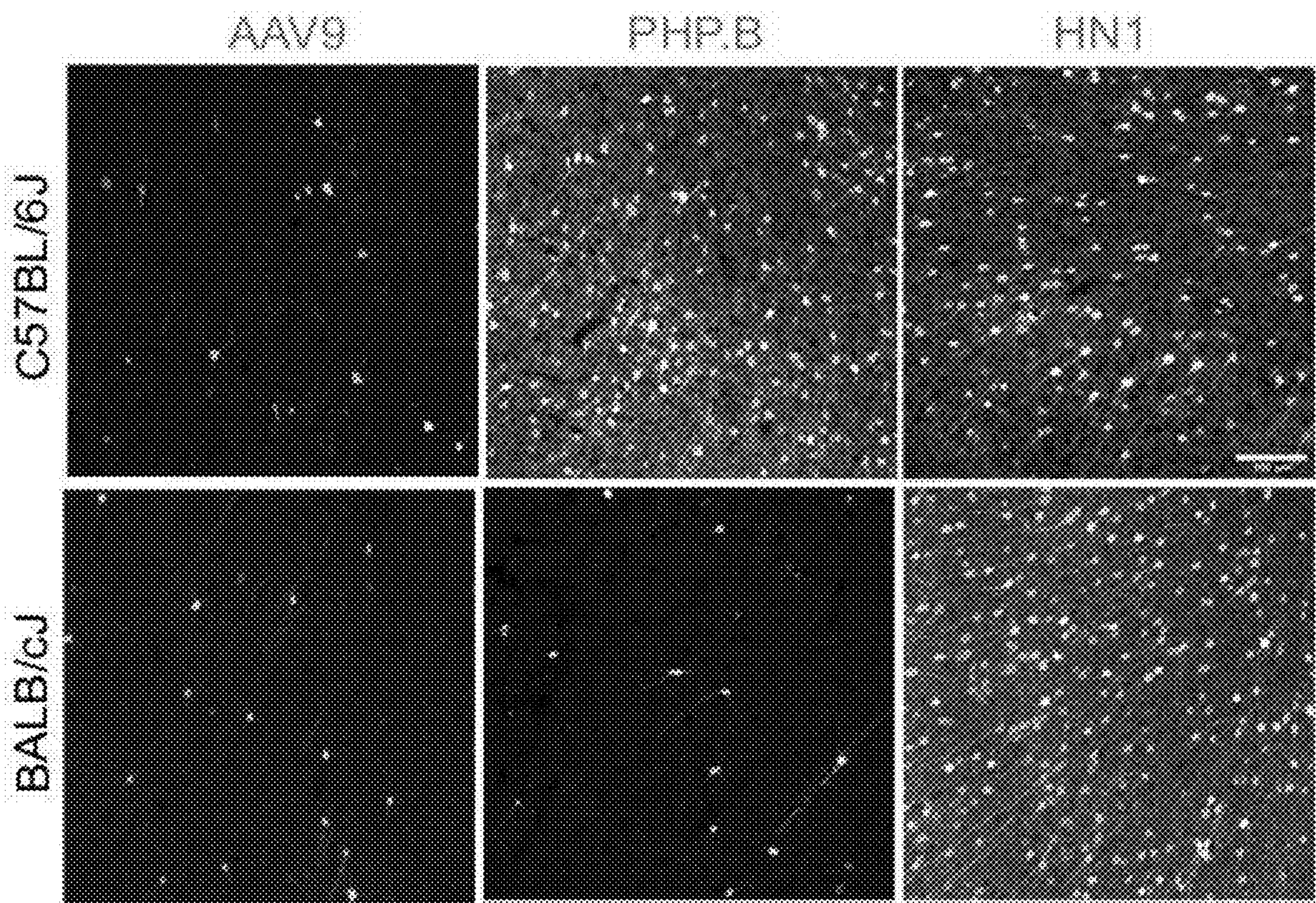


FIG. 15D

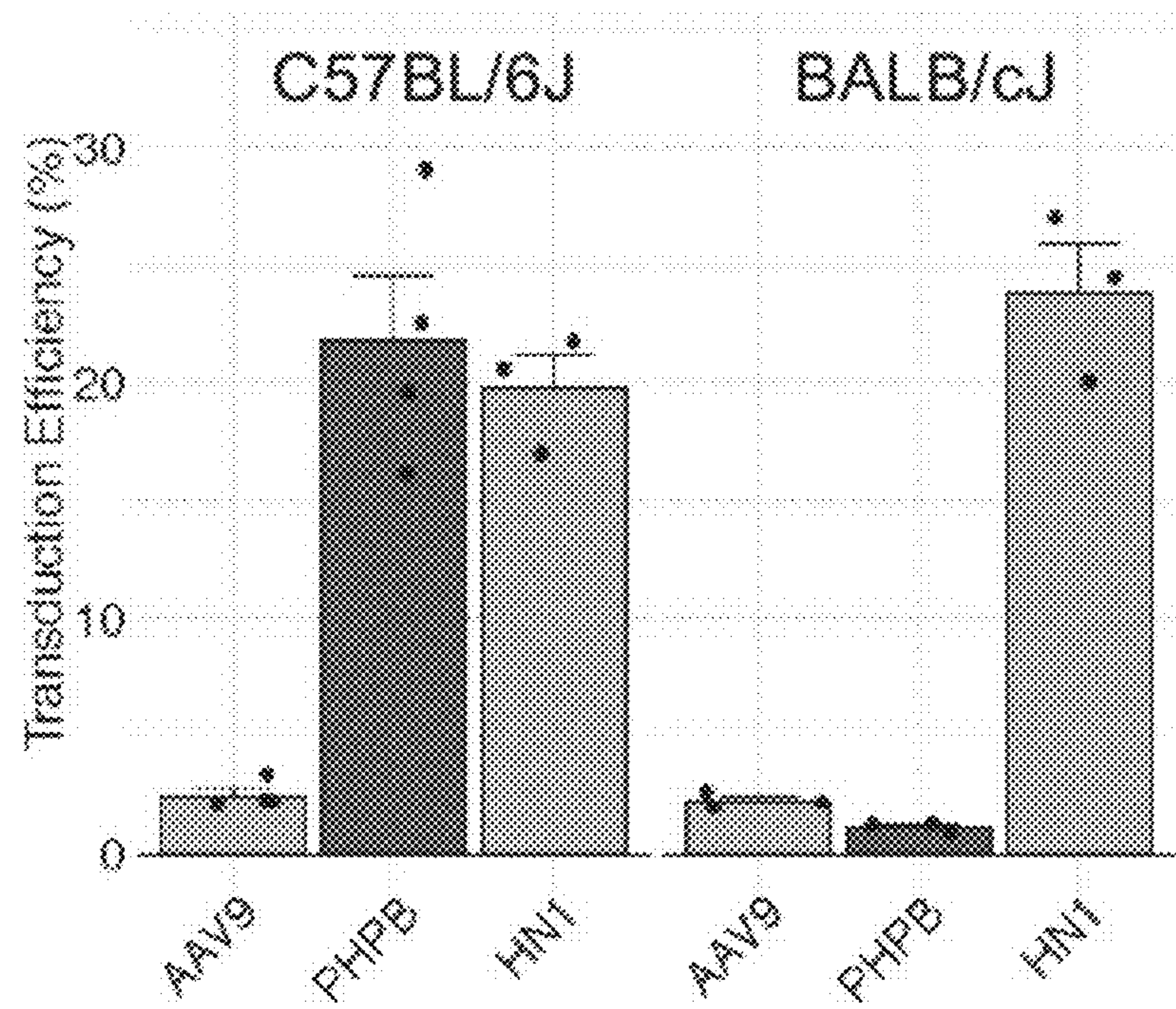


FIG. 15E

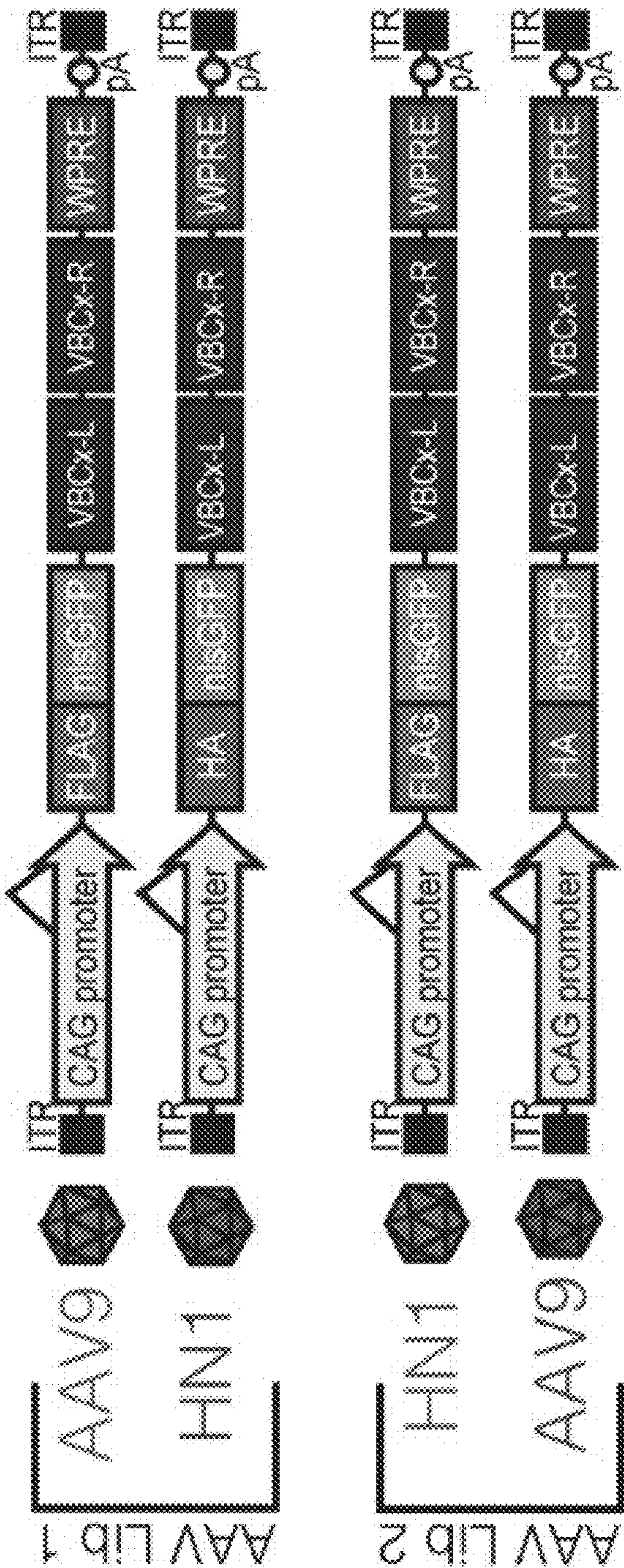


FIG. 16A

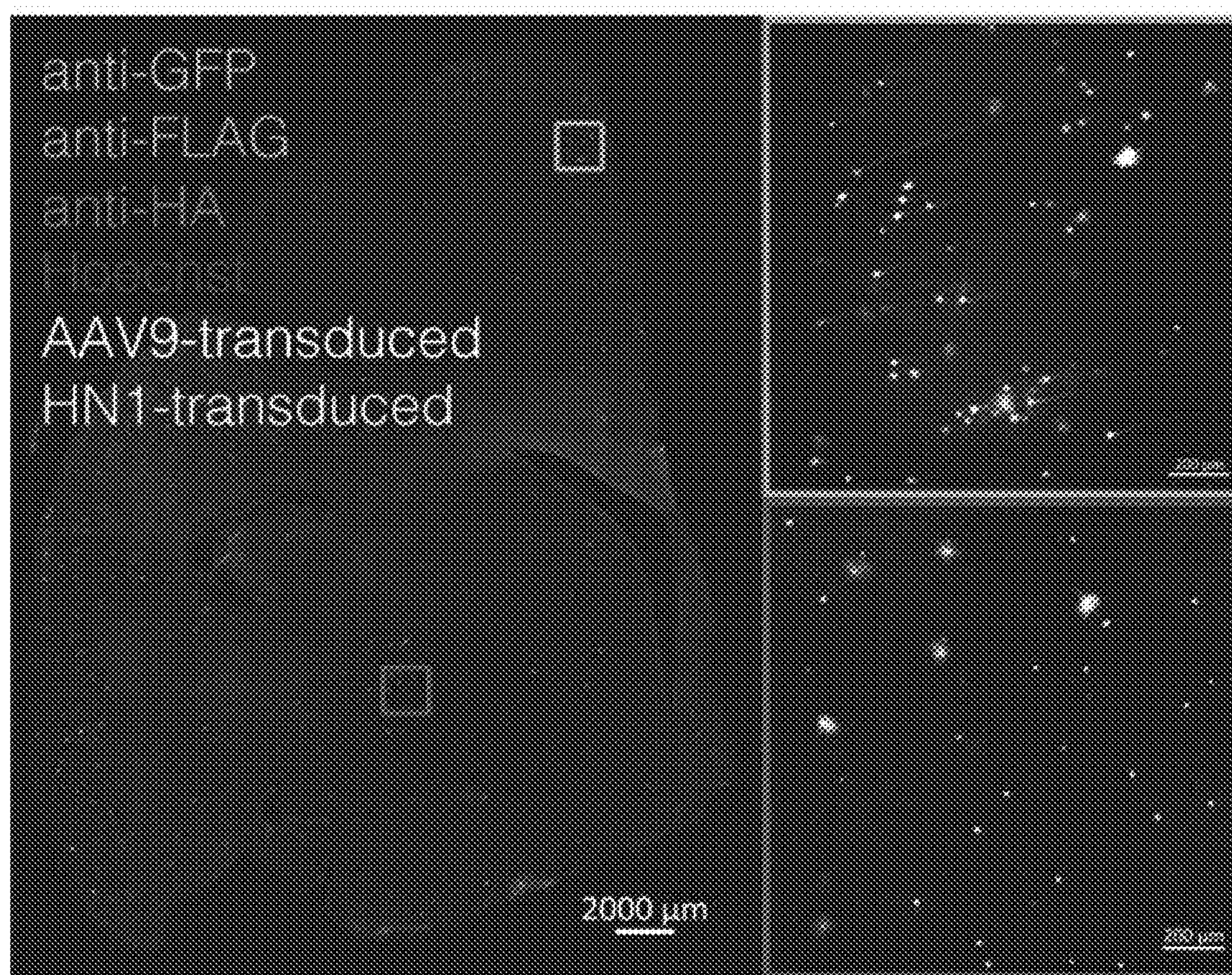


FIG. 16B

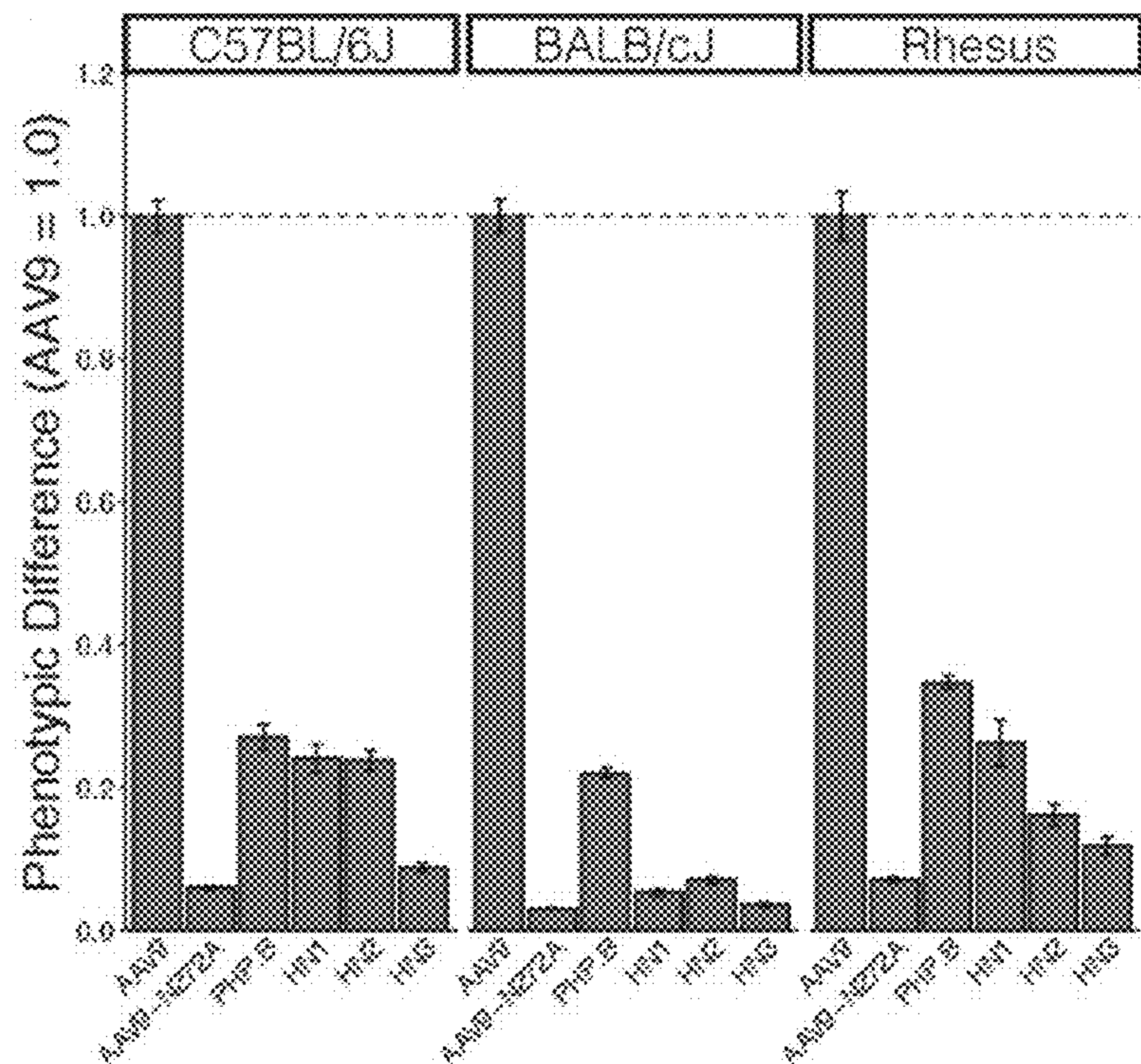


FIG. 17A

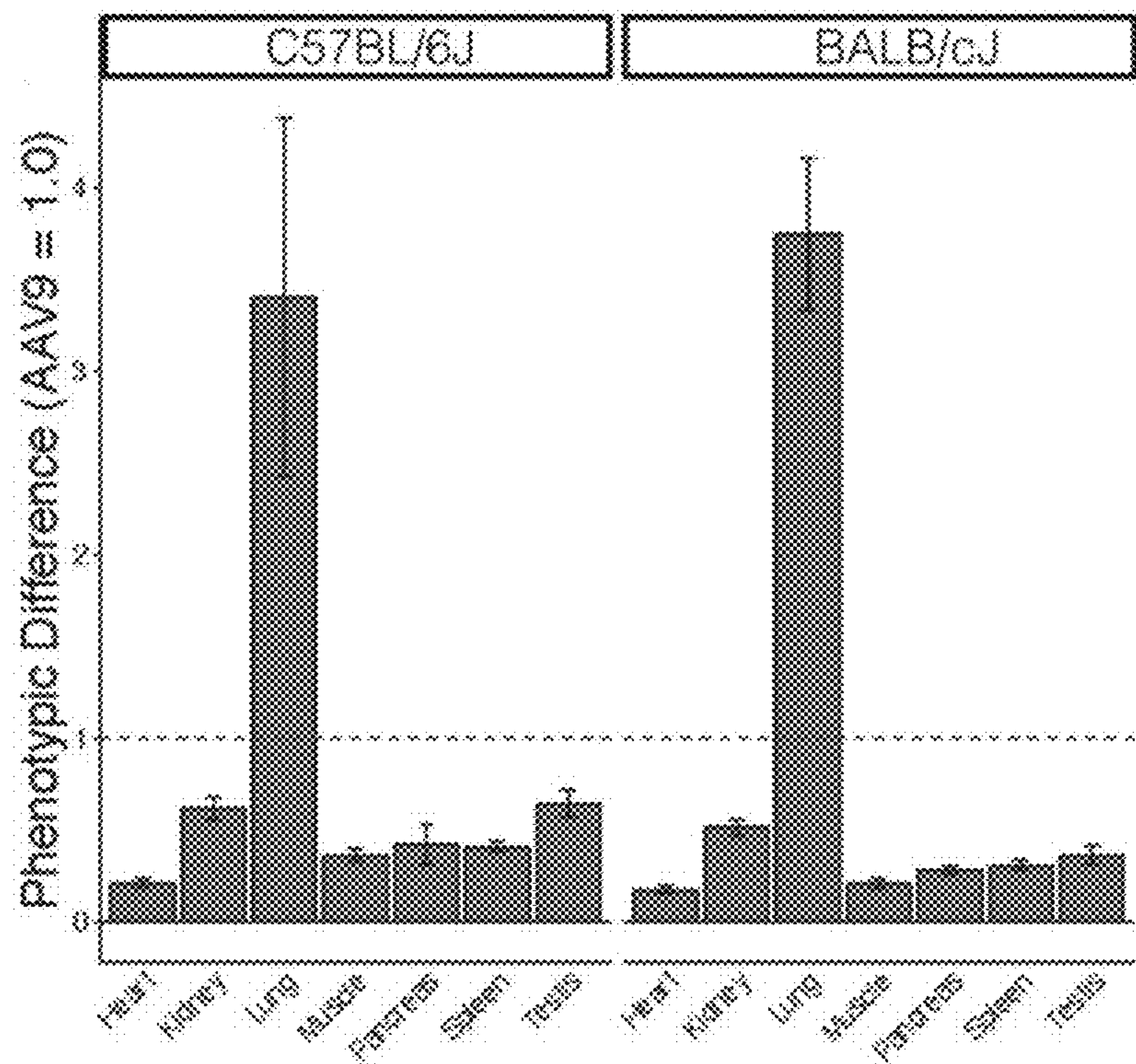


FIG. 17B

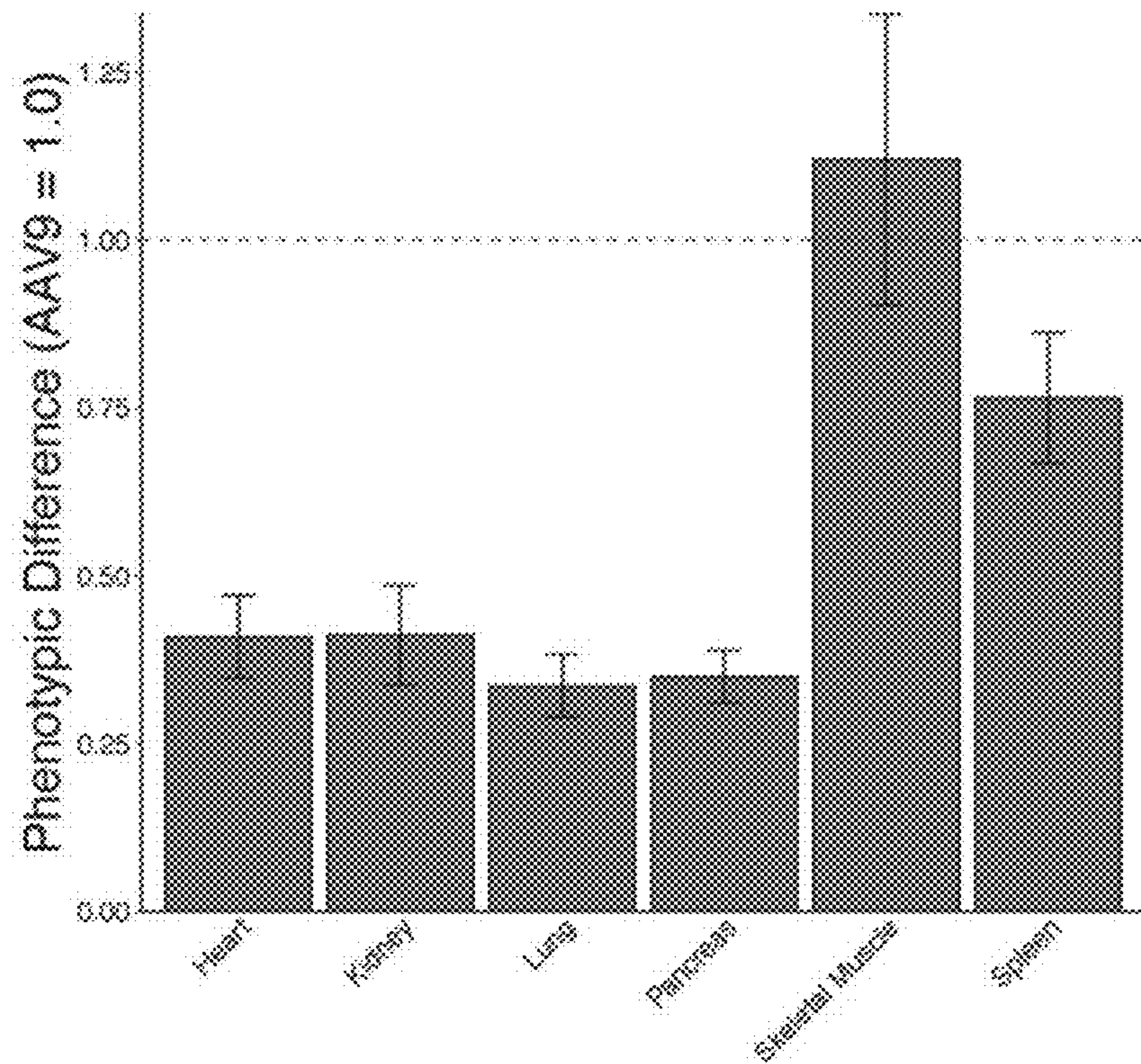


FIG. 17C

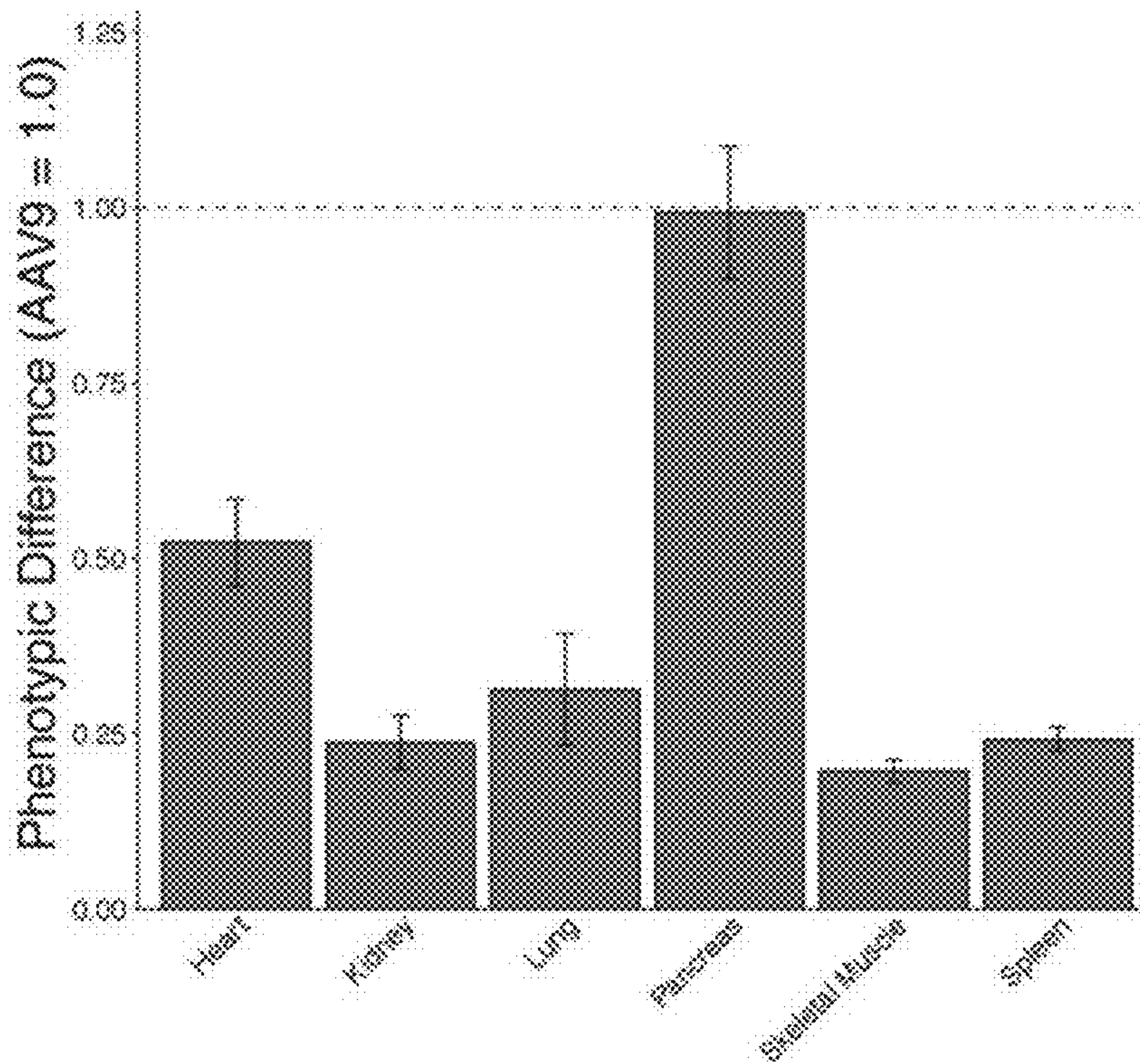


FIG. 17D

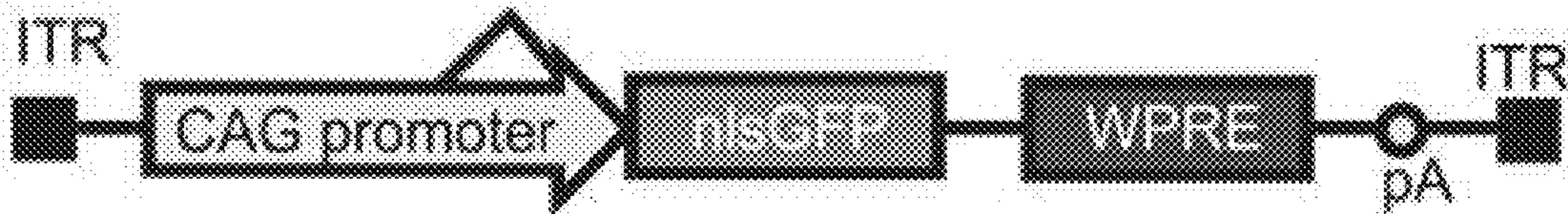


FIG. 18A

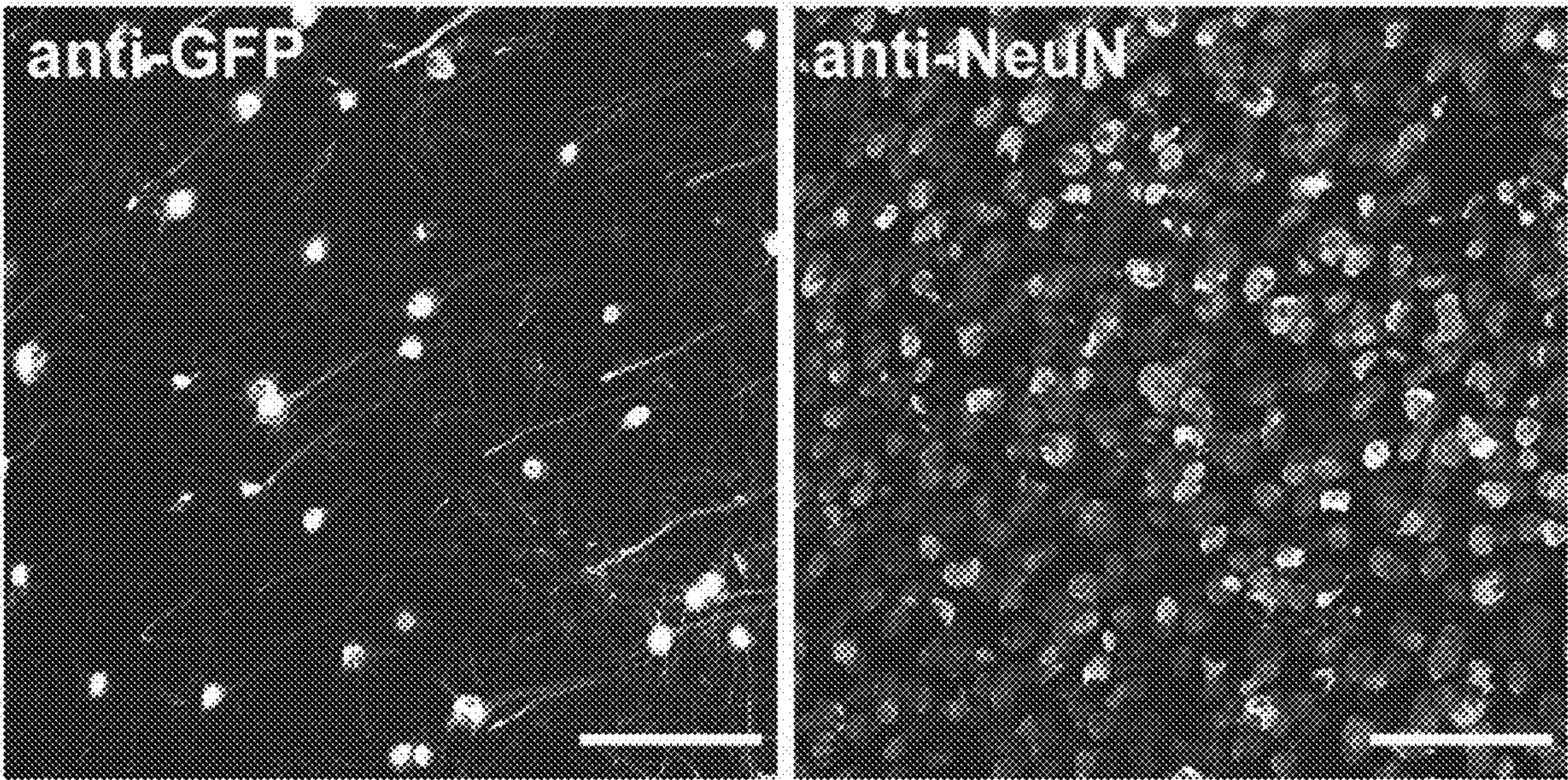


FIG. 18B

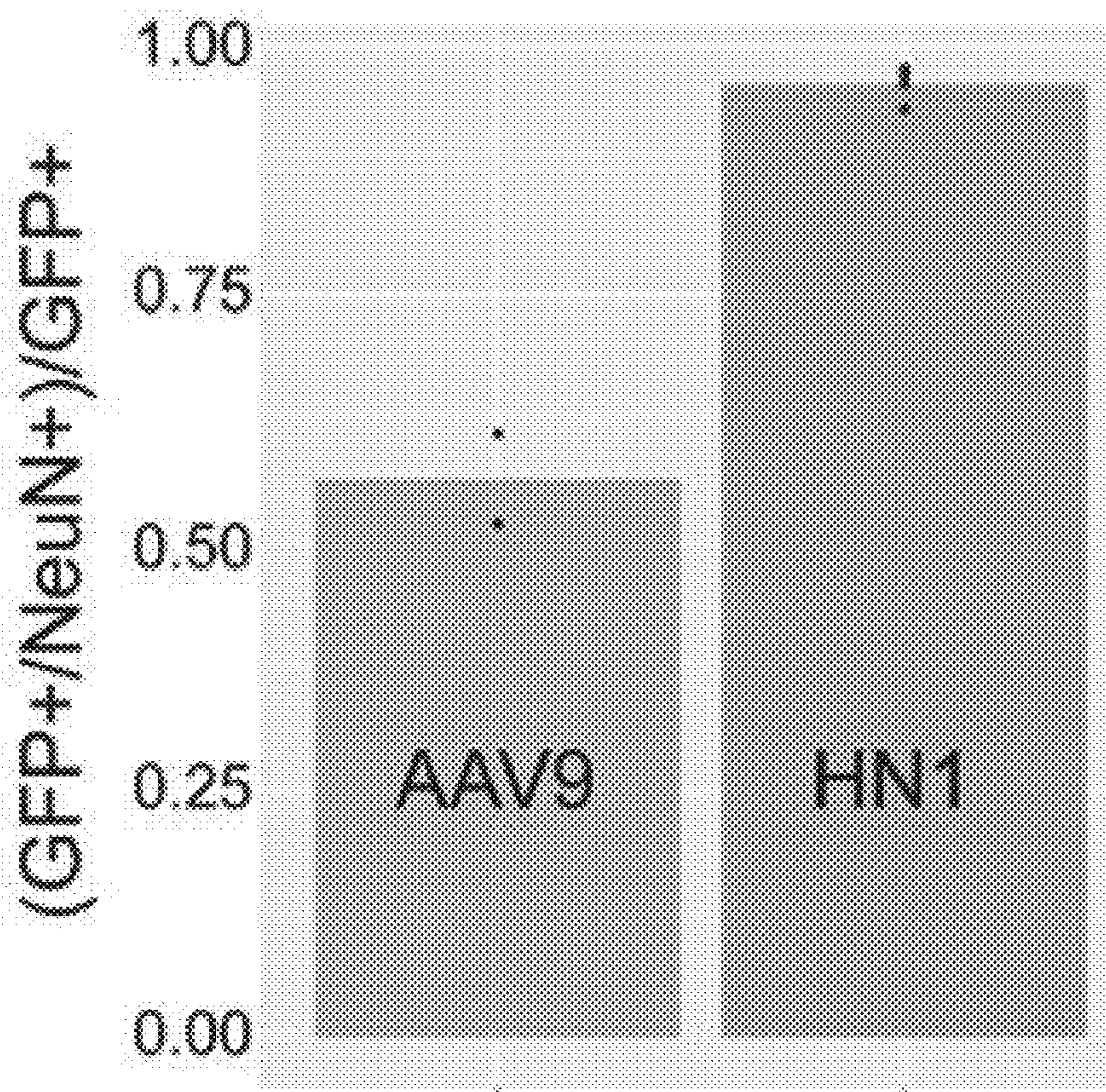


FIG. 18C

METHODS FOR USING TRANSCRIPTION-DEPENDENT DIRECTED EVOLUTION OF AAV CAPSIDS

RELATED CASES

[0001] This application is a continuation of U.S. patent application Ser. No. 17/337,341, filed on Jun. 2, 2021, and entitled “METHODS FOR USING TRANSCRIPTION-DEPENDENT DIRECTED EVOLUTION OF AAV CAPSIDS,” which is a continuation of International Patent Application No. PCT/US2020/016273 filed on Jan. 31, 2020, and entitled “METHODS FOR USING TRANSCRIPTION-DEPENDENT DIRECTED EVOLUTION OF AAV CAPSIDS,” which claims priority to U.S. Provisional Patent Application No. 62/799,603, filed on Jan. 31, 2019, and entitled “TRANSCRIPTION-DEPENDENT DIRECTED EVOLUTION OF AAV CAPSIDS,” each of which are hereby incorporated by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. NS088399 awarded by the National Institutes of Health/National Institute of Neurological Disorders and Stroke. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Aug. 24, 2022, is named 60255-701_302_SL.xml and is 163,102 bytes in size.

TECHNICAL FIELD

[0004] This disclosure relates to viral vectors used in gene delivery. More specifically, this disclosure relates to a method for transcription-dependent directed evolution and adeno-associated virus (“AAV”) vectors that are selected by using this method.

BACKGROUND

[0005] Recombinant adeno-associated virus (“AAV”) vectors are among the most promising for in vivo gene delivery. The usefulness of AAV vectors has been expanded since a number of naturally occurring new serotypes and subtypes were isolated from human and non-human primate tissues. Gao et al., *J Virol* 78, 6381-6388 (2004) and Gao et al., *Proc Natl Acad Sci USA* 99, 11854-11859 (2002). Among the newly-identified AAV isolates, AAV serotype 8 (AAV8) and AAV serotype 9 (AAV9) have gained attention because AAV vectors derived from these two serotypes can transduce a variety of organs including the liver, heart, skeletal muscles and central nervous system with high efficiency following systemic administration. Ghosh et al., *Mol Ther* 15, 750-755 (2007); Pacak et al., *Circ Res* 99, 3-9 (2006); Inagaki et al., *Mol Ther* 14, 45-53 (2006); Zhu et al., *Circulation* 112, 2650-2659 (2005); Wang et al., *Nat Biotechnol* 23, 321-328 (2005); Nakai et al., *J Virol* 79, 214-224 (2005); and Foust et al., *Nature Biotechnol* 23, 321-328 (2009). This robust transduction by AAV8 and 9 vectors has been ascribed to strong tropism for these cell types, efficient cellular uptake

of vectors, and/or rapid uncoating of virion shells in cells. Thomas et al., *J Virol* 78, 3110-3122 (2004). In addition, emergence of capsid-engineered AAV vectors with better performance has significantly broadened the utility of AAV as a vector toolkit. Asokan et al., *Mol Ther* 20, 699-708 (2012).

[0006] A proof-of-concept using AAV-mediated gene therapy has been shown in many preclinical animal models of human diseases. Phase I/II clinical studies have shown promising results for the treatment for hemophilia B (Nathwani et al., *N Engl J Med* 71, 1994-2004 (2014)), lipoprotein lipase deficiency (Carpentier et al., *J Clin Endocrinol Metab* 97, 1635-1644 (2012)), Leber congenital amaurosis (Jacobson et al., *Arch Ophthalmol* 130, 9-24 (2012) and Pierce and Bennett, *Cold Spring Harb Perspect Med* 5, a017285 (2015)), among others (reviewed in Mingozzi and High, *Nat Rev Genet* 12, 341-355 (2011) and Wang et al., *Nat Rev Drug Discov* 18, 358-378 (2019)).

[0007] Despite this promise, human studies have also revealed unexpected issues and potential challenges in AAV-mediated gene therapy. Manno et al., *Nat Med* 12, 342-347 (2006). In addition, despite rapid progress in our understanding of AAV biology and capsid-phenotype relationships (Adachi et al., *Nat Commun* 5, 3075, (2014); Grimm et al., *Hum Gene Ther* 28, 1075-1086, (2017); and Ogden et al., *Science* 366, 1139-1143, (2019)), there remain many desirable properties for clinical AAV vectors that we cannot rationally design.

[0008] To this end, high throughput screening methods for identifying novel AAV capsids with such desirable phenotypes have been employed. In particular, the development of in vivo AAV library selection strategies have produced a variety of designer AAV variants capable of highly efficient transduction of previously refractory cell types (reviewed in Kotterman and Schaffer, *Nat Rev Genet* 15, 445-451 (2014) and Grimm et al., *Mol Ther* 23, 1819-1831 (2015)).

[0009] The earliest attempts at in vivo library selection (1st Generation) relied on recovery of vector genome DNA from dissected tissue. Theoretically, this strategy results in recovery of both effective AAV variants, as well as AAV variants that mediate some, but not all of the steps required for vector-mediated transgene expression (FIG. 1). Thus, screening a diverse library of synthetic AAV variants potentially leads to a high background recovery of AAV variants that are completely ineffective gene therapy vectors. Furthermore, targeting a specific cell type requires further processing, such as fluorescence-activated cell sorting or laser capture microdissection. Nonetheless, there have been several reports of successfully employing this technology. Excoffon et al., *Proc Natl Acad Sci USA* 106, 3865-3870 (2009); Grimm et al., *J Virol* 82, 5887-5911 (2008); Lisowski et al., *Nature* 506, 382-386 (2014); and Dalkara et al., *Sci Transl Med* 5, 189ra176 (2013). However, a landmark study in 2016 by Deverman et al. showed that this process could be greatly improved upon by using a Cre-dependent selection strategy (2nd Generation). Deverman et al., *Nat Biotechnol* 34, 204-209 (2016). Cre-dependent library selection takes advantage of the selective ability of Cre recombinase to act on double-stranded DNA, but not single-stranded DNA, in order to invert vector genome DNA containing a primer binding sequence. Inversion of this sequence allows for direction-selective PCR to specifically amplify viral DNA delivered to cells by AAV variants that are able to undergo the late stage of transduction at which

double stranded DNA is formed from single-stranded AAV genomes. In addition, the use of Cre driver lines facilitates selective expression of Cre recombinase in a cell type-specific manner, allowing for selection of novel AAV variants that efficiently transduce. Indeed, the use of Cre-dependent selection allowed the authors to develop an AAV9 variant, AAV-PHP.B, that is capable of 40 times greater transduction than the parental AAV9 following systemic administration in C57BL/6J mice. Deverman et al., *Nat Biotechnol* 34, 204-209 (2016). Unfortunately, it has recently become clear that the enhancement exhibited by AAV-PHP.B in mice does not translate to the non-human primate context (Matsuzaki et al. 2018 and Hordeaux et al. 2019). Surprisingly, the enhancement does not even extend to all commonly used mouse strains (Matsuzaki et al. 2018 and Hordeaux et al. 2019). There is, therefore, a strong impetus to accelerate the development of clinically relevant AAV vectors by performing AAV library selection experiments in primate models. However, unlike the AAV variant selection in mice where a plethora of cell type-specific transgenic Cre driver lines are already established, Cre-dependent selection is not tractable in clinically relevant large animals, including non-human primates, because Cre transgenic animals are not readily available.

[0010] We therefore sought to develop a next-generation selection strategy (3rd Generation) with similar or better selective stringency as that provided by Cre-dependent selection, but without the need for Cre recombinase. In order to accomplish this goal, we developed the TRAnscription-dependent Directed Evolution system, or TRADE. In the transcription-dependent selection, we express the AAV cap gene as a non-coding antisense mRNA driven by a cell type-specific enhancer-promoter. Recovery of this antisense transcript by RT-PCR allows for stringent recovery of AAV cap genes at the level of vector-mediated mRNA expression in a specific cell type without the use of Cre recombinase. Targeting of different cell types merely requires cloning of a different cell type-specific enhancer-promoter into the plasmid construct. Thus, TRADE is a highly flexible system that can be applied in a wide variety of contexts, including the non-human primate context for development of enhanced AAV vectors for clinical gene therapy. Note that the same principle can be used for expressing AAV cap gene in an sense orientation. However, the sense strand approach results in expression of immunogenic capsid proteins in target cells and is therefore less ideal than the antisense strand approach employed by the TRADE system.

SUMMARY

[0011] This disclosure provides a next-generation directed evolution strategy, termed TRAnscription-dependent Directed Evolution (“TRADE”), that selects for AAV capsid transduction at the level of cell type-specific or ubiquitous mRNA expression. The method described herein provides the following advantages over Cre recombination-based AAV targeted evolution (“CREATE”), the most contemporary methods for AAV capsid directed evolution reported in the literature. Deverman et al., *Nat Biotech* 34, 204-209 (2016). First, the CREATE system requires Cre expression, which can be attained either by exogenously-delivered Cre expression or by the use of Cre-transgenic animals. In contrast, the TRADE system does not require Cre-transgenic animals; therefore, it can be applied to animals and cultured cells derived from any animal species and can be readily

adapted to large animals, including non-human primates. Second, unlike the CREATE system, in which the cell-type specific selection is applied at the level of AAV viral genome conversion from single-stranded DNA to double-stranded DNA, TRADE allows for cell type-specific selection at the level of AAV genome transcription. Therefore, the TRADE system can provide greater selective pressure than the CREATE system. Third, multiple directed evolution schemes (e.g., neuron-specific, astrocyte-specific, oligodendrocyte-specific, and microglia-specific) can be integrated into one AAV capsid library and selection for AAV vectors targeting each cell type can be performed in a single animal. Fourth, any cell type-specific or tissue/organ-specific enhancers/promoters or ubiquitous enhancers/promoters can be readily used for AAV capsid directed evolution aimed at identification of cell type-specific or ubiquitous novel AAV capsids with enhanced potency. Fifth, the TRADE methodology is not limited to the genus Dependoparvovirus, including the common AAVs that have been used for gene delivery, but can also be applied more broadly to the family Parvoviridae, including in the genera Bocaparvoviruses and Erythroparvoviruses other than AAV (e.g., bocaviruses), and even more broadly to an DNA virus.

[0012] This disclosure also provides novel AAV capsid mutants. TRADE technology was used to identify novel AAV vectors that mediate neuronal transduction in the brain following intravenous administration. Application of TRADE in C57BL/6J mice and a rhesus macaque resulted in the identification of new AAV capsids that can transduce neurons more efficiently and more specifically than AAV9 in the mouse and non-human primate brain following intravenous administration. In addition, we identified a novel AAV capsid that can transduce an undefined cell population or populations, that reside in the lung and are potentially of neuronal origin, 5 to 18 times better than the AAV9.

[0013] The present disclosure also provides a method to prevent splicing of antisense mRNA of the AAV capsid gene. Antisense pre-mRNA transcribed from the AAV cap gene open reading frame (“ORF”) can be spliced making (a) truncated mRNA species. To our knowledge, this is a new discovery that has never previously been reported. Such splicing has the potential to hinder effective recovery of full-length antisense mRNA of the AAV cap ORF, which is essential for TRADE when a wide region of the cap ORF is mutagenized. This disclosure provides a novel strategy to prevent splicing of antisense mRNA of the cap gene.

[0014] The TRADE system described herein uses antisense mRNA to recover capsid sequence information, TRADE using sense strand mRNA (i.e., sense strand TRADE) is also feasible using the same principle. However, it should be noted that the sense strand TRADE approach results in expression of immunogenic capsid proteins in target cells and therefore is presumably less ideal than the antisense strand approach.

BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1 An overview of in vivo library selection strategies utilized for directed evolution of the AAV capsid. AAV vector-mediated transduction is a multi-step process that requires the virion to overcome extracellular barriers, bind receptors on the target cell, enter the cell via endocytosis, escape the endosome, traffic to the nucleus, uncoat, achieve a double-stranded DNA configuration, and finally undergo transcription/translation. The earliest strategies for

in vivo library selection (1st Gen) recovered all vector genome DNA from a tissue sample. Theoretically, this strategy would recover both effective AAV variants, as well as AAV variants that mediate some, but not all of the steps required for vector-mediated transgene expression. In addition, this strategy would also recover AAV vector genome DNA from AAV vector particles that do not enter cells and stay in the extracellular matrix. Thus, screening a diverse library of synthetic AAV variants would lead to a relatively high background recovery of AAV variants that are completely ineffective gene therapy vectors. Furthermore, focusing on a specific cell type requires further processing, such as fluorescence-activated cell sorting (FACS) or laser capture microdissection (LCM). The second generation of library selection (2nd Gen) substantially increased selection stringency by utilizing Cre-dependent recovery of only those AAV variants that are able to achieve the double-strand DNA stage of transduction. Furthermore, driving the expression of Cre with a cell type-specific enhancer-promoter allows for targeting of a specific cell type while retaining the benefits of processing bulk tissue samples. The third generation of library selection (3rd Gen) further builds on AAV directed evolution technology by employing transcription-dependent recovery of AAV variants that are able to mediate transgene mRNA expression from a cell type-specific enhancer-promoter, without the requirement of Cre expression.

[0016] FIGS. 2A-2B: Principle of TRADE. (FIG. 2A) A map of the AAV vector genome in a TRADE configuration (AAV-TRADE). A cell type-specific enhancer-promoter is placed in an antisense orientation to drive AAV cap gene transcription expression as antisense mRNA. A polyadenylation signal (pA) derived from the simian virus 40 (SV40) genome is placed within the AAV genome intron in an antisense orientation to terminate antisense AAV cap gene mRNA transcription. The eGFP open-reading frame (ORF) can be placed as depicted to serve as a reporter or facilitate enrichment of transduced cells by FACS; however, such a marker gene is not strictly necessary for TRADE. A ubiquitous promoter such as the CAG promoter can be also used in TRADE in place of cell type-specific enhancers-promoters to identify AAV capsids that can transduce a variety of cell types. A cell type-specific enhancer-promoter can be placed upstream of the AAV cap gene ORF to drive expression of the AAV cap gene mRNA transcripts in a sense orientation (i.e., sense strand TRADE). However, this approach may not be ideal for TRADE because AAV capsid protein would be expressed in target cells, which may result in undesired biological consequences in the directed evolution process. (FIG. 2B) During AAV vector production in HEK293 cells, and in the presence of the adenoviral helper functions, the AAV2 viral p40 promoter drives cap gene expression (forward transcription) and cell type-specific transcripts are suppressed, leading to successful production of recombinant AAV vectors containing the AAV-TRADE vector genome. Following transduction of a specific cell type, the cell type-specific enhancer-promoter is activated, driving expression of eGFP and the antisense cap mRNA sequence, while the transcriptional activity of the p40 promoter remains inactive in transduced cells due to a lack of adenoviral helper functions. The entire cap gene ORF can be recovered by reverse transcription (RT)-PCR using antisense cap gene mRNA as a template that is expressed in a cell type-specific manner. We have observed that recombi-

nant AAV vectors can be produced successfully at high levels even in the presence of antisense mRNA transcripts expressed due to leaky expression from the human synapsin I gene (hSynI) enhancer-promoter in HEK293 cells. We have also observed that recombinant AAV vectors can be produced successfully at high titers even when we use the CAG promoter that drives expression of antisense AAV cap mRNA transcripts at high levels.

[0017] FIGS. 3A-3G: Validation of the TRADE system targeting brain neurons. (FIG. 3A) A map of the AAV-PHP.B-hSynI-GFP-TRADE vector genome. (FIG. 3B) To verify the TRADE system, this AAV vector genome was packaged into the AAV-PHP.B capsid as a single-stranded DNA genome and the resulting AAV vector was injected into two 8-week-old C57BL/6J mice intravenously at a dose of 3×10^{11} vector genomes (vg) per mouse. Brain tissue was harvested 12 days post-injection. The brain tissue from one animal was fixed with 4% paraformaldehyde and used for immunofluorescence microscopy and the brain tissue from the other animal was unfixed and used for molecular analysis of AAV vector genome DNA and RNA. (FIG. 3C) Immunofluorescence microscopy image of brain sections stained with anti-GFP antibody confirmed expression of the cell type-specific enhancer-promoter-driven transcript. (FIG. 3D) hSynI enhancer-promoter-driven GFP expression was observed specifically in neurons (anti-HuC/D+). (FIG. 3E) RT-PCR was used to recover the full-length cap ORF sequence (RT+). RT-, a no reverse transcriptase control; Plas, a positive control obtained with DNA-PCR using a plasmid template containing the AAV-PHP.B-hSynI-GFP-TRADE vector genome sequence; NT, a no template PCR control. (FIG. 3F) Sanger sequencing of the RT-PCR product revealed expected splicing of the MVM intron in the antisense transcripts expressed by the hSynI enhancer-promoter (SEQ ID NO:190). The exon-exon junction is highlighted with gray. (FIG. 3G) Sanger sequencing confirmed the insertion of the PHP.B peptide (highlighted with gray) (SEQ ID NO:191).

[0018] FIG. 4 Splicing of the antisense mRNA of the AAV9 cap ORF. Two cell lines, HEK293 and Neuro2a, were transfected with plasmids containing the AAV9 cap ORF in the TRADE configuration, with or without a GFP reporter. They are indicated as "GFP TRADE" and "TRADE", respectively, in the figure. Cells were harvested 3 days post-transfection, RNA was extracted, and RT-PCR was performed with a set of PCR primers that amplify the full cap ORF sequence. Instead of recovering the expected amplicon size of 2.4 kb as shown in the positive control (PC) lane, we consistently recovered amplicons of approximately 0.7 kb. Sanger sequencing of these RT-PCR products identified a truncation consistent with splicing of a 1.7 kb region of the AAV9 cap ORF indicated in FIG. 5. PC, a positive control using a plasmid template containing the AAV-PHP.B-hSynI-GFP-TRADE vector genome sequence; NC, a no template PCR control.

[0019] FIG. 5 An intron identified in antisense mRNA derived from the AAV9 cap gene (SEQ ID NO:192). When the AAV-PHP.B cap gene sequence was transcribed in an antisense orientation in HEK293 cells or Neuro2a cells under the control of the neuron-specific human synapsin I (hSynI) enhancer-promoter, a splicing event was identified with cryptic splice donor and splice acceptor sites (please refer to FIGS. 6A-6F as well). The underlined sequence indicates the intron found within the AAV9 cap ORF. This

splicing event was not observed in mouse brain neurons. It should be noted that (1) although the hSynI enhancer-promoter has been used as a neuron-specific element, it has been shown to drive leaky expression in HEK293 cells; and (2) the AAV9 cap ORF sequence used for the intron splicing experiment had the following silent mutations near the C-terminus: gaaccccgccccattggcacGCgT-tacCTGACTCGTAATCTGTAA (SEQ ID NO:1). The intron sequence is underlined, and the silent mutations that have been introduced into the intron to create an MluI (ACGCGT) recognition site are indicated in uppercase.

[0020] FIGS. 6A-6F: Cryptic splice donor (SD) and splice acceptor (SA) sites with the common features of exon-intron junctions present in the AAV cap ORFs in an antisense orientation. Nucleotide sequences of the cap genes derived from 122 naturally occurring AAV strains (serotypes and variants) are aligned using a multiple sequence alignment program (SEQ ID NO 223-316). The exon-intron junctions identified in the AAV9 cap ORF-derived antisense mRNA are indicated with solid lines. The dashed line in the splice acceptor region indicates putative splice acceptor sites in the AAV cap ORFs devoid of the splice acceptor AG/TC sequence at the position expected from the sequence conservation. The dashed line in the splice donor region indicates the splice donor site identified in the AAV3 cap ORF-derived antisense mRNA (please refer to FIGS. 7A-7B). The GT/CA splice donor sites and the AG/TC splice acceptor motifs, followed by a stretch of T's, are the common features of exon-intron junctions and are very well-conserved across many AAV strains. The splice donor and acceptor sites identified in the AAV9 cap ORF shown in this figure have also been identified in the AAV1 cap ORF. For serotypes other than AAV1, 3, 5 and 9, splicing events in antisense mRNA of the AAV cap ORFs are currently under investigation. The highlighted variants are common AAV serotypes.

[0021] FIGS. 7A-7B: Introns identified in antisense mRNA derived from the AAV3 cap gene. pAAV3-hnLSP-MCS-TRADE2 is a plasmid carrying the wild-type AAV3 cap ORF placed under a liver-specific enhancer-promoter with an MVM intron (hnLSP). The nucleotide sequence of the AAV3 cap ORF is the same as that of the naturally identified AAV3. HepG2 cells, a human hepatoma cell line, were transfected with plasmid pAAV3-hnLSP-MCS-TRADE2. Antisense mRNA derived from the AAV3 cap ORF was then analyzed by RT-PCR. Sequences of two truncated RT-PCR products were determined by Sanger sequencing following blunt-end TOPO cloning of the PCR products, which revealed introns found within the antisense AAV3 cap ORF (Panels A and B, SEQ ID NO:193). Intron sequences are in lowercase letters with underline. The most upstream splice donor site is found to be only 3 bp away from the splice donor site identified in the AAV9 cap ORF, which is indicated in a dashed line in FIGS. 6B-6F. The most downstream splice acceptor site is found approximately 80 bp upstream of that of the AAV9 cap ORF. Please note that all the splice donor and acceptor sites identified in the AAV3 cap ORF have also been identified in the AAV1 cap ORF.

[0022] FIGS. 8A-8F. Additional cryptic splice acceptor sites present in the AAV cap ORFs. (FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D, FIG. 8E, and FIG. 8F) Nucleotide sequences of the cap genes derived from 122 naturally occurring AAV strains (serotypes and variants) are aligned using a multiple sequence alignment program (SEQ ID NO:317-420). The

exon-intron junctions at the splice acceptor sites identified in the AAV3 cap ORF-derived antisense mRNA are indicated with solid thin lines. The dashed line in Panel A indicates alternative putative splice acceptor sites near the experimentally determined splice acceptor site. The AG/TC splice acceptor sites, followed by a stretch of T's, are a common feature of exon-intron junctions at splice acceptor sites and are very well conserved across many AAV strains. The AAV3 cap ORF is highlighted. The splice acceptor sites identified in the AAV3 cap ORF shown in Panels A and B have also been identified in the AAV1 cap ORF. As for the AAV5 cap ORF, no splicing events have been observed at any sites in antisense mRNA transcription. For serotypes other than AAV1, 3, 5 and 9, splicing events in antisense mRNA of the AAV cap ORFs are currently under investigation.

[0023] FIGS. 9A-9C: Additional potential splice donor sites present in the AAV cap ORFs. Nucleotide sequences of the cap genes derived from 122 naturally occurring AAV strains (serotypes and variants) are aligned using a multiple sequence alignment program (SEQ ID NO:421-461). The exon-intron junctions at the splice donor sites identified in the AAV3 cap ORF-derived antisense mRNA are indicated with a solid line. The GT/CA splice donor consensus sequence at this position is retained by only half of AAV strains. This splice donor site has been identified in the AAV1 cap ORF.

[0024] FIG. 10 Splice donor and splice acceptor sites identified in the AAV1 cap ORF. The nucleotide sequence of the AAV1 cap ORF is shown (SEQ ID NO:194). The AAV1 cap ORF was expressed by the hSynI enhancer-promoter in human embryonic kidney (HEK) 293 cells or Neuro2a cells in an antisense orientation. Antisense mRNA derived from the AAV1 cap ORF was then analyzed by RT-PCR. Sequences of RT-PCR products were determined by Sanger sequencing following blunt-end TOPO cloning of the PCR products, which revealed introns found within the AAV1 cap ORF. Exon-intron junctions identified in antisense AAV1 cap mRNA are indicated with AG/TC for the splice donor sites and GT/CA for the splice acceptor sites. AG/TC and GT/CA in uppercase are the consensus two nucleotides at the 5' end and the 3' end of an intron, respectively. Since the splicing occurs in antisense mRNA of the ORF, intron sequences are between CT (splice acceptor) and AC (splice donor) in various combinations in the above sequence. The detailed information about the observed combinations of the splice donors and acceptors is not shown. The two conserved nucleotides at exon-intron junctions (CT or AC) indicated in boldface are those that are highly conserved across different AAV serotypes. The two conserved nucleotides at exon-intron junctions (CT or AC) that are underlined are those that have also been identified in antisense AAV3 or AAV9 cap mRNA transcripts.

[0025] FIG. 11 Splicing-suppressing mutagenesis of the AAV9 cap ORF. Silent mutations are introduced around the splice acceptor (SA) site and/or the splice donor (SD) site in the AAV9 cap ORF to suppress the splicing observed on the antisense mRNA transcripts. The spliced-out intron from the native sequence (SEQ ID NO:195, SEQ ID NO:196) is indicated with underlines. The AAV9NS1 genome (SEQ ID NO:197) has a set of mutations around the SA site while the AAV9NS2 genome (SEQ ID NO:198) has a set of mutations around the SD site. The AAV9NS3 genome has both sets of

mutations. The numbers to the right indicate the nucleotide position relative to the first nucleotide of the AAV9 cap ORF.

[0026] FIG. 12 Mutations introduced around the splice donor and/or acceptor site(s) effectively suppress the splicing of antisense mRNA derived from the AAV9 cap ORF. Neuro2a cells were transfected with plasmids containing the AAV9 cap ORF and various potentially splicing-suppressing mutations in the TRADE configuration (NS1-3). RNA was harvested 3 days post-transfection and RT-PCR was performed with a set of PCR primers that can recover the full cap ORF sequence. In stark contrast to results seen in FIG. 4, full-length amplicons were successfully recovered. NS1, the AAV9-TRADE vector genome with a codon-modified splice acceptor. NS2, the AAV9-TRADE vector genome with a codon-modified splice donor. NS3, the AAV9-TRADE vector genome with codon-modified splice acceptor and splice-donor. PC, a positive control using a plasmid template containing the AAV-PHP.B-hSynI-GFP-TRADE vector genome sequence; NC, a no template PCR control.

[0027] FIGS. 13A-13B: Study design for application of TRADE to identify enhanced AAV variants for brain neuron transduction following systemic AAV vector injection. (A) A map of the AAV9-N272A-hSynI-GFP-TRADE-PepLib vector genome. The hSynI enhancer-promoter is utilized to drive expression specifically in neurons. The liver-detargeted AAV9-N272A cap (PCT/US2017/068050) serves as the platform for AAV library generation. A randomized 8 amino acid peptide encoded by (NNK)₈ and flanked by glycine-serine linkers (SEQ ID NO:2) was substituted for Q588 of the AAV9-N272A cap sequence (SEQ ID NO:222). (B) The plasmid library was used to produce an AAV library using a triple transfection protocol. The library was purified through PEG precipitation and two rounds of CsCl ultracentrifugation, then injected via tail vein at a dose of 3×10^{11} vg/mouse. Brain tissue was harvested 12 days post-injection. RNA was recovered using TRIzol and RT-PCR was used to recover a fragment of cap containing the peptide insertion, which was subsequently cloned back into the AAV vector plasmid backbone. This was repeated for 3 rounds of selection in C57BL/6J mice. In parallel, a single round of selection was performed in rhesus macaque using a dose of 2.7×10^{12} vg/kg.

[0028] FIGS. 14A-14D: Validation of neuronal transduction of the 26 novel AAV capsids in mice and a nonhuman primate by AAV RNA Barcode-Seq. (FIG. 14A) A map of the double-stranded (ds) AAV-hSynI-GFP-BC vector. A pair of two 12 nucleotide-long DNA barcodes (VBCx-L and VBCx-R) are placed under the human synapsin I (hSynI) gene enhancer-promoter. These two virus barcodes (VBCs) can be expressed as transcripts specifically in cells where the hSynI enhancer-promoter is active (i.e., neurons). (FIG. 14B) Neuronal transduction of 26 novel AAV variants, HN1 to HN26, identified by TRADE (5 variants identified in mice and 21 variants identified in a non-human primate) and 3 control AAV capsids (AAV9, AAV9-N272A and AAV-PHP.B) in C57BL/6J and BALB/cJ mice. A DNA/RNA-barcoded dsAAV-hSynI-GFP-BC library (dsAAV-hSynI-GFP-BCLib) containing 26 novel AAV variants identified by TRADE (5 variants identified by TRADE in mice and 21 variants identified by TRADE in a non-human primate) and control AAV capsids (AAV9, AAV9-N272A and AAV-PHP.B) was injected intravenously into three adult male C57BL/6J mice and three adult male BALB/cJ mice at a dose of 5×10^{11} vg per mouse. Two weeks post-injection, various tissues were

harvested and analyzed for transduction at AAV vector genome transcripts levels by AAV RNA Barcode-Seq. Transduction levels are expressed as phenotypic difference (PD) values relative to the reference control, AAV9. For the AAV capsid amino acid sequence information of the HN1 to HN26 variants, please refer to Table 3. (FIG. 14C) Neuronal transduction of the 26 novel AAV variants and 3 control AAV capsids in the hippocampus of a rhesus macaque. The same DNA/RNA-barcoded AAV library was injected intravenously into one juvenile male rhesus macaque at a dose of 2×10^{13} vg/kg. Two weeks post-injection, various brain regions were harvested and analyzed for transduction by AAV RNA Barcode-Seq. (FIG. 14D) Relative neuronal transduction efficiencies of 3 TRADE variants, HN1, HN2 and HN3, and AAV-PHP.B were analyzed by AAV RNA Barcode-Seq in 12 different brain regions in the single rhesus macaque used for Panel C. In Panels B, C and D, dashed lines indicate the PD value of AAV9 (i.e., 1.0).

[0029] FIGS. 15A-15E: Validation of enhanced neuronal transduction of AAV9-N272A-HN1 in mice using conventional eGFP reporter vectors and histological quantification. We produced AAV9, AAV-PHP.B, and AAV9-N272A-HN1 vectors containing self-complementary AAV genomes expressing eGFP under the control of the hSynI enhancer-promoter (dsAAV-hSynI-eGFP). Purified vectors were administered via the tail vein at a dose of 3×10^{11} vg/mouse into 8-week old male C57BL/6J or BALB/cJ mice (n=4 mice/vector/mouse strain). Three weeks post-injection, mice were transcardially perfused with 4% paraformaldehyde and brain tissue was processed for immunohistochemistry. (FIG. 15A) A map of the self-complementary hSynI-eGFP vector genome. (FIG. 15B) Representative tilescan images of sagittal sections stained with anti-GFP antibody. (FIG. 15C) Quantification of neuronal transduction in (FIG. 15B) based on automated counts of cells expressing eGFP and NeuN in four brain regions. (FIG. 15D) Validation of the automated counting process in (FIG. 15B) and (FIG. 15C). Representative 20 $\times$ confocal images from visual cortex are shown. Scale bar=100 μ m. (FIG. 15E) Quantification of neuronal transduction in (FIG. 15D) based on hand counts of cells expressing eGFP and NeuN by a blinded observer. Error bars represent mean $\pm$ SEM. ***p<0.001.

[0030] FIGS. 16A-16B: Validation of enhanced AAV9-N272A-HN1 transduction relative to AAV9 in rhesus macaques using epitope-tagged eGFP reporter vectors. (FIG. 16A) AAV-CAG-nlsGFP vectors used for this study. We produced 4 AAV vectors: AAV9-CAG-FLAGnlsGFP-BCLib, AAV9-CAG-HAnlsGFP-BCLib, AAV9-N272A-HN1-CAG-FLAGnlsGFP-BCLib and AAV9-N272A-HN1-CAG-HAnlsGFP-BCLib. The nlsGFP (eGFP with the nuclear localization signal derived from the SV40 large T antigen) was tagged with either the FLAG tag or the HA tag at the N-terminus. Each vector was a DNA/RNA-barcoded library containing an approximately 1 to 1 mixture of 9 different DNA/RNA-barcoded viral clones; however, this feature was not used in this study. The two vectors in the top half depicted in Panel A were mixed at a ratio of 1:1 to make AAV Library 1 (AAVLib1) and the two vectors in the bottom half were mixed at a ratio of 1:1 to make AAV Library 2 (AAVLib2). In this experimental scheme, AAVLib1 and AAVLib2 each contain AAV9 and AAV9-N272A-HN1 vectors expressing epitope-tagged nlsGFP at a ratio of 1:1, but the capsid-epitope relationship is inverted in order to avoid potential antibody bias in downstream analyses. (FIG. 16B)

Representative tile-scanned brain section from one animal receiving AAVLib. Each AAV library was administered intravenously into a juvenile rhesus macaque at a dose of 3×10^{13} vg/kg. Tissue was harvested 3-weeks post-injection, cut into 4 mm slabs, fixed in 4% paraformaldehyde, and processed for immunohistochemical analysis with anti-GFP, anti-FLAG and anti-HA antibodies. eGFP expression indicates that a cell was transduced by either AAV9 or AAV9-N272A-HN1 or both. FLAG staining indicates that the AAV9 capsid mediated transduction, while HA staining indicates that AAV9-N272A-HN1 mediated transduction. Top-right inset, motor cortex; bottom-right inset, putamen. This experiment revealed that AAV9-N272A-HN1 transduced the brain cells better than AAV9 by several fold with strong neuronal tropism compared to AAV9. Therefore, as far as neuronal transduction is concerned, AAV9-N272A-HN1 mediates much higher neuronal transduction than AAV9.

[0031] FIGS. 17A-17D: Biodistribution of AAV9-N272A-HN1 to major peripheral organs following systemic delivery in mice and rhesus macaques. We used AAV DNA Barcode-Seq to determine relative abundance of AAV vector genome DNAs in each peripheral organ, delivered by each AAV capsid contained in the dsAAV-hSynI-GFP-BCLib library (Panels FIG. 17A, FIG. 17B and FIG. 17C). As explained earlier, the dsAAV-hSynI-GFP-BCLib library contained 26 AAV variants identified by TRADE in mice and in a non-human primate together with the controls, AAV9, AAV9-N272A and AAV-PHP.B. DNA was extracted from various tissues following administration of the dsAAV-hSynI-GFP-BCLib library (see Table 3) and subjected to AAV DNA Barcode-Seq analysis. We also used AAV RNA Barcode-Seq to determine relative transduction efficiency compared to AAV9 in each peripheral organ of rhesus macaques intravenously injected with the ssAAV-CAG-nlsGFP-BCLib library depicted in FIG. 16A (Panel D). (FIG. 17A) Biodistribution of AAV9, AAV9-N272A, AAV-PHP.B, and TRADE variants to the liver, relative to AAV9, in C57BL/6J mice, BALB/cJ mice and rhesus macaques. (FIG. 17B) Biodistribution of AAV9-N272A-HN1 to major peripheral organs besides the liver in C57BL/6J mice and BALB/cJ mice (n=3 mice/strain). (FIG. 17C) Biodistribution of AAV9-N272A-HN1 to major peripheral organs besides the liver in a rhesus macaque (n=1) based on dsAAV-hSynI-GFP-BC analysis. For this experiment, AAV DNA Barcode-Seq analysis was performed on the samples collected from one rhesus macaque injected with the dsAAV-hSynI-GFP-BCLib library shown in FIG. 14D. (FIG. 17D) Biodistribution of AAV9-N272A-HN1 to major peripheral organs besides the liver in rhesus (n=2) based on ssAAV-CAG-nlsGFP-BC analysis. For this experiment, AAV RNA Barcode-Seq analysis was performed on the samples collected from rhesus macaques injected with the ssAAV-GAG-nlsGFP-BCLib vectors shown in FIG. 16A. Error bars represent mean $\pm$ SEM. AAV9-N272A-HN1 capsid transduced peripheral organs to a lesser degree compared to AAV9 capsid.

[0032] FIGS. 18A-18C: AAV9-N272A-HN1 is highly neurotropic following systemic administration in mice. AAV9 and AAV9-N272A-HN1 vectors expressing nlsGFP under the control of the strong, ubiquitous CAG promoter were injected intravenously into 8-week old male BALB/cJ mice at a dose of 3×10^{11} vg/mouse. Tissues were harvested 12 days post-injection and analyzed by immunostaining

with anti-GFP and anti-NeuN antibodies. (FIG. 18A) A map of the single-stranded (ss) AAV-CAG-nlsGFP vector genomes used in this study. (FIG. 18B) Representative image from mouse cerebral cortex transduced with AAV9-N272A-HN1-CAG-nlsGFP. The vast majority of cells transduced with AAV9-N272A-HN1-CAG-nlsGFP are also positive for the neuronal marker NeuN. Scale bar=100 μ m. (FIG. 18C) Neuronal specificity of AAV9 and AAV9-N272A-HN1 capsids. Quantification of neuronal specificity was determined by dividing the number of double-positive cells (eGFP+/NeuN+) by the total number of GFP+ cells. AAV9-N272A-HN1 is highly specific to neurons (96%) compared to AAV9 (56%).

DETAILED DESCRIPTION

[0033] In some embodiments, the present disclosure provides a TRADE system that allows directed evolution of the AAV capsid using antisense mRNA of the cap ORF expressed in a cell type-specific or ubiquitous manner. Such a system does not require Cre-transgenic animals. Therefore, it can be applied to cell type-specific AAV capsid evolution in large animals, including non-human primates, for which Cre-transgenic strains are not readily available. Any cell type-specific or tissue/organ-specific enhancers/promoters or ubiquitous enhancers/promoters can be readily applied to the system with no requirement of transgenesis. The cell type-specific selection is given at the mRNA level. In certain embodiments, multiple directed evolution schemes may be combined into one directed evolution scheme. For example, selection of neuron-specific AAV capsids, astrocyte-specific AAV capsids, oligodendrocyte-specific AAV capsids and microglia-specific AAV capsids based on cell type-specific transgene mRNA expression can be performed simultaneously in a single animal.

[0034] In some embodiments, the present disclosure provides a sense strand TRADE system that allows directed evolution of the AAV capsid using mRNA of the cap ORF expressed in a cell type-specific or ubiquitous manner that is capable of expressing AAV capsid proteins in target cells. The sense strand TRADE has the same advantages of those antisense strand TRADE presented with data here in that it does not require Cre-transgenic animals, cell type-specific selection is given at the mRNA level, and it is capable of combining multiple directed evolution schemes into one directed evolution round done in a single animal. However, the possible disadvantage is that immunogenic AAV capsid proteins may be unavoidably expressed persistently in target cells, which may result in undesired consequences in the capsid selection process.

[0035] In some embodiments, the present disclosure also provides novel AAV capsids. In certain embodiments, these novel AAV capsids can transduce brain neurons several times better than AAV9 in C57BL/6J mice following intravenous injection. In certain embodiments, the novel AAV capsids transduced up to 8 times better than AAV9 in C57BL/6J mice following intravenous injection. The neuronal transduction levels may be greatly enhanced compared to AAV9 although they may not attain the levels obtained with AAV PHP.B. In certain embodiments, the novel AAV capsids may transduce brain neurons more efficiently than AAV PHP.B.

[0036] In some embodiments, this disclosure provides novel AAV capsids that can transduce brain neurons several times better than AAV9 following intravenous injection in

BALB/cJ mice. In certain embodiments, the novel AAV capsids can transduce brain neurons up to 7 times better than AAV9 following intravenous injection in BALB/cJ mice. The transduction levels are much higher than AAV PHP.B.

[0037] In some embodiments, this disclosure provides novel AAV capsids that can transduce brain neurons several times better than AAV9 in rhesus macaques following intravenous injection. In certain embodiments, the novel AAV capsids can transduce brain neurons up to 4 times better than AAV9 in rhesus macaques following intravenous injection. These transduction levels are better than AAV PHP.B.

[0038] In some embodiments, the disclosure provides AAV capsids that can transduce the pulmonary cells with neuronal cell marker expression several times better than AAV9. In certain embodiments, the AAV capsids can transduce such cells up to 17 times better than AAV9.

[0039] In some embodiments, the novel AAV capsids exhibit a liver-detargeting phenotype.

[0040] In some embodiments, the disclosure provides codon-modified AAV cap sequences that are not spliced when expressed in an antisense direction. We have observed that unmodified AAV cap ORFs are spliced when expressed in an antisense direction (e.g., AAV1, AAV3 and AAV9). In contrast, some of the codon-modified AAV cap ORFs described in this disclosure are not spliced. Based on the knowledge we have developed about the putative splice donor and acceptor sites, it has become possible to design such non-spliced versions of AAV cap ORFs. The use of such non-spliced cap ORFs may be used for directed evolution using the TRADE system when mutagenesis of the cap gene takes place over a wide region of the cap ORF.

[0041] The term “AAV vector” as used herein means any vector that comprises or derives from components of AAV and is suitable to infect mammalian cells, including human cells, of any of a number of tissue types, such as brain, heart, lung, skeletal muscle, liver, kidney, spleen, or pancreas, whether in vitro or in vivo. The term “AAV vector” may be used to refer to an AAV type viral particle (or virion) comprising at least a nucleic acid molecule encoding a protein of interest.

[0042] Additionally, the AAVs disclosed herein may be derived from various serotypes, including combinations of serotypes (e.g., “pseudotyped” AAV) or from various genomes (e.g., single-stranded or self-complementary). In particular embodiments, the AAV vectors disclosed herein may comprise desired proteins or protein variants. A “variant” as used herein refers to an amino acid sequence that is altered by one or more amino acids. The variant may have “conservative” changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. Alternatively, a variant may have “nonconservative” changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also include amino acid deletions or insertions, or both.

[0043] Nucleotide sequences, such as polynucleotides, encoding proteins of the present disclosure are provided herein. The nucleotides of the present disclosure can be composed of either RNA or DNA. The disclosure also encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein.

[0044] Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode the proteins of the present disclosure. In addition, it is well within the skill of a person trained in the art to create

alternative polynucleotide sequences encoding the same, or essentially the same, proteins disclosed herein. These variant or alternative polynucleotide sequences are within the scope of the current disclosure. As used herein, references to “essentially the same sequence” refers to one or more sequences that encode amino acid substitutions, deletions, additions, or insertions that do not eliminate the detectability of the polypeptide encoded by the polynucleotides of the present disclosure.

[0045] The current disclosure also includes variants of the polynucleotides and polypeptides disclosed herein. Variant sequences include those sequences wherein one or more peptides or nucleotides of the sequence have been substituted, deleted, and/or inserted.

[0046] Polynucleotide and polypeptide sequences of the current disclosure can also be defined in terms of particular identity and/or similarity with certain polynucleotides and polypeptides described herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical as compared to a sequence disclosed herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990). BLAST searches can be performed with the NBLAST program, score=100, wordlength=12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul et al. (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used.

[0047] Methods of producing AAV vectors as disclosed herein are well known in the art, including methods, for example, of using packaging cells, auxiliary viruses or plasmids, and/or baculovirus systems. See, e.g., Samulski et al., *J. Virology* 63, 3822 (1989); Xiao et al., *J. Virology* 72, 2224 (1998); Inoue et al., *J. Virology* 72, 7024 (1998); WO1998/022607; and WO2005/072364.

[0048] Methods of producing pseudotyped AAV vectors are also known (see, e.g., WO00/28004), as well as various modifications or formulations of AAV vectors, to reduce their immunogenicity upon in vivo administration (see, e.g., WO01/23001; WO00/73316; WO04/112727; WO05/005610; and WO99/06562). In some embodiments, AAV vectors may be prepared or derived from various serotypes of AAVs which may be mixed together or mixed with other types of viruses to produce chimeric (e.g., pseudotyped) AAV viruses.

[0049] In particular embodiments, the AAV vector may be a human serotype AAV vector. In such embodiments, a human AAV may be derived from any known serotype, e.g., from any one of serotypes 1-11, for instance from AAV1, AAV2, AAV4, AAV6, or AAV9.

[0050] The AAV vectors disclosed herein may include a nucleic acid encoding a protein of interest. In various embodiments, the nucleic acid also may include one or more regulatory sequences allowing expression and, in some embodiments, secretion of the protein of interest, such as e.g., a promoter, enhancer, polyadenylation signal, an internal ribosome entry site (“IRES”), a sequence encoding a protein transduction domain (“PTD”), a 2A peptide, and the like. Thus, in some embodiments, the nucleic acid may comprise a promoter region operably linked to the coding sequence to cause or improve expression of the protein of interest in infected cells. Such a promoter may be ubiquitous, cell- or tissue-specific, strong, weak, regulated, chimeric, etc., for example, to allow efficient and stable production of the protein in the infected tissue. The promoter may be homologous to the encoded protein, or heterologous, although generally promoters of use in the disclosed methods are functional in human cells. Examples of regulated promoters include, without limitation, Tet on/off element-containing promoters, rapamycin-inducible promoters, tamoxifen-inducible promoters, and metallothionein promoters. Other promoters that may be used include promoters that are tissue specific for tissues such as kidney, spleen, and pancreas. Examples of ubiquitous promoters include viral promoters, particularly the CMV promoter, the RSV promoter, the SV40 promoter, etc., and cellular promoters such as the phosphoglycerate kinase (PGK) promoter and the β -actin promoter.

[0051] In some embodiments of the AAV vectors disclosed herein, one or more feedback elements may be used to dampen over-expression of the protein of interest. For example, some embodiments of the AAV vectors may include one or more siRNA sequences that would target the exogenous transcript. In other embodiments, the AAV vector may include one or more additional promoters that may be recognized by inhibitory transcription factors. In various embodiments, the AAV vectors disclosed herein may comprise a construct that may create a homeostatic feedback loop that may maintain expression levels of the protein of interest at a physiological level.

[0052] In some embodiments of the AAV vectors disclosed herein, genome editing machinery may be used to genetically modify cellular genome DNA or mRNA transcripts at a site-specific manner. Komor et al., *Cell* 168, 20-36 (2017); and Katrekar et al., *Nature Methods* 16:239-242, 2019. For example, some embodiments of the AAV vectors may include a CRISPR-associated enzyme such as Cas9, a DNA base editor, an RNA editase and/or guide RNA (gRNA) to modify nucleic acid in cells in a site-specific manner. In addition, AAV vectors may contain a homology repair template (HDR) for genome editing.

[0053] In various embodiments, the AAV vectors disclosed herein can comprise a nucleic acid that may include a leader sequence allowing secretion of the encoded protein. In some embodiments, fusion of the transgene of interest with a sequence encoding a secretion signal peptide (usually located at the N-terminal of secreted polypeptides) may allow the production of the therapeutic protein in a form that can be secreted from the transduced cell. Examples of such signal peptides include the albumin, the β -glucuronidase, the alkaline protease or the fibronectin secretory signal peptides.

[0054] As described herein, effective and long-term expression of therapeutic proteins of interest in brain, heart, lung, skeletal muscle, kidney, spleen, or pancreas can be

achieved with non-invasive techniques, through peripheral administration of certain AAV vectors, such as a non-AAV9 vector with AAV9 sequences. Such peripheral administration may include any administration route that does not necessitate direct injection into brain, heart, lung, skeletal muscle, kidney, spleen, or pancreas. More particularly, peripheral administration may include systemic injections, such as intramuscular, intravascular (such as intravenous) intraperitoneal, intra-arterial, or subcutaneous injections. In some embodiments, peripheral administration also may include oral administration (see, e.g., WO96/40954), delivery using implants, (see, e.g., WO01/91803), or administration by instillation through the respiratory system, e.g., using sprays, aerosols or any other appropriate formulations.

[0055] In various embodiments, the desired doses of the AAV vectors may be adapted by the skilled artisan, e.g., depending on the disease condition, the subject, the treatment schedule, etc. In some embodiments, from 10^5 to 10^{12} viral genomes are administered per dose, for example, from 10^6 to 10^{11} , from 10^7 to 10^{11} , or from 10^8 to 10^{11} . In other embodiments, exemplary doses for achieving therapeutic effects may include virus titers of at least about 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} or 10^{11} viral genomes or more. Virus titer may also be expressed in terms of transducing units, which may be readily calculated by those of skill in the art.

[0056] In various embodiments, the AAV vectors disclosed herein may be administered in any suitable form, for instance, either as a liquid solution or suspension, as a solid form suitable for solution or suspension in liquid prior to injection, as a gel or as an emulsion. The vectors may be formulated with any appropriate and pharmaceutically acceptable excipient, carrier, adjuvant, diluent, etc. For instance, for injection, a suitable carrier or diluent may be an isotonic solution, a buffer, sterile and pyrogen-free water, or, for instance, a sterile and pyrogen-free phosphate-buffered saline solution. For inhalation, the carrier may be in particulate form.

[0057] The vectors may be administered in a “therapeutically-effective” amount, e.g., an amount that is sufficient to alleviate (e.g., decrease, reduce) at least one of the symptoms associated with a disease state, or to provide improvement in the condition of the subject. In some embodiments, repeated administrations may be performed, for instance using the same or a different peripheral administration route and/or the same vector or a distinct vector.

ENUMERATED EMBODIMENTS

Embodiment 1

[0058] A nucleic acid comprising: a Parvoviridae genome flanked by ITR sequences, wherein the Parvoviridae genome comprises a Parvoviridae intron, a Parvoviridae cap gene, and a first polyadenylation signal in a first orientation; a first promoter in the first orientation that drives expression of the Parvoviridae cap gene in the presence of adenoviral helper functions; and a second promoter and a second polyadenylation signal in a second orientation that is antisense with respect to the first orientation, and wherein the second polyadenylation signal is located at a position that causes termination of antisense mRNA transcription of the Parvoviridae cap gene.

Embodiment 2

[0059] The nucleic acid of embodiment 1, wherein the second promoter is a cell type-specific promoter.

Embodiment 3

[0060] The nucleic acid of embodiment 1, wherein the second promoter is a ubiquitous promoter.

Embodiment 4

[0061] The nucleic acid of any of embodiments 1-3, wherein the Parvoviridae genome is an AAV genome comprising an AAV intron and an AAV cap gene

Embodiment 5

[0062] The nucleic acid of embodiment 4, wherein the AAV cap gene is a wild-type AAV cap gene.

Embodiment 6

[0063] The nucleic acid of embodiment 5, wherein the AAV cap gene sequence is the AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or other natural AAV isolate cap gene sequence.

Embodiment 7

[0064] The nucleic acid of embodiment 4, wherein the AAV cap gene is an engineered AAV cap gene.

Embodiment 8

[0065] The nucleic acid of embodiment 4, wherein the AAV cap gene is one of a library of diverse AAV cap genes.

Embodiment 9

[0066] A nucleic acid library comprising a plurality of nucleic acids of embodiment 4, wherein the nucleic acids comprise a plurality of unique AAV cap gene sequences.

Embodiment 10

[0067] The nucleic acid library of embodiment 9, wherein the nucleic acid library comprises greater than about 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 or 10^8 unique AAV cap gene sequences.

Embodiment 11

[0068] The nucleic acid of any of embodiments 1-8, further comprising a gene of interest in the second orientation.

Embodiment 12

[0069] The nucleic acid of any of embodiments 4-8 or 11, wherein the second polyadenylation signal is located within the AAV intron.

Embodiment 13

[0070] The nucleic acid of any of embodiments 4-8 or 11, wherein the second polyadenylation signal is located such that the cap gene is correctly translated into a full-length capsid protein in the first orientation and the cap gene is correctly transcribed into anti-sense mRNA that contains a full-length AAV cap gene coding sequence.

Embodiment 14

[0071] A method for identifying an AAV vector with a cap gene sequence that has increased ability to transduce cells from a tissue of interest when compared to at least one other AAV vector with a different cap gene sequence, the method comprising: Preparing a first-round AAV TRADE vector library by introducing the nucleic acid library of embodiment 9 or 10 into an AAV packaging cell line and recovering the first round AAV TRADE vector library from the packaging cell line; Injecting one or more animals with the first-round AAV TRADE vector library; Recovering cap gene sequences of AAV vectors that are enriched in cells of the tissue of interest in the animals; Preparing a second-round AAV TRADE nucleic acid library comprising recovered cap gene sequences of the enriched AAV vectors and introducing this library into an AAV packaging cell line and recovering the second round AAV TRADE vector library from the packaging cell line; Performing a second round of enrichment by injecting one or more animals with the second-round AAV TRADE vector library and recovering cap gene sequence that are enriched in cells of the tissue of interest in the animals; and Identifying enriched AAV cap gene sequences after the first-round enrichment, after the second-round enrichment, and after any subsequent rounds of enrichment.

Embodiment 15

[0072] A method for producing an AAV TRADE vector or an AAV TRADE vector library comprising: Introducing the nucleic acid of any of embodiments 4-8 or 11-13, or the nucleic acid library of embodiment 9 or 10, into an AAV packaging cell line and recovering the AAV TRADE vector or AAV TRADE vector library from the packaging cell line.

Embodiment 16

[0073] A method for determining a sequence of a novel cap gene of an AAV vector that has increased ability to transduce cells from a tissue of interest comprising: Identifying the AAV vector according to the method of embodiment 14; Recovering antisense mRNA comprising the cap gene sequence; and determining the novel cap gene sequence.

Embodiment 17

[0074] The method of embodiment 16, wherein the anti-sense mRNA is recovered using RT-PCR.

Embodiment 18

[0075] The method of either embodiment 16 or 17, further comprising the step of determining the cap gene sequence

Embodiment 19

[0076] An AAV vector comprising the nucleic acid of any of embodiments 4-8 or 11-13.

Embodiment 20

[0077] A nucleic acid comprising: a Parvoviridae genome flanked by ITR sequences, wherein the Parvoviridae genome comprises a Parvoviridae intron, a Parvoviridae cap gene, and a first polyadenylation signal in a first orientation; a first promoter in the first orientation that drives expression of the

Parvoviridae cap gene in the presence of adenoviral helper functions; and a second promoter in the first orientation that drives expression of the Parvoviridae cap gene in the absence of adenoviral helper functions.

Embodiment 21

[0078] The nucleic acid of embodiment 20, wherein the second promoter is a cell type-specific promoter.

Embodiment 22

[0079] The nucleic acid of embodiment 20, wherein the second promoter is a ubiquitous promoter.

Embodiment 23

[0080] The nucleic acid of any of embodiments 20-22, wherein the Parvoviridae cap gene is a wild-type AAV cap gene.

Embodiment 24

[0081] The nucleic acid of embodiment 23, wherein the AAV cap gene sequences is the AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13 or other natural AAV isolate cap gene sequence.

Embodiment 25

[0082] The nucleic acid of any of embodiments 20-22, wherein the Parvoviridae cap gene is an engineered AAV cap gene.

Embodiment 26

[0083] The nucleic acid of any of embodiments 20-25, wherein the Parvoviridae cap gene is one of a library of diverse AAV cap genes.

Embodiment 27

[0084] A nucleic acid library comprising a plurality of nucleic acids of embodiment 20, wherein the nucleic acids comprise a plurality of unique Parvoviridae cap gene sequences.

Embodiment 28

[0085] The nucleic acid library of embodiment 27, wherein the nucleic acid library comprises greater than about 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 or 10^8 unique AAV cap gene sequences.

Embodiment 29

[0086] The nucleic acid of any of embodiments 20-26, further comprising a gene of interest.

Embodiment 30

[0087] A method for identifying an AAV vector with a cap gene sequence that has increased ability to transduce cells from a tissue of interest when compared to at least one other AAV vector with a different cap gene sequence, the method comprising: Preparing a first-round AAV TRADE vector library by introducing the nucleic acid library of embodiment 27 or 28 into an AAV packaging cell line and recovering the first round AAV TRADE vector library from the

packaging cell line; Injecting one or more animals with the first-round AAV TRADE vector library; Recovering cap gene sequences of AAV vectors that are enriched in cells of the tissue of interest in the animals; preparing a second-round AAV TRADE nucleic acid library comprising recovered cap gene sequences of the enriched AAV vectors and introducing this library into an AAV packaging cell line and recovering the second round AAV TRADE vector library from the packaging cell line; Performing a second round of enrichment by injecting one or more animals with the second-round AAV TRADE vector library and recovering cap gene sequence that are enriched in cells of the tissue of interest in the animals; and Identifying enriched AAV cap gene sequences after the first-round enrichment, after the second-round enrichment, and after any subsequent rounds of enrichment.

Embodiment 31

[0088] A method for producing an AAV TRADE vector or an AAV TRADE vector library comprising: Introducing the nucleic acid of any of embodiments 23-26 or 29, or the nucleic acid library of embodiment 27 or 28, into an AAV packaging cell line and recovering the AAV TRADE vector or AAV TRADE vector library from the packaging cell line.

Embodiment 32

[0089] A method for determining a sequence of a novel cap gene of an AAV vector that has increased ability to transduce cells from a tissue of interest comprising: Identifying the AAV vector according to the method of embodiment 30; Recovering sense mRNA comprising the cap gene sequence; and Determining the novel cap gene sequence.

Embodiment 33

[0090] The method of embodiment 32, wherein the sense mRNA is recovered using RT-PCR.

Embodiment 34

[0091] The method of either embodiment 32 or 33, further comprising the step of determining the cap gene sequence.

Embodiment 35

[0092] An AAV vector comprising the nucleic acid of any of embodiments 23-26 or 29.

Embodiment 36

[0093] The nucleic acid of any of embodiments 1-8 or 11-13, further comprising at least one mRNA splicing suppressing mutation in the second orientation.

Embodiment 37

[0094] The nucleic acid of embodiment 36, wherein the at least one mRNA splicing suppressing mutation comprises an alteration of one or more nucleotides located within ten nucleotides of the splice donor and/or splice acceptor site.

Embodiment 38

[0095] The nucleic acid of embodiment 36 or 37, wherein the alteration does not change the amino acid sequence encoded by the AAV cap gene.

Embodiment 39

[0096] An AAV cap ORF sequence comprising one or more following mutations in the exon-intron junctions at splicing donor sites:

AAV1 VP1 cap ORF (SEQ ID NO: 199)
1009-CTTAC(junction) CAGCA-1018*

AAV3 VP1 cap ORF (SEQ ID NO: 199)
1006-CTTAC(junction) CAGCA-1015*

AAV1 VP1 cap ORF (SEQ ID NO: 200)
1228-TTTAC(junction) CTTC-1237

AAV3 VP1 cap ORF (SEQ ID NO: 201)
1237-TATAC(junction) CTTTC-1246

AAV1 VP1 cap ORF (SEQ ID NO: 202)
1331-ATTAC(junction) CTGAA-1340

AAV1 VP1 cap ORF (SEQ ID NO: 203)
1434-GCTAC(junction) CTGGA-1443

AAV1 VP1 cap ORF (SEQ ID NO: 204)
1502-TTTAC(junction) CTGGA-1510

AAV1 VP1 cap ORF (SEQ ID NO: 205)
1803-ATTAC(junction) CTGGC-1812

AAV3 VP1 cap ORF (SEQ ID NO: 206)
1803-CTTAC(junction) CTGGC-1812

AAV1 VP1 cap ORF (SEQ ID NO: 207)
1835-TGTAC(junction) CTGCA-1844

AAV1 VP1 cap ORF (SEQ ID NO: 208)
2189-GTTAC(junction) CTTAC-2198

AAV9 VP1 cap ORF (SEQ ID NO: 209)
2189-GATAC(junction) CTGAC-2198

AAV1 VP1 cap ORF (SEQ ID NO: 210)
2194-CTTAC(junction) CCGTC-2203

AAV3 VP1 cap ORF (SEQ ID NO: 211)
2194-CTCAC(junction) ACGAA-2203.

(*Although the nucleotide numbers are different, they are corresponding nucleotides of the AAV cap ORFs in sequence alignment.)

Embodiment 40

[0097] An AAV cap ORF sequence comprising one or more following mutations in the exon-intron junctions at splicing donor sites:

AAV1 VP1 cap ORF (SEQ ID NO: 212)
305-AGCGT(junction) CTGCA-314

AAV1 VP1 cap ORF (SEQ ID NO: 213)
414-GGCTC(junction) CTGGA-423

AAV3 VP1 cap ORF (SEQ ID NO: 213)
414-GGCTC(junction) CTGGA-423

AAV1 VP1 cap ORF (SEQ ID NO: 214)
495-GCCCG(junction) CTAAA-504

AAV9 VP1 cap ORF (SEQ ID NO: 214)
495-GCCCG(junction) CTAAA-504

AAV3 VP1 cap ORF (SEQ ID NO: 215)
1133-TCACC(junction) CTGAA-1142

AAV1 VP1 cap ORF (SEQ ID NO: 216)
1181-ACTGC(junction) CTGGA-1190

AAV1 VP1 cap ORF (SEQ ID NO: 202)
1331-ATTAC(junction) CTGAA-1340**

AAV3 VP1 cap ORF (SEQ ID NO: 217)
1328-ACTAC(junction) CTGAA-1337**

AAV1 VP1 cap ORF (SEQ ID NO: 218)
1464-CGTTT(junction) CTAAA-1473

AAV1 VP1 cap ORF (SEQ ID NO: 219)
1653-AAACA(junction) CTGCA-1662

AAV1 VP1 cap ORF (SEQ ID NO: 220)
2054-GGGAG(junction) CTGCA-2063

AAV3 VP1 cap ORF (SEQ ID NO: 463)
2054-GGGAG(junction) CTACA-2063

(*Although the nucleotide numbers are different, they are corresponding nucleotides of the AAV cap ORFs in sequence alignment.)

Embodiment 41

[0098] An AAV cap ORF sequence comprising one or more following mutations in the exon-intron junctions at splicing donor or splicing acceptor sites:

Splice donors
AAV1 VP1 cap ORF (SEQ ID NO: 199)
1009-CTTAC(junction) CAGCA-1018*

AAV3 VP1 cap ORF (SEQ ID NO: 199)
1006-CTTAC(junction) CAGCA-1015*

-continued

AAV1 VP1 cap ORF (SEQ ID NO: 200)
1228-TTTAC(junction)CTTCA-1237

AAV3 VP1 cap ORF (SEQ ID NO: 201)
1237-TATAC(junction)CTTCG-1246

AAV1 VP1 cap ORF (SEQ ID NO: 202)
1331-ATTAC(junction)CTGAA-1340

AAV1 VP1 cap ORF (SEQ ID NO: 203)
1434-GCTAC(junction)CTGGA-1443

AAV1 VP1 cap ORF (SEQ ID NO: 204)
1502-TTTAC(junction)CTGGA-1510

AAV1 VP1 cap ORF (SEQ ID NO: 205)
1803-ATTAC(junction)CTGGC-1812

AAV3 VP1 cap ORF (SEQ ID NO: 206)
1803-CTTAC(junction)CTGGC-1812

AAV1 VP1 cap ORF (SEQ ID NO: 207)
1835-TGTAC(junction)CTGCA-1844

AAV1 VP1 cap ORF (SEQ ID NO: 208)
2189-GTTAC(junction)CTTAC-2198

AAV9 VP1 cap ORF (SEQ ID NO: 209)
2189-GATAC(junction)CTGAC-2198

AAV1 VP1 cap ORF (SEQ ID NO: 210)
2194-CTTAC(junction)CCGTC-2203

AAV3 VP1 cap ORF (SEQ ID NO: 211)
2194-CTCAC(junction)ACGAA-2203

Splice acceptor

AAV1 VP1 cap ORF (SEQ ID NO: 212)
305-AGCGT(junction)CTGCA-314

AAV1 VP1 cap ORF (SEQ ID NO: 213)
414-GGCTC(junction)CTGGA-423

AAV3 VP1 cap ORF (SEQ ID NO: 213)
414-GGCTC(junction)CTGGA-423

AAV1 VP1 cap ORF (SEQ ID NO: 214)
495-GCCCG(junction)CTAAA-504

AAV9 VP1 cap ORF (SEQ ID NO: 214)
495-GCCCG(junction)CTAAA-504

AAV3 VP1 cap ORF (SEQ ID NO: 215)
1133-TCACC(junction)CTGAA-1142

AAV1 VP1 cap ORF (SEQ ID NO: 216)
1181-ACTGC(junction)CTGGA-1190

-continued

AAV1 VP1 cap ORF (SEQ ID NO: 202)
1331-ATTAC(junction)CTGAA-1340**

AAV3 VP1 cap ORF (SEQ ID NO: 217)
1328-ACTAC(junction)CTGAA-1337**

AAV1 VP1 cap ORF (SEQ ID NO: 218)
1464-CGTTT(junction)CTAAA-1473

AAV1 VP1 cap ORF (SEQ ID NO: 219)
1653-AAACA(junction)CTGCA-1662

AAV1 VP1 cap ORF (SEQ ID NO: 220)
2054-GGGAG(junction)CTGCA-2063

AAV3 VP1 cap ORF (SEQ ID NO: 463)
2054-GGGAG(junction)CTACA-2063.

Examples

[0099] The following examples are for illustration only. In light of this disclosure, those of skill in the art will recognize that variations of these examples and other embodiments of the disclosed subject matter are enabled without undue experimentation.

[0100] We applied the TRADE system in both C57BL/6J mice and a rhesus macaque in order to identify novel AAV capsids that efficiently transduce brain neurons following systemic delivery. The TRADE system utilizes a plasmid construct containing an overlapping bicistronic AAV genome flanked by ITR sequences (FIG. 2A). In the sense direction, the AAV2 p40 promoter drives expression of the AAV cap gene to facilitate efficient production of viral particles (FIG. 2B). In the antisense direction, a cell type-specific enhancer-promoter (e.g. the human synapsin I (hSynI) enhancer-promoter) drives expression of transcripts encoding GFP and the antisense cap sequence (FIG. 2B), terminating at a polyadenylation signal (poly A) embedded in the intron present in the AAV2 genome. Utilizing the TRADE construct as a cloning backbone, we generated an AAV library based on the liver-detargeted AAV9-N272A (PCT/US2017/068050) cap gene platform that contained random 8-mer peptides with glycine-serine linkers (5'-GGGS; 3'-GGGS) substituted at the position Q588 in the AAV9 capsid. In vivo selection in a specific cell type (e.g. neurons) was performed by recovering capsid sequences as antisense cap ORF mRNA from brain tissue by RT-PCR. This method ensures that recovered sequences are only derived from AAV variants that are capable of mediating RNA expression in infected cells of our interest. When the hSynI enhancer-promoter is used, only sequences of AAV capsids that are capable of transducing neurons can be retrieved, thus enabling neuron-specific selection of AAV capsids.

[0101] We first tested the ability of the TRADE system to recover the sequence of the AAV cap gene from cell type-specific antisense mRNA using an AAV-PHP.B-hSynI-GFP-TRADE vector (FIGS. 3A-3G). A hSynI enhancer-promoter-driven GFP expression cassette was incorporated in the AAV-PHP. B capsid gene-containing AAV vector genome in the TRADE configuration (FIG. 3A). This vector genome was packaged into the AAV-PHP.B capsid, and the

resulting AAV vector was injected intravenously into two 8-week-old male C57BL/6J mice (FIG. 3B). Twelve days after injection, brain tissue was harvested. Tissue fixed with 4% paraformaldehyde was analyzed by immunofluorescence microscopy. Unfixed tissue was utilized for RNA extraction and RT-PCR analysis. We confirmed that eGFP was expressed only in neurons (FIG. 3C and FIG. 3D), indicating that the antisense mRNA transcribed from the cap gene is expressed in a cell type-specific manner. We recovered antisense mRNA of the cap gene efficiently by RT-PCR (FIG. 3E). Sanger sequencing of a splice junction unique to the antisense mRNA confirmed that RT-PCR products were indeed derived from the hSynI enhancer-promoter-driven antisense mRNA (FIG. 3F). In addition, Sanger sequencing confirmed the sequence of the PHP.B peptide insertion (FIG. 3F). Together, these observations established the ability of the TRADE system to successfully recover the AAV cap sequence from the hSynI enhancer-promoter-driven antisense mRNA expressed in AAV vector-transduced brain neurons.

[0102] With the successful establishment of the TRADE system, we performed two AAV capsid directed evolution experiments; one used 8-week-old male C57BL/6J mice and the other used one 8-month-old male rhesus macaque. We produced an AAV9-N272A-hSynI-GFP-TRADE-Lib library composed of AAV9-derived mutant capsids that have a GGGS(N₈)GGGS (SEQ ID NO:2) peptide insertion at the position of Q588 where N₈ represents a random 8-mer peptide encoded by (NNK)₈. For the peptide insertion, Q588 was substituted with each peptide sequence. The diversity of the AAV library was at least 10⁷. In the mouse directed evolution experiment, we infused the AAV library via the tail vein at a dose of 3×10¹¹ vector genomes (vg) per mouse. For the second round of selection, we injected the AAV library at a dose of 1×10¹², 1×10¹¹, 1×10¹⁰, or 1×10⁹ vector genomes (vg) using two mice. For the third round of selection, we injected the AAV library at a dose of 1×10¹¹ vg using two mice. We harvested brain tissues twelve days after injection, and separated them into three regions, i.e., the cerebrum, the cerebellum and the brain stem. Only the cerebrum samples were used for the directed evolution experiments. We extracted total RNA from the cerebrum, reverse-transcribed the RNA using an oligo dT primer, and amplified the peptide region including the flanking regions by a pair of the cap gene-specific PCR primers. The RT-PCR products were then used to create the next AAV9-N272A-hSynI-TRADE-Lib plasmid library, which was subsequently used to produce the next AAV9-N272A-hSynI-TRADE-Lib virus library. For the second and third round selection, we packaged an AAV9-N272A-hSynI-TRADE-Lib genome that was devoid of the GFP ORF. In the non-human primate directed evolution experiment, we infused the AAV9-N272A-hSynI-GFP-TRADE-Lib library via the saphenous vein at a dose of 2.0×10¹² vg per kg. Twelve days post-injection, the whole brain was harvested and sliced using a brain matrix, treated with RNAlater (Thermo Fisher Scientific), and stored frozen. Total RNA was then extracted from the following brain regions: frontal cortex, occipital cortex, cerebellum (Purkinje and granular layers), medulla, pons, frontal cortex, hypothalamus, thalamus, cingulate gyrus, caudate nucleus, putamen, hippocampus, and preoptic area. We retrieved the peptide sequences by RT-PCR in the same manner as described above except that we performed nested PCR to obtain PCR products

sufficient for the downstream Illumina and Sanger sequencing procedures. For some samples, we cloned the first PCR products directly into a plasmid backbone without performing nested PCR for Sanger sequencing. Following three rounds of selection in mice (Table 1) and one round of selection in non-human primate, we identified a number of potentially transduction-enhancing peptides inserted into the AAV9 capsids (Table 2). We then generated a barcoded AAV library and utilized DNA/RNA Barcode-Seq technology, previously developed in the Nakai lab (Adachi et al. *Nat Commun* 5, 3075 (2014); and PCT/US2017/068050), to compare the transduction efficiency, tropism/biodistribution, and pharmacokinetics of 26 selected novel AAV variants (Table 3) following intravenous administration in two commonly used mouse lines (C57BL/6J and BALB/cJ) and one rhesus macaque. As a result, we have found: (1) Some of the novel variants identified by TRADE technology, in particular AAV9-N272A-TTNLAKNS (HN1) and AAV9-N272A-QQNGTRPS (HN2), performed up to 8 times better than AAV9 in the brain of C57BL/6J mice (FIG. 14B and FIG. 14C). For HN_x designation, please refer to Table 3. (2) As previously reported by Hordeaux et al. (Hordeaux et al. 2018), AAV-PHP.B transduced the brain of BALB/cJ mice only at a level comparable to or lower than that of AAV9 (FIG. 14B and FIG. 14C), demonstrating a mouse strain dependency for AAV-PHP.B's robust neurotropic enhancement. (3) In contrast, AAV9-N272A-TTNLAKNS (HN1) and AAV9-N272A-QQNGTRPS (HN2) retained robust neuronal transduction in BALB/cJ mice showing up to 7 times better transduction than AAV9 (FIG. 14B). (4) In a rhesus macaque, many of the novel AAV mutants showed enhanced neuronal transduction, up to 4-fold greater than AAV9 in certain brain regions, while AAV-PHP.B transduced non-human primate brain similarly to or lower than AAV9. In particular, AAV9-N272A-TTNLAKNS (HN1) transduced the non-human primate brain best in multiple brain regions (FIG. 14C and FIG. 14D). (5) All of the AAV9-N272A-derived variants including HN1, HN2 and HN3 showed varying degrees of liver-detargeting properties in mice and rhesus macaques (FIG. 17A). (6) AAV9-N272A-TTNLAKNS (HN1) and AAV9-N272A-QQNGTRPS (HN2) can transduce cells with the hSynI enhancer-promoter transcriptional activity in the lung up to 17 times better than AAV9 in mice (FIG. 17B, Tables 4 and 6). (7) AAV9-N272A-TTNLAKNS (HN1) exhibits vector genome dissemination to peripheral organs to a lesser degree compared to AAV9 (FIG. 17C and FIG. 17D). The AAV Barcode-Seq data are summarized in Tables 4 to 9. Representative data presented in Tables 4 to 9 are also shown in a graph format in FIG. 14B, FIG. 14C and FIG. 17A, FIG. 17B, FIG. 17C, and FIG. 17D.

TABLE 1

Peptide sequences identified by the hSynI-TRADE system using an AAV9-N272N-GGGS (N ₈) GGGS library targeting mouse brain neurons.					
1st round		2nd round		3rd round	
ADKPPGLS	SEQ ID NO: 3	APTNFHP	SEQ ID NO: 97	AGAAYTPA	SEQ ID NO: 150
AGEDGSSR	SEQ ID NO: 4	AQTNLAA	SEQ ID NO: 98	APSVSREK	SEQ ID NO: 151

TABLE 1-continued

Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272N-GGGS (N ₈) GGGGS library targeting mouse brain neurons.					
1st round		2nd round		3rd round	
ALGTATQR	SEQ ID NO: 5	ASLPNLGQ	SEQ ID NO: 99	DYMHKTGL	SEQ ID NO: 152
ALNTALVE	SEQ ID NO: 6	DYMHNTGL	SEQ ID NO: 100	EEDAQLLI (2)	SEQ ID NO: 14
AMVRLTHN	SEQ ID NO: 7	DYMHTTGL	SEQ ID NO: 101	ENKSAPLP	SEQ ID NO: 18
ASRDPSAT	SEQ ID NO: 8	ERNAWHAG	SEQ ID NO: 102	GDYTVQRP	SEQ ID NO: 107
DANDARQR	SEQ ID NO: 9	ETQATPMP	SEQ ID NO: 103	GGMNETTR	SEQ ID NO: 153
DLARMAAA	SEQ ID NO: 10	EWEDSARS	SEQ ID NO: 104	GGSAFVTG	SEQ ID NO: 154
DQGSITAH	SEQ ID NO: 11	FTGDTDTL	SEQ ID NO: 105	GGSPLAHP	SEQ ID NO: 21
DRTPGVNV	SEQ ID NO: 12	FTNRTSTT	SEQ ID NO: 106	GNSHTGSS	SEQ ID NO: 155
DTDTLSPG	SEQ ID NO: 13	GDYTVQRP	SEQ ID NO: 107	GPQEGSER (2)	SEQ ID NO: 109
EEDAQLLI	SEQ ID NO: 14	GGLRTDYG	SEQ ID NO: 108	GQRGLPIA	SEQ ID NO: 27
EKLNDWPT	SEQ ID NO: 15	GGSPLAHP	SEQ ID NO: 21	GSNHTQSL	SEQ ID NO: 110
ELNSARQV	SEQ ID NO: 16	GKQPVQPY	SEQ ID NO: 24	HQVTSSGA (4)	SEQ ID NO: 33
ELQSFAGL	SEQ ID NO: 17	GPQEGSER	SEQ ID NO: 109	LEQQRGAS	SEQ ID NO: 113
ENKSAPLP	SEQ ID NO: 18	GSNHTQSL	SEQ ID NO: 110	LERNRDS	SEQ ID NO: 39
ERTAVKGN	SEQ ID NO: 19	GTPQTTKE	SEQ ID NO: 29	LLVTARSH (3)	SEQ ID NO: 44
GGIQTVVT	SEQ ID NO: 20	HDRDTRQA	SEQ ID NO: 111	MESQRANS (2)	SEQ ID NO: 117
GGSPLAHP	SEQ ID NO: 21	LDQNRPPQ	SEQ ID NO: 112	MSGQGYQA (2)	SEQ ID NO: 50
GGTAAQGV	SEQ ID NO: 22	LEQQRGAS	SEQ ID NO: 113	NSARTQLS	SEQ ID NO: 156
GKMASGSL	SEQ ID NO: 23	LERNRDS	SEQ ID NO: 39	PLTILNRH	SEQ ID NO: 157
GKQPVQPY	SEQ ID NO: 24	LGGNAQGL	SEQ ID NO: 114	QGTRTNPP	SEQ ID NO: 158
GNPHTGST	SEQ ID NO: 25	LLVTTRSH	SEQ ID NO: 115	QQNGTRPS (4)	SEQ ID NO: 128
GPTLGGSG	SEQ ID NO: 26	LVTNTTR	SEQ ID NO: 116	QSGDSALN (3)	SEQ ID NO: 67
GQRGLPIA	SEQ ID NO: 27	MESQRANS	SEQ ID NO: 117	QSSAMPRN (2)	SEQ ID NO: 159
GREPRRLH	SEQ ID NO: 28	MISQTLMA	SEQ ID NO: 118	SATISLQV	SEQ ID NO: 136

TABLE 1-continued

Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272N-GGGS (N ₈) GGGGS library targeting mouse brain neurons.					
1st round		2nd round		3rd round	
GTPQTTKE	SEQ ID NO: 29	MMSQSLRA	SEQ ID NO: 119	SHNSQPVA	SEQ ID NO: 160
GVTERPNR	SEQ ID NO: 30	NNVQSALN	SEQ ID NO: 120	SHTNLRDT	SEQ ID NO: 137
HLGDNLAR	SEQ ID NO: 31	NSARTQLS	SEQ ID NO: 121	SSGYLTAN	SEQ ID NO: 139
HPGSGAGP	SEQ ID NO: 32	PQWNRTPL	SEQ ID NO: 122	TAQGAAFR (4)	SEQ ID NO: 161
HQVTSSGA	SEQ ID NO: 33	PRFNNSSL	SEQ ID NO: 123	TPGLNNAR	SEQ ID NO: 162
HVGSQMHA	SEQ ID NO: 34	PRPTVVGT	SEQ ID NO: 60	TSLGTPEA	SEQ ID NO: 163
IG*TVPMQ	SEQ ID NO: 35	PVDGGRHL	SEQ ID NO: 124	TTNLAKNS (6)	SEQ ID NO: 164
KFTRDGPY	SEQ ID NO: 36	PWFNKSSL	SEQ ID NO: 125	VVQGEQKR (4)	SEQ ID NO: 146
KGPAEQGH	SEQ ID NO: 37	QDMNSQRS	SEQ ID NO: 126	WSPDAVEG	SEQ ID NO: 165
LAHSPRLW	SEQ ID NO: 38	QGASNSQL	SEQ ID NO: 127	WSQDAVKG (2)	SEQ ID NO: 148
LERNRDS	SEQ ID NO: 39	QQNGTRPS	SEQ ID NO: 128	WTGGSGT (3)	SEQ ID NO: 149
LEHTSLT	SEQ ID NO: 40	QRSAYPTS	SEQ ID NO: 129	WTGGRHL	SEQ ID NO: 166
LHDGKYST	SEQ ID NO: 41	QRTPSITP	SEQ ID NO: 130		
LKATGRGK	SEQ ID NO: 42	QWMKEQAG	SEQ ID NO: 131		
LLPGSADG	SEQ ID NO: 43	RDGRHPSE	SEQ ID NO: 132		
LLVTARSH	SEQ ID NO: 44	RGTVTVEQ	SEQ ID NO: 133		
LPEVEPTN	SEQ ID NO: 45	RPAHSTA	SEQ ID NO: 134		
LPWENSSQ	SEQ ID NO: 46	RQGDADTL	SEQ ID NO: 135		
LQRNSDAN	SEQ ID NO: 47	SATISLQV	SEQ ID NO: 136		
LQSAPRAT	SEQ ID NO: 48	SHTNLRDT	SEQ ID NO: 137		
MLGSQVPT	SEQ ID NO: 49	SRMGETPQ	SEQ ID NO: 138		
MSGQGYQA	SEQ ID NO: 50	SSGYLTAN	SEQ ID NO: 139		
NPGRDFRD	SEQ ID NO: 51	SSVVSQGP	SEQ ID NO: 79		

TABLE 1-continued					
Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272N-GGGS(N ₈)GGGS library targeting mouse brain neurons.					
1st round		2nd round		3rd round	
NQPSDYVS	SEQ ID NO: 52	TGNSPEQA	SEQ ID NO: 140		
NSVGSADK	SEQ ID NO: 53	THSQGRLA	SEQ ID NO: 141		
NVQRTQRG	SEQ ID NO: 54	TPIVGSNV	SEQ ID NO: 142		
PAQLNGPR	SEQ ID NO: 55	TPPKSPSM	SEQ ID NO: 143		
PERERLPR	SEQ ID NO: 56	TRMDERSP	SEQ ID NO: 144		
PGNGSHTM	SEQ ID NO: 57	TTATTSIT	SEQ ID NO: 145		
PIPGTPQP	SEQ ID NO: 58	VVQGEQKR	SEQ ID NO: 146		
PMSVPASN	SEQ ID NO: 59	WNDRSGER	SEQ ID NO: 147		
PRPTWGT	SEQ ID NO: 60	WSQDAVKG	SEQ ID NO: 148		
PRTNRGPE	SEQ ID NO: 61	WTGGSGT	SEQ ID NO: 149		
PVANPTTA	SEQ ID NO: 62				
PVLGGPPK	SEQ ID NO: 63				
QGSRQGSS	SEQ ID NO: 64				
QMAETPIS	SEQ ID NO: 65				
QMLGIGRS	SEQ ID NO: 66				
QSGDSALN	SEQ ID NO: 67				
RAGLTSSE	SEQ ID NO: 68				
RLDNTGVG	SEQ ID NO: 69				
RMPGKPYS	SEQ ID NO: 70				
RVAGASQP	SEQ ID NO: 71				
RVESSQLE	SEQ ID NO: 72				
SARTGASE	SEQ ID NO: 73				
SERNRASM	SEQ ID NO: 74				
SIDVRMAA	SEQ ID NO: 75				

TABLE 1-continued					
Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272N-GGGS(N ₈)GGGS library targeting mouse brain neurons.					
1st round		2nd round		3rd round	
SRDGHILR	SEQ ID NO: 76				
SRQVVLPG	SEQ ID NO: 77				
SSRGYTST	SEQ ID NO: 78				
SSVVSQGP	SEQ ID NO: 79				
SVAESGRE	SEQ ID NO: 80				
TALTANTQ	SEQ ID NO: 81				
TESSVGNL	SEQ ID NO: 82				
TGREGANL	SEQ ID NO: 83				
TLSEPPKK	SEQ ID NO: 84				
TNAVSGKS	SEQ ID NO: 85				
TRAPTIHL	SEQ ID NO: 86				
TRESTDRG	SEQ ID NO: 87				
TVAAAPNL	SEQ ID NO: 88				
TYHNNTPR	SEQ ID NO: 89				
VSNSTRTS	SEQ ID NO: 90				
VTLQIDTK	SEQ ID NO: 91				
WMSRPGPT	SEQ ID NO: 92				
WPYRGLTQ	SEQ ID NO: 93				
WRRQGSRA	SEQ ID NO: 94				
YAQRFAKM	SEQ ID NO: 95				

TABLE 1-continued		
Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272N-GGGS(N ₈)GGGGS library targeting mouse brain neurons.		
1st round	2nd round	3rd round
YNSPRQTV SEQ ID NO: 96		
The table lists peptide insertions on AAV9-N272A after each of three rounds of selection. The numbers in parentheses indicate the frequency of each peptide among a total of 69 peptides identified after the three round of selection. Peptides with no number were found only once. The sequences of the peptide region were determined by Sanger sequencing. Actual peptide sequences were randomized octapeptides flanked by glycine-serine linkers such that position Q588 was substituted with GGGS(N₈)GGGGS. For example, "-TNHQSAGGGSTTNLAKNSGGGSAQAQTG-" for TTNLAKNS and "-TNHQSAGGGSQQNGTRPSGGGSAQAQTG-" for QQNGTRPS.		

TABLE 2	
Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272A- GGGS(N ₈)GGGGS library targeting rhesus macaque brain neurons.	
1st round	
AVAGDRLL SEQ ID NO: 167	
DLLTRSVS SEQ ID NO: 168	
EWKTQLAL SEQ ID NO: 169	
GNINVVPH SEQ ID NO: 170	
GSPAASSW SEQ ID NO: 171	
KHSLTLES SEQ ID NO: 172	
KPVSTDTF SEQ ID NO: 173	
LDRSGSTG SEQ ID NO: 174	

TABLE 2-continued	
Peptide sequences identified by the hSyn1-TRADE system using an AAV9-N272A- GGGS(N ₈)GGGGS library targeting rhesus macaque brain neurons.	
1st round	
LGAQNHVV SEQ ID NO: 175	
LMATDYGP SEQ ID NO: 176	
LRATDYGP SEQ ID NO: 177	
MERTEPLG SEQ ID NO: 178	
NDGLRLHL SEQ ID NO: 179	
NLSAHSHA SEQ ID NO: 180	
NLSAHSHD SEQ ID NO: 181	
RALDLVTR SEQ ID NO: 182	
SAGMARNS SEQ ID NO: 183	
SGQRVGSA SEQ ID NO: 184	
SGQRVGSD SEQ ID NO: 185	
TAQGAAFR SEQ ID NO: 161	
TGRPEQPK SEQ ID NO: 186	
THSPIKLP SEQ ID NO: 187	
TQFSQAQR SEQ ID NO: 188	
VGDSANLR SEQ ID NO: 189	
The sequences of the peptide region were determined either by Illumina sequencing or Sanger sequencing. Actual peptide sequences were randomized octapeptides flanked by glycine-serine linkers such that position Q588 was substituted with GGGS(N)GGGGS. These peptides were recovered from frontal cortex, occipital cortex, hypothalamus and thalamus.	

TABLE 3			
A list of the 29 AAV capsids contained in the DNA/RNA-barcoded dsAAV-hSyn1-GFP-BCLib library used for phenotype determination of each AAV strain.			
AAV strain (AAV capsid)	Abbreviation	Number of viral clones in the AAV library	Note
AAV9	AAV9	15	Reference
AAV9-N272A	AAV9-N272A	5	Reference
AAV-PHP.B	AAV-PHP.B	2	Reference
AAV9-N272A-TTNLAKNS (peptide insertion site SEQ ID NO: 164)	HN1	2	TRADE variant (C57BL/6J)
AAV9-N272A-QQNGTRPS (peptide insertion site SEQ ID NO: 128)	HN2	2	TRADE variant (C57BL/6J)
AAV9-N272A-SGQRVGSD (peptide insertion site SEQ ID NO: 185)	HN3	2	TRADE variant (rhesus macaque)
AAV9-N272A-AVAGDRLL (peptide insertion site SEQ ID NO: 167)	HN4	2	TRADE variant (rhesus macaque)

TABLE 3-continued

A list of the 29 AAV capsids contained in the DNA/RNA-barcoded dsAAV-hSyn1-GFP-BCLib library used for phenotype determination of each AAV strain.			
AAV strain (AAV capsid)	Abbreviation	Number of viral clones in the AAV library	Note
AAV9-N272A-DLLTRSVS (peptide insertion site SEQ ID NO: 168)	HN5	2	TRADE variant (rhesus macaque)
AAV9-N272A-EWKTQLAL (peptide insertion site SEQ ID NO: 169)	HN6	2	TRADE variant (rhesus macaque)
AAV9-N272A-GNINWPH (peptide insertion site SEQ ID NO: 170)	HN7	2	TRADE variant (rhesus macaque)
AAV9-N272A-GSPAASSW (peptide insertion site SEQ ID NO: 171)	HN8	2	TRADE variant (rhesus macaque)
AAV9-N272A-KHSLTLES (peptide insertion site SEQ ID NO: 172)	HN9	2	TRADE variant (rhesus macaque)
AAV9-N272A-KPVSTDTF (peptide insertion site SEQ ID NO: 173)	HN10	2	TRADE variant (rhesus macaque)
AAV9-N272A-LDRSGSTG (peptide insertion site SEQ ID NO: 174)	HN11	2	TRADE variant (rhesus macaque)
AAV9-N272A-LGAQNHVV (peptide insertion site SEQ ID NO: 175)	HN12	2	TRADE variant (rhesus macaque)
AAV9-N272A-LRATDYGP (peptide insertion site SEQ ID NO: 177)	HN13	2	TRADE variant (rhesus macaque)
AAV9-N272A-MERTEPLG (peptide insertion site SEQ ID NO: 178)	HN14	2	TRADE variant (rhesus macaque)
AAV9-N272A-NDGLRLHL (peptide insertion site SEQ ID NO: 179)	HN15	2	TRADE variant (rhesus macaque)
AAV9-N272A-NLSAHSKD (peptide insertion site SEQ ID NO: 181)	HN16	2	TRADE variant (rhesus macaque)
AAV9-N272A-RALDLVTR (peptide insertion site SEQ ID NO: 182)	HN17	2	TRADE variant (rhesus macaque)
AAV9-N272A-SAGMARN (peptide insertion site SEQ ID NO: 183)	HN18	2	TRADE variant (rhesus macaque)
AAV9-N272A-TAQGAAGR (peptide insertion site SEQ ID NO: 161)	HN19	2	TRADE variant (rhesus macaque)
AAV9-N272A-TGRPEQPK (peptide insertion site SEQ ID NO: 186)	HN20	2	TRADE variant (rhesus macaque)
AAV9-N272A-THSPIKLP (peptide insertion site SEQ ID NO: 187)	HN21	2	TRADE variant (rhesus macaque)

TABLE 3-continued

A list of the 29 AAV capsids contained in the DNA/RNA-barcoded dsAAV-hSyn1-GFP-BCLib library used for phenotype determination of each AAV strain.			
AAV strain (AAV capsid)	Abbreviation	Number of viral clones in the AAV library	Note
AAV9-N272A-TQFSQAQR (peptide insertion site SEQ ID NO: 188)	HN22	2	TRADE variant (rhesus macaque)
AAV9-N272A-VGDSANLR (peptide insertion site SEQ ID NO: 189)	HN23	2	TRADE variant (rhesus macaque)
AAV9-N272A-HQVTSSGA (peptide insertion site SEQ ID NO: 33)	HN24	2	TRADE variant (mouse)
AAV9-N272A-LLVTARSH (peptide insertion site SEQ ID NO: 44)	HN25	2	TRADE variant (mouse)
AAV9-N272A-VVQGEQKR (peptide insertion site SEQ ID NO: 146)	HN26	2	TRADE variant (mouse)

The novel AAV9-hSyn1-TRADE-derived capsid variants were selected from those identified following three rounds of selection in mice (Table 1) and one round of selection in a rhesus macaque (Table 2). Each recovered AAV variant was assigned an abbreviation, HN_x. A DNA/RNA-barcoded dsAAV-hSyn1-GFP-BCLib library containing was constructed such that each AAV variant packaged a unique dsAAV-hSyn1-GFP-BC viral genome expressing AAV variant-specific RNA barcodes. The number of unique AAV barcode clones for each variant, including critical reference variants, is presented in this table.

TABLE 4

Brain neuronal transduction efficiency and biodistribution of the TRADE-identified AAV variants in C57BL/6J mice following intravenous administration.										
	Brain (RNA)	Lung (RNA)	Heart (DNA)	Kidney (DNA)	Liver (DNA)	Lung (DNA)	Muscle (DNA)	Pancreas (DNA)	Spleen (DNA)	Testis (DNA)
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	1.93	0.22	0.82	2.11	0.06	0.51	0.17	0.25	4.49	1.42
AAV-PHP.B	9.42	0.64	0.92	2.35	0.27	0.81	0.36	0.62	2.69	1.45
HN1	8.01	2.74	0.21	0.62	0.24	3.40	0.36	0.42	0.41	0.64
HN2	6.52	1.19	0.36	0.56	0.24	2.55	0.32	0.33	0.66	0.91
HN3	2.35	0.41	0.95	1.29	0.09	0.45	0.20	0.25	2.57	1.45
HN4	0.87	0.15	0.65	2.96	0.02	0.48	0.11	0.21	4.02	1.22
HN5	0.46	0.10	0.57	2.66	0.01	0.47	0.09	0.21	3.90	1.10
HN6	0.37	0.22	0.64	3.13	0.01	0.52	0.08	0.27	4.27	1.22
HN7	1.03	0.21	0.40	0.54	0.25	0.17	0.23	0.10	0.75	0.43
HN8	0.74	0.11	0.61	2.61	0.02	0.45	0.09	0.16	3.27	1.06
HN9	1.47	0.28	0.62	1.49	0.08	0.30	0.15	0.16	2.41	0.82
HN10	1.40	0.18	0.64	1.48	0.04	0.24	0.12	0.16	2.64	0.97
HN11	1.38	0.21	0.73	1.37	0.05	0.29	0.15	0.17	3.17	1.12
HN12	0.80	0.17	0.26	0.42	0.24	0.16	0.20	0.06	0.49	0.21
HN13	1.59	0.28	0.77	1.17	0.10	0.28	0.19	0.18	2.28	0.93
HN14	0.45	0.05	0.47	1.31	0.01	0.20	0.07	0.14	1.86	0.65
HN15	0.50	0.21	0.68	3.48	0.01	0.58	0.10	0.24	4.70	1.30
HN16	1.43	0.24	0.58	1.28	0.02	0.32	0.11	0.22	3.70	1.07
HN17	0.29	0.07	0.50	2.80	0.01	0.46	0.08	0.19	3.73	1.05
HN18	1.46	0.12	0.68	1.78	0.11	0.28	0.17	0.14	2.10	0.92
HN19	0.56	0.10	0.57	3.07	0.01	0.52	0.09	0.18	3.98	1.16
HN20	1.68	0.35	0.90	1.10	0.18	0.29	0.24	0.18	1.89	0.89
HN21	0.26	0.08	0.50	2.53	0.01	0.46	0.06	0.14	3.44	1.01
HN22	0.82	0.06	0.51	2.55	0.02	0.42	0.10	0.19	3.32	1.05
HN23	2.45	0.26	0.72	1.02	0.05	0.30	0.15	0.21	2.25	1.12
HN24	1.33	0.22	0.63	1.31	0.05	0.28	0.14	0.15	2.83	0.90

TABLE 4-continued

Brain neuronal transduction efficiency and biodistribution of the TRADE-identified AAV variants in C57BL/6J mice following intravenous administration.										
	Brain (RNA)	Lung (RNA)	Heart (DNA)	Kidney (DNA)	Liver (DNA)	Lung (DNA)	Muscle (DNA)	Pancreas (DNA)	Spleen (DNA)	Testis (DNA)
HN25	0.37	0.12	0.64	3.25	0.02	0.53	0.09	0.22	4.35	1.23
HN26	0.73	0.14	0.63	2.53	0.06	0.40	0.12	0.17	2.86	1.07

A DNA/RNA-barcoded dsAAV-hSynl-GFP-BC library (dsAAV-hSynl-GFP-BCLib) containing 26 novel AAV variants identified by TRADE and control AAV capsids was injected intravenously into 3 C57BL/6J mice at a dose of 5×10^{11} vg per mouse (for the library, see Table 3).
Two weeks post-injection, various tissues were harvested and analyzed for brain transduction by AAV RNA Barcode-Seq and biodistribution to peripheral organs by AAV DNA Barcode-Seq.
All the values are normalized with those of AAV9 (AAV9 = 1.0).

TABLE 5

Pharmacokinetic profiles of TRADE-identified AAV variants in C57BL/6J mice following intravenous administration.								
	1 m	10 m	30 m	1 h	4 h	8 h	24 h	72 h
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	1.16	1.17	1.28	1.34	1.77	2.02	3.14	0.13
AAV-PHP.B	1.38	1.42	1.44	1.62	1.88	2.15	3.14	0.39
HN1	0.87	0.47	0.29	0.29	0.27	0.31	0.37	0.02
HN2	0.79	0.60	0.49	0.55	0.59	0.66	0.91	0.03
HN3	1.12	1.15	1.21	1.32	1.65	1.88	3.05	0.04
HN4	1.32	1.50	1.43	1.52	2.01	2.37	3.78	0.03
HN5	1.14	1.30	1.37	1.42	1.84	2.03	3.37	0.02
HN6	1.21	1.34	1.43	1.60	1.99	2.38	3.90	0.02
HN7	0.92	0.88	0.93	0.93	1.09	1.21	1.22	0.03
HN8	1.20	1.29	1.30	1.44	1.83	2.10	3.42	0.03
HN9	0.94	0.91	0.97	0.99	1.26	1.36	1.97	0.04
HN10	0.98	0.98	1.00	1.06	1.31	1.36	1.89	0.02
HN11	1.00	1.04	1.04	1.14	1.39	1.48	2.11	0.02
HN12	0.93	0.93	0.84	0.79	0.71	0.61	0.62	0.01
HN13	0.95	0.90	0.95	0.95	1.20	1.28	1.60	0.03
HN14	0.94	0.95	1.00	1.08	1.41	1.58	2.56	0.01

TABLE 5-continued

Pharmacokinetic profiles of TRADE-identified AAV variants in C57BL/6J mice following intravenous administration.								
	1 m	10 m	30 m	1 h	4 h	8 h	24 h	72 h
HN15	1.39	1.56	1.67	1.66	2.20	2.76	4.26	0.03
HN16	0.98	1.00	1.04	1.15	1.46	1.63	2.77	0.02
HN17	1.32	1.28	1.27	1.31	1.94	2.13	4.03	0.04
HN18	1.10	1.06	0.96	0.93	0.82	0.84	1.27	0.01
HN19	1.39	1.39	1.51	1.49	2.04	2.50	4.09	0.03
HN20	1.15	1.09	1.19	1.14	1.41	1.70	1.97	0.06
HN21	1.25	1.19	1.34	1.38	1.90	1.99	3.30	0.02
HN22	1.16	1.24	1.32	1.35	1.74	2.13	3.74	0.02
HN23	1.03	1.02	1.04	1.14	1.43	1.64	2.56	0.03
HN24	0.99	1.01	1.05	1.16	1.45	1.58	2.38	0.03
HN25	1.29	1.40	1.44	1.49	1.93	2.49	3.74	0.03
HN26	1.21	1.19	1.29	1.30	1.74	2.03	3.09	0.03

AAV DNA Barcode-Seq analysis was performed on the blood samples obtained from the mice injected with 1×10^{13} vg/kg of the DNA/RNA-barcoded dsAAV-hSynl-GFP-BCLib library (see Table 3, n = 2) All the values are normalized with those of AAV9 (AAV9 = 1.0).
All the values are normalized to AAV9 (AAV9 = 1.0).

TABLE 6

Brain neuronal transduction efficiency and biodistribution of the TRADE-identified AAV variants in BALB/cJ mice following intravenous administration.										
	Brain (RNA)	Lung (RNA)	Heart (DNA)	Kidney (DNA)	Liver (DNA)	Lung (DNA)	Muscle (DNA)	Pancreas (DNA)	Spleen (DNA)	Testis (DNA)
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	1.00	0.30	0.45	1.23	0.03	0.64	0.29	1.23	2.75	0.17
AAV-PHP.B	1.06	0.44	0.56	1.30	0.22	0.67	0.40	1.12	1.69	0.30
HN1	7.59	17.84	0.18	0.52	0.05	3.75	0.21	0.28	0.31	0.36
HN2	3.26	4.71	0.31	0.52	0.07	3.13	0.25	0.39	0.69	0.44
HN3	1.00	0.35	0.51	1.04	0.04	0.41	0.20	0.60	1.38	0.14
HN4	0.41	0.41	0.29	1.58	0.01	0.44	0.16	1.18	2.50	0.09
HN5	0.28	0.15	0.25	1.34	0.00	0.38	0.13	1.05	2.25	0.07
HN6	0.25	0.06	0.30	1.60	0.01	0.45	0.17	1.17	2.69	0.07
HN7	0.54	0.21	0.27	0.65	0.12	0.22	0.16	0.32	0.75	0.11
HN8	0.34	0.03	0.28	1.53	0.01	0.38	0.14	0.94	2.10	0.07
HN9	0.49	0.19	0.28	1.18	0.02	0.22	0.11	0.45	1.08	0.07
HN10	0.54	0.19	0.33	1.25	0.01	0.30	0.14	0.50	1.82	0.08
HN11	0.23	0.12	0.23	1.15	0.03	0.17	0.09	0.62	0.95	0.05
HN12	0.34	0.13	0.19	0.98	0.03	0.12	0.07	0.23	0.48	0.04
HN13	0.43	0.25	0.36	1.25	0.03	0.25	0.15	0.41	1.01	0.09
HN14	0.22	0.08	0.20	0.93	0.02	0.14	0.08	0.38	0.68	0.04
HN15	0.25	0.26	0.33	1.80	0.01	0.46	0.19	1.39	3.03	0.10
HN16	0.62	0.25	0.32	0.93	0.01	0.41	0.16	0.71	1.55	0.10
HN17	0.18	0.12	0.22	1.40	0.00	0.37	0.13	0.96	2.29	0.07
HN18	0.75	0.16	0.40	1.46	0.04	0.29	0.15	0.59	1.36	0.08
HN19	0.28	0.10	0.28	1.57	0.01	0.44	0.15	1.14	2.51	0.08
HN20	0.69	0.11	0.42	0.56	0.08	0.31	0.13	0.53	0.85	0.12
HN21	0.14	0.15	0.25	1.38	0.00	0.34	0.11	0.91	2.34	0.08

TABLE 6-continued

Brain neuronal transduction efficiency and biodistribution of the TRADE-identified AAV variants in BALB/cJ mice following intravenous administration.										
	Brain (RNA)	Lung (RNA)	Heart (DNA)	Kidney (DNA)	Liver (DNA)	Lung (DNA)	Muscle (DNA)	Pancreas (DNA)	Spleen (DNA)	Testis (DNA)
HN22	0.41	0.09	0.22	1.33	0.01	0.36	0.12	1.01	2.09	0.07
HN23	0.79	0.32	0.33	0.99	0.02	0.36	0.17	0.43	1.24	0.10
HN24	0.56	0.25	0.34	1.17	0.02	0.34	0.14	0.53	1.33	0.08
HN25	0.19	0.02	0.30	1.65	0.01	0.49	0.17	1.15	2.68	0.09
HN26	0.31	0.11	0.33	1.52	0.02	0.35	0.17	0.84	1.77	0.09

A DNA/RNA-barcoded dsAAV-hSynl-GFP-BC library (dsAAV-hSynl-GFP-BCLib) containing 26 novel AAV variants identified by TRADE and control AAV capsids was injected intravenously into 3 BALB/cJ mice at a dose of 5×10^{11} vg per mouse (for the library, see Table 3).
Two weeks post-injection, various tissues were harvested and analyzed for brain transduction by AAV RNA Barcode-Seq and biodistribution to peripheral organs by AAV DNA Barcode-Seq.
All the values are normalized with those of AAV9 (AAV9 = 1.0).

TABLE 7

Transduction efficiency of hSynl-TRADE-derived AAV variants in various brain regions of one <i>rhesus macaque</i> following intravenous administration as determined by AAV hSynl-RNA Barcode-Seq analysis												
	Cerebellum (Granular layer)	Cere- bellum (Purkinje)	Cingu- late Gyrus	Frontal Cortex	Hippo- campus	Hypo- thalamus	Medulla	Occipital Cortex	Pons	Pre- optic Area	Put- amen	Thala- mus
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	1.28	1.63	1.76	1.75	2.08	1.00	1.45	1.51	1.45	0.66	1.99	1.19
AAV-PHP.B	0.78	1.21	1.27	1.50	1.39	0.94	1.33	1.23	1.62	0.35	1.29	0.69
HN1	1.99	1.78	2.68	2.18	4.07	2.05	1.39	1.54	1.99	1.99	2.54	2.84
HN2	0.81	0.96	1.06	1.13	1.45	0.78	0.77	0.84	0.79	0.72	1.15	1.16
HN3	1.75	2.03	2.14	2.28	3.54	1.48	1.87	1.67	1.71	1.28	2.20	1.87
HN4	0.27	0.61	0.70	0.68	0.37	0.39	0.58	0.54	0.27	0.30	0.49	0.62
HN5	0.15	0.27	0.35	0.22	0.15	0.01	0.27	0.17	0.27	0.22	0.31	0.15
HN6	0.06	0.28	0.36	0.12	0.10	0.27	0.04	0.12	0.12	0.01	0.26	0.10
HN7	0.96	1.40	1.44	1.45	1.64	1.13	1.48	1.18	1.01	0.87	1.39	1.29
HN8	0.38	0.61	0.59	0.67	0.73	0.42	0.56	0.42	0.45	0.35	0.57	0.50
HN9	1.17	1.45	1.91	1.66	2.15	0.92	1.65	1.30	1.27	1.10	1.96	1.48
HN10	1.08	1.24	1.40	1.45	1.78	0.87	1.33	1.16	1.11	0.76	1.50	1.00
HN11	0.96	1.22	1.37	1.42	1.62	1.00	1.28	1.15	1.12	0.65	1.41	1.19
HN12	1.04	1.43	1.64	1.70	1.98	0.97	1.49	1.26	1.09	0.73	1.74	1.44
HN13	1.77	1.74	1.86	1.82	2.17	1.20	2.38	1.54	1.97	1.36	2.14	1.77
HN14	0.13	0.45	0.27	0.26	0.28	0.14	0.30	0.32	0.19	0.04	0.23	0.17
HN15	0.38	0.43	0.19	0.46	0.44	0.04	0.28	0.19	0.23	0.63	0.36	0.09
HN16	0.57	0.65	0.79	0.82	0.89	0.35	0.75	0.77	0.64	0.18	0.72	0.54
HN17	0.05	0.18	0.24	0.14	0.08	0.28	0.16	0.11	0.07	0.01	0.19	0.03
HN18	1.21	1.23	1.62	1.70	2.60	1.17	1.47	1.15	1.09	0.69	1.46	1.13
HN19	0.24	0.24	0.21	0.59	0.50	0.13	0.14	0.26	0.26	0.01	0.31	0.17
HN20	1.08	1.42	1.60	1.81	2.28	1.45	1.51	1.22	1.41	1.32	2.27	1.34
HN21	0.19	0.11	0.05	0.15	0.04	0.49	0.27	0.19	0.08	0.01	0.10	0.14
HN22	0.27	0.17	0.48	0.59	0.49	0.24	0.24	0.27	0.19	0.01	0.23	0.12
HN23	0.60	1.01	1.21	1.11	1.55	0.61	1.23	0.92	0.95	0.49	1.25	0.76
HN24	0.99	1.18	1.19	1.33	1.71	0.70	1.17	1.06	1.04	0.57	1.39	1.21
HN25	0.13	0.14	0.06	0.32	0.21	0.12	0.23	0.24	0.07	0.01	0.28	0.08
HN26	0.35	0.52	0.42	0.60	0.88	0.40	0.50	0.44	0.36	0.27	0.61	0.28

AAV RNA Barcode-Seq analysis was performed on RNAs extracted from various brain regions of one *rhesus macaque* (n = 1) intravenously injected with 2.0×10^{13} vg/kg of a DNA/RNA-barcoded dsAAV-hSynl-GFP-BCLib library that expresses RNA barcodes under the control of the hSynl enhancer-promoter.
All the values are normalized with those of AAV9 (AAV9 = 1.0).

TABLE 8

Pharmacokinetic profiles of hSynl-TRADE-derived AAV variants in <i>rhesus macaque</i> following intravenous administration.								
	1 m	10 m	30 m	1 h	4 h	8 h	24 h	72 h
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	0.95	0.96	1.06	1.05	1.24	1.79	2.64	1.18
AAV-PHP.B	1.00	1.10	1.16	1.11	1.27	1.99	2.94	1.69
HN1	0.78	0.66	0.71	0.65	0.67	0.88	0.65	0.30
HN2	0.69	0.72	0.69	0.62	0.75	0.92	1.24	0.25
HN3	0.85	0.86	0.96	0.77	1.12	1.70	2.60	0.60

TABLE 8-continued

Pharmacokinetic profiles of hSynl-TRADE-derived AAV variants in <i>rhesus macaque</i> following intravenous administration.								
	1 m	10 m	30 m	1 h	4 h	8 h	24 h	72 h
HN4	1.07	1.07	1.15	1.15	1.30	1.88	3.39	2.15
HN5	0.99	0.98	1.14	1.06	1.19	1.62	2.83	1.39
HN6	1.13	1.09	1.18	1.09	1.32	1.97	3.32	1.90
HN7	0.75	0.78	0.81	0.79	0.95	1.33	1.78	0.27
HN8	1.05	1.06	1.02	1.07	1.27	1.82	3.02	1.67
HN9	0.83	0.76	0.87	0.78	0.92	1.36	1.91	0.29
HN10	0.84	0.87	0.90	0.87	1.14	1.32	2.32	0.28
HN11	0.88	0.89	0.90	0.86	1.20	1.55	2.42	0.61
HN12	0.81	0.80	0.86	0.80	0.99	1.41	1.90	0.27
HN13	0.76	0.71	0.82	0.76	0.90	1.31	1.86	0.29
HN14	0.76	0.75	0.83	0.78	0.99	1.40	2.21	0.18
HN15	1.31	1.07	1.27	1.23	1.36	2.37	4.46	2.03
HN16	0.76	0.80	0.91	0.84	0.98	1.41	1.90	0.26
HN17	1.02	1.07	1.21	1.00	1.29	1.88	2.91	1.84
HN18	0.88	0.88	0.96	0.90	1.10	1.57	2.44	0.58
HN19	1.08	1.04	1.15	1.14	1.26	2.04	3.69	2.05
HN20	0.89	0.82	0.88	0.81	0.91	1.62	2.28	0.39
HN21	1.00	1.00	1.11	0.98	1.20	1.54	2.41	2.10
HN22	0.96	0.97	1.09	1.03	1.24	1.74	2.90	1.82
HN23	0.76	0.76	0.86	0.80	0.97	1.50	2.14	0.35
HN24	0.93	1.00	0.96	1.00	1.41	1.48	2.31	1.41
HN25	1.05	1.16	1.08	1.19	1.18	2.00	3.52	1.54
HN26	1.03	0.98	1.08	1.07	1.18	1.77	2.72	1.56

AAV DNA Barcode-Seq analysis was performed on the blood samples obtained from a single *rhesus macaque* injected with 2×10^{13} vg/kg of the DNA/RNA-barcoded dsAAV-hSynl-GFP-BCLib library (the same animal as in Table 7).
All the values are normalized with those of AAV9 (AAV9 = 1.0).

TABLE 9

Biodistribution of hSynl-TRADE-derived AAV variants to peripheral tissues of a <i>rhesus macaque</i> following intravenous administration as determined by AAV DNA Barcode-Seq analysis											
	Liver	Heart	Lung	Kidney	Pancreas	Spleen	Gastrocnemius muscle	Soleus muscle	Intestine	Bone marrow	Smooth muscle (Stomach)
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9-N272A	0.07	0.20	0.79	1.29	2.91	4.67	0.26	0.50	0.64	0.06	0.82
AAV-PHP.B	0.35	0.42	1.09	1.22	2.55	2.14	0.54	0.77	0.40	0.43	0.80
HN1	0.26	0.41	0.34	0.41	0.35	0.76	1.04	1.12	0.24	0.33	0.33
HN2	0.16	0.17	0.35	0.32	0.51	0.71	0.32	0.52	0.11	0.07	0.30
HN3	0.12	0.21	0.78	0.91	2.19	2.48	0.25	0.58	0.18	0.05	0.63
HN4	0.02	0.08	0.78	1.12	2.25	3.42	0.17	0.41	0.15	0.03	0.39
HN5	0.01	0.05	0.73	0.98	2.24	2.85	0.10	0.30	0.07	0.01	0.37
HN6	0.01	0.05	0.92	1.11	2.15	3.50	0.11	0.40	0.12	0.01	0.30
HN7	0.27	0.19	0.36	0.43	0.47	0.51	0.18	0.23	0.16	0.12	0.37
HN8	0.04	0.08	0.71	0.98	2.28	2.57	0.12	0.38	0.11	0.02	0.44
HN9	0.12	0.13	0.32	0.36	0.71	0.79	0.13	0.31	0.11	0.04	0.29
HN10	0.08	0.13	0.63	0.63	1.39	2.53	0.16	0.38	0.11	0.02	0.28
HN11	0.06	0.16	0.63	0.74	1.62	1.68	0.18	0.44	0.16	0.03	0.47
HN12	0.19	0.11	0.28	0.38	0.56	0.61	0.10	0.19	0.09	0.05	0.30
HN13	0.18	0.23	0.58	0.44	0.88	1.12	0.21	0.38	0.18	0.06	0.49
HN14	0.00	0.04	0.30	0.49	0.59	0.83	0.06	0.28	0.05	0.01	0.34
HN15	0.01	0.06	1.01	1.43	2.43	3.80	0.13	0.46	0.10	0.02	0.69
HN16	0.02	0.07	0.37	1.00	0.70	0.87	0.08	0.17	0.06	0.02	0.28
HN17	0.01	0.04	0.85	1.01	2.38	3.25	0.08	0.29	0.10	0.01	0.32
HN18	0.15	0.13	0.36	0.52	0.90	1.17	0.13	0.31	0.09	0.01	0.33
HN19	0.06	0.08	0.93	1.14	2.98	3.41	0.15	0.42	0.10	0.02	0.36
HN20	0.36	0.22	0.34	0.50	0.88	0.96	0.21	0.48	0.14	0.05	0.31
HN21	0.02	0.05	0.74	1.03	2.17	3.08	0.12	0.33	0.06	0.01	0.57
HN22	0.06	0.07	0.72	0.89	1.73	2.68	0.14	0.31	0.09	0.02	0.25
HN23	0.04	0.08	0.61	0.28	0.74	0.71	0.09	0.30	0.10	0.01	0.24
HN24	0.06	0.14	0.66	0.74	1.99	1.98	0.18	0.43	0.13	0.03	0.50
HN25	0.06	0.07	0.90	1.21	2.76	3.28	0.22	0.43	0.10	0.03	0.56
HN26	0.17	0.10	0.64	0.90	1.84	2.38	0.12	0.35	0.12	0.02	0.56

AAV DNA Barcode-Seq analysis was performed on DNA extracted from various peripheral tissues of one *rhesus macaque* (n = 1, the same animal as presented in Table 7) intravenously injected with 2×10^{13} vg/kg of a DNA/RNA-barcoded dsAAV-hSynl-GFP-BCLib library.
All values are normalized to AAV9 (AAV9 = 1.0).

TABLE 9

Splice donor and splice acceptor sites identified in antisense AAV cap ORF transcripts.				
SEQ ID	AAV serotype	SD or SA	Exon-intron junction sequence (Introns are underlined)	
SEQ ID NO: 199	AAV1	SD	1009- <u>CTTACCAGCA</u> -1018	
SEQ ID NO: 199	AAV3	SD	1006- <u>CTTACCAGCA</u> -1015	
SEQ ID NO: 200	AAV1	SD	1228- <u>TTTACCTTCA</u> -1237	
SEQ ID NO: 201	AAV3	SD	1237- <u>TATACCTTCG</u> -1246	
SEQ ID NO: 202	AAV1	SD	1331- <u>ATTACCTGAA</u> -1340	
SEQ ID NO: 203	AAV1	SD	1434- <u>GCTACCTGGA</u> -1443	
SEQ ID NO: 204	AAV1	SD	1502- <u>TTTACCTGGA</u> -1510	
SEQ ID NO: 205	AAV1	SD	1803- <u>ATTACCTGGC</u> -1812	
SEQ ID NO: 206	AAV3	SD	1803- <u>CTTACCTGGC</u> -1812	
SEQ ID NO: 207	AAV1	SD	1835- <u>TGTACCTGCA</u> -1844	
SEQ ID NO: 208	AAV1	SD	2189- <u>GTTACCTTAC</u> -2198	
SEQ ID NO: 209	AAV9	SD	2189- <u>GATACCTGAC</u> -2198	
SEQ ID NO: 210	AAV1	SD	2194- <u>CTTACCCGTC</u> -2203	
SEQ ID NO: 211	AAV3	SD	2194- <u>CTCACACGAA</u> -2203	
SEQ ID NO: 212	AAV1	SA	305-AGCGT <u>CTGCA</u> -314	
SEQ ID NO: 213	AAV1	SA	414-GGCTC <u>CTGGA</u> -423	
SEQ ID NO: 213	AAV3	SA	414-GGCTC <u>CTGGA</u> -423	
SEQ ID NO: 214	AAV1	SA	495-GCCCG <u>CTAAA</u> -504	
SEQ ID NO: 214	AAV9	SA	495-GCCCG <u>CTAAA</u> -504	
SEQ ID NO: 215	AAV3	SA	1133-TCACC <u>CTGAA</u> -1142	
SEQ ID NO: 216	AAV1	SA	1181-ACTGC <u>CTGGA</u> -1190	
SEQ ID NO: 202	AAV1	SA	1331-ATTAC <u>CTGAA</u> -1340	
SEQ ID NO: 217	AAV3	SA	1328-ACTAC <u>CTGAA</u> -1337	
SEQ ID NO: 218	AAV1	SA	1464-CGTTT <u>CTAAA</u> -1473	
SEQ ID NO: 219	AAV1	SA	1653-AAAC <u>ACTGCA</u> -1662	
SEQ ID NO: 220	AAV1	SA	2054-GGGAG <u>CTGCA</u> -2063	
SEQ ID NO: 463	AAV3	SA	2054-GGGAG <u>CTACA</u> -2063	

Ten nucleotides around exon-intron junctions identified in antisense AAV cap mRNA are presented with the junction at the center.
Letters with underlines represent intron sequences.
Letters with no underline represent exon sequences.
Numbers indicate nucleotide positions of the AAV cap ORF.
SD, splice donor;
SA, splice acceptor.
Please note that SEQ ID NO: 199 of AAV1 and SEQ ID NO: 199 of AAV3 are corresponding to each other in sequence alignment. Likewise, SEQ ID NO: 213 of AAV1 and SEQ ID NO: 213 of AAV3 are corresponding to each other in sequence alignment.

[0103] In the course of the experiment, when the AAV9 cap gene ORF was expressed in an antisense orientation in HEK293 cells or Neuro2a cells, the majority of the antisense

AAV9 cap gene mRNA-derived RT-PCR products were truncated by approximately 1.7 kb (FIG. 4), although this was not the case with the RNA recovered from the AAV-PHP.B-hSynI-GFP-TRADE-transduced mouse brain tissue (FIG. 3C, FIG. 3D). Sequencing of the truncated RT-PCR products revealed that a 1694 bp-long region was missing within the AAV9 cap ORF (FIG. 5). Without being bound by any particular theory, it appears that the truncation results from a splicing event, based on the observation that we could identify splice donor and acceptor sites in the PCR products that have the common features of exon-intron junctions. Intriguingly, a sequence alignment study revealed that the cryptic splice donor and acceptor sites with the common features of exon-intron junctions can also be identified in many naturally occurring AAV serotypes at the regions corresponding to the splice donor and acceptor sites identified in the AAV9 cap gene and they are highly conserved (FIGS. 6A-6F). This indicates that splicing could potentially take place in the cap ORF-derived antisense mRNA of not only AAV9 but also many other AAV strains. To date, we have found that splicing occurs on the AAV3 cap ORF-derived antisense mRNA when it is expressed under the control of a human liver-specific promoter (LSP) in HepG2 cells. Although full characterization has not yet been completed, a preliminary RT-PCR using an antisense mRNA-specific RT primer yielded truncated RT-PCR products in addition to the full-length, non-spliced product. The sequencing analysis of two truncated RT-PCR products revealed that there were multiple splicing events on the antisense mRNA (FIGS. 7A-7B). A sequencing alignment study has identified additional potential splice donor and acceptor sites (FIGS. 8A-8F and FIGS. 9A-9C). We also found splicing events in the antisense mRNA derived from the AAV1 cap ORF when antisense mRNA was transcribed by the hSynI enhancer-promoter in HEK293 cells or Neuro2a cells (FIG. 10). Many of the identified splice donor sites (GT/CA) and splice acceptor sites (AG/TC) are highly conserved across different serotypes, indicating the possibility that these sites are also utilized as splicing donor and acceptor sites in the AAV serotypes that have yet to be investigated. Indeed, we have found that splicing of antisense mRNA transcripts of the AAV1, AAV3 and AAV9 cap ORFs uses several common splice donor and acceptor sites (FIG. 10). To date, we have not yet observed splicing of antisense mRNA transcripts of the AAV5 cap ORF. For serotypes other than AAV1, 3, 5 and 9, splicing events in antisense mRNA of the AAV cap ORFs have not yet been investigated.

[0104] Potential splicing of the cap ORF-derived antisense mRNA is scientifically intriguing, but may hinder the TRADE system when the full-length cap ORF sequence needs to be recovered from antisense mRNA. To overcome this potential issue, we introduced silent mutations that presumably disrupt the conserved sequences at exon-intron junctions and branching points. To demonstrate proof of principle of this approach, we introduced silent mutations into the AAV9 cap ORF contained in the plasmid, pAAV9-N272A-PHP.B-hSyn1-GFP-TRADE, that disrupt the splice acceptor (SA) consensus sequence (pAAV9NS1 construct), the splice donor (SD) consensus sequence (pAAV9NS2 construct), and both the splice acceptor and donor consensus sequences (pAAV9NS3 construct). Please note that NS stands for “non-spliced.” The method we use to disrupt these consensus sequences is described below.

[0105] We codon-optimize the AAV cap ORF sequence for human cell expression.

[0106] To identify potential splice donor and acceptor sites on antisense mRNA derived from the cap ORFs, we develop and use our proprietary database of potential splice donor and acceptor sites on antisense mRNA based on our experimental and bioinformatics observations (i.e., FIGS. 5, 6A-6F, 7A-7B, 8A-8F, 9A-9C and 10).

[0107] We destroy the GT (splice donor) and/or AG (splice acceptor) consensus sequence by changing at least one nucleotide using the codon-optimized sequence. If the codon-optimized sequence is not applicable, we use an alternative nucleotide(s) that can destroy the consensus sequence.

[0108] We remove a stretch of T's upstream of the splice acceptor sites by introducing silence mutations based on the codon-optimized sequence. If the codon-optimized sequence is not sufficient to destroy a stretch of T's, we use alternative nucleotides.

[0109] We also avoid G at the exon termini as much as possible.

[0110] Using several programs that can predict branching points (e.g., Human Splicing Finder (Desmet, Hamroun et al. 2009)), we identify potential branching points and replace them with the codon-optimized sequence. If the degree of nucleotide changes attainable by this method is not sufficient, we introduce alternative nucleotides to disrupt potential branching points.

[0111] With this method, we have created AAV9NS1 (SA, destroyed), AAV9NS2 (SD, destroyed) and AAV9NS3 (both SD and SA, destroyed) cap ORFs (FIGS. 9A-9C). We expressed these ORFs in an antisense orientation under the control of the hSynI enhancer-promoter in Neuro2a cells by transient plasmid transfection, and analyzed the antisense transcripts by RT-PCR. This experiment revealed that the splicing could be effectively suppressed in all of the NS1, NS2 and NS3 cap ORFs (FIG. 10). It should be noted that even if splicing takes place on the cap ORF-derived antisense mRNA, it would still be possible to recover the relatively small peptide insertion region of the cap ORF by RT-PCR from pre-mRNA.

[0112] The TRADE method described herein uses antisense mRNA for viral protein evolution to establish the proof-of-principle and to show successful reduction of the method to practice. The TRADE system can also utilize mRNA in a sense orientation as long as the viruses can be produced and potential expression of viral proteins in target cells during the directed evolution procedure does not hinder successful evolution of novel capsids.

[0113] Additional information related to nucleic acid splicing and AAV may be found in Desmet et al., *Nucleic Acids Res* 37, e67 (2009); Matsuzaki et al., *Neurosci Lett* 665, 182-188 (2018); and Hordeaux et al., *Mol Ther* 26, 664-668 (2018).

[0114] All references cited in this disclosure are incorporated by reference in their entirety.

SEQUENCE LISTING

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 misc_feature 1..27
 source 1..45
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 organism = synthetic construct

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 organism = synthetic construct

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 PEPTIDE 1..8
 source 1..8
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 3
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SEQ ID NO: 4 moltype = AA length = 8
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 note = peptide

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SEQUENCE: 18		
ENKSAPLP		8
SEQ ID NO: 19	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 19		
ERTAVKGN		8
SEQ ID NO: 20	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 20		
GGIQTVVT		8
SEQ ID NO: 21	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 21		
GGSPLAHP		8
SEQ ID NO: 22	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 22		
GGTAAQGV		8
SEQ ID NO: 23	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 23		
GKMASGSL		8
SEQ ID NO: 24	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 24		
GKQPVQPY		8
SEQ ID NO: 25	moltype = AA length = 8	

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FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 25		
GNPHTGST		8
SEQ ID NO: 26	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 26		
GPTLGGSG		8
SEQ ID NO: 27	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 27		
GQRGLPIA		8
SEQ ID NO: 28	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 28		
GREPRRLH		8
SEQ ID NO: 29	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 29		
GTPQTTKE		8
SEQ ID NO: 30	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 30		
GVTERPNR		8
SEQ ID NO: 31	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 31		
HLGDNLAR		8

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SEQ ID NO: 32	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 32		
HPGSGAGP		8
SEQ ID NO: 33	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 33		
HQVTSSGA		8
SEQ ID NO: 34	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 34		
HVGSQMHA		8
SEQ ID NO: 35	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
UNSURE	3	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 35		
IGXTVPMQ		8
SEQ ID NO: 36	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 36		
KFTRDGPY		8
SEQ ID NO: 37	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 37		
KGPAEQGH		8
SEQ ID NO: 38	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 38		

-continued

LAHSPRLW		8
SEQ ID NO: 39	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 39		
LERNRDS		8
SEQ ID NO: 40	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 40		
LEHTSLT		8
SEQ ID NO: 41	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 41		
LHDGKYST		8
SEQ ID NO: 42	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 42		
LKATGRGK		8
SEQ ID NO: 43	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 43		
LLPGSADG		8
SEQ ID NO: 44	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 44		
LLVTARSH		8
SEQ ID NO: 45	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	

-continued

SEQUENCE: 45		
LPEVEPTN		8
SEQ ID NO: 46	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 46		
LPWENSSQ		8
SEQ ID NO: 47	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 47		
LQRNSDAN		8
SEQ ID NO: 48	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 48		
LQSAPRAT		8
SEQ ID NO: 49	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 49		
MLGSQVPT		8
SEQ ID NO: 50	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 50		
MSGQGYQA		8
SEQ ID NO: 51	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 51		
NPGRDFRD		8
SEQ ID NO: 52	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	

-continued

SEQUENCE: 52	organism = synthetic construct	
NQPSDYVS		8
SEQ ID NO: 53	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 53		
NSVGSADK		8
SEQ ID NO: 54	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 54		
NVQRTQRG		8
SEQ ID NO: 55	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 55		
PAQLNGPR		8
SEQ ID NO: 56	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 56		
PERERLPR		8
SEQ ID NO: 57	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 57		
PGNGSHTM		8
SEQ ID NO: 58	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 58		
PIPGTPQP		8
SEQ ID NO: 59	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	

-continued

	mol_type = protein organism = synthetic construct	
SEQUENCE: 59 PMSVPASN		8
SEQ ID NO: 60 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 60 PRPTVVGT		8
SEQ ID NO: 61 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 61 PRTNRGPE		8
SEQ ID NO: 62 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 62 PVANPTTA		8
SEQ ID NO: 63 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 63 PVLGGPPK		8
SEQ ID NO: 64 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 64 QGSRQGSS		8
SEQ ID NO: 65 FEATURE REGION PEPTIDE source	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 65 QMAETPIS		8
SEQ ID NO: 66 FEATURE REGION PEPTIDE	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide 1..8	

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source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 66		
QMLGIGRS		8
SEQ ID NO: 67	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 67		
QSGDSALN		8
SEQ ID NO: 68	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 68		
RAGLTSSE		8
SEQ ID NO: 69	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 69		
RLDNTGVG		8
SEQ ID NO: 70	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 70		
RMPGKPYS		8
SEQ ID NO: 71	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 71		
RVAGASQP		8
SEQ ID NO: 72	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 72		
RVESQLE		8
SEQ ID NO: 73	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	

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PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 73		
SARTGASE		8
SEQ ID NO: 74	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 74		
SERNRASM		8
SEQ ID NO: 75	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 75		
SIDVRMAA		8
SEQ ID NO: 76	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 76		
SRDGHILR		8
SEQ ID NO: 77	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 77		
SRQVVLPG		8
SEQ ID NO: 78	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 78		
SSRGYTST		8
SEQ ID NO: 79	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 79		
SSVVSQGP		8
SEQ ID NO: 80	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	

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PEPTIDE	note = peptide	
source	1..8	
	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 80		
SVAESGRE		8
SEQ ID NO: 81	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 81		
TALTANTQ		8
SEQ ID NO: 82	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 82		
TESSVGNL		8
SEQ ID NO: 83	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 83		
TGREGANL		8
SEQ ID NO: 84	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 84		
TLSEPPKK		8
SEQ ID NO: 85	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 85		
TNAVSGKS		8
SEQ ID NO: 86	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 86		
TRAPTIHL		8
SEQ ID NO: 87	moltype = AA length = 8	
FEATURE	Location/Qualifiers	

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REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 87		
TRESTDRG		8
SEQ ID NO: 88	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 88		
TVAAAPNL		8
SEQ ID NO: 89	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 89		
TYHNNTPR		8
SEQ ID NO: 90	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 90		
VSNSTRTS		8
SEQ ID NO: 91	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 91		
VTLQIDTK		8
SEQ ID NO: 92	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 92		
WMSRPGPT		8
SEQ ID NO: 93	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 93		
WPYRGLTQ		8
SEQ ID NO: 94	moltype = AA length = 8	

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FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 94		
WRRQGSRA		8
SEQ ID NO: 95	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 95		
YAQRFAKM		8
SEQ ID NO: 96	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 96		
YNSPRQTV		8
SEQ ID NO: 97	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 97		
APTNF AHP		8
SEQ ID NO: 98	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 98		
AQTNLAAG		8
SEQ ID NO: 99	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 99		
ASLPNLGQ		8
SEQ ID NO: 100	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 100		
DYMHNTGL		8

-continued

SEQ ID NO: 101	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 101		
DYMHTTGL		8
SEQ ID NO: 102	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 102		
ERNAWHAG		8
SEQ ID NO: 103	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 103		
ETQATPMP		8
SEQ ID NO: 104	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 104		
EWEDSARS		8
SEQ ID NO: 105	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 105		
FTGDTDTL		8
SEQ ID NO: 106	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 106		
FTNRTSTT		8
SEQ ID NO: 107	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 107		
GDYTVQRP		8

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SEQ ID NO: 108	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 108	
GGLRTDYG	8
SEQ ID NO: 109	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 109	
GPQEGSER	8
SEQ ID NO: 110	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 110	
GSNHTQSL	8
SEQ ID NO: 111	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 111	
HDRDTRQA	8
SEQ ID NO: 112	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 112	
LDQNRRPQ	8
SEQ ID NO: 113	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 113	
LEQQRGAS	8
SEQ ID NO: 114	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = peptide
PEPTIDE	1..8
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 114	

-continued

LGGNAQGL		8
SEQ ID NO: 115	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 115		
LLVTTRSH		8
SEQ ID NO: 116	moltype = AA length = 7	
FEATURE	Location/Qualifiers	
REGION	1..7	
	note = peptide	
PEPTIDE	1..7	
source	1..7	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 116		
LVTNTTR		7
SEQ ID NO: 117	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 117		
MESQRANS		8
SEQ ID NO: 118	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 118		
MISQTLMA		8
SEQ ID NO: 119	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 119		
MMSQSLRA		8
SEQ ID NO: 120	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 120		
NNVQSALN		8
SEQ ID NO: 121	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	

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SEQUENCE: 121		
NSARTQLS		8
SEQ ID NO: 122	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 122		
PQWNRTPPL		8
SEQ ID NO: 123	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 123		
PRFNNSSL		8
SEQ ID NO: 124	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 124		
PVDGGRHL		8
SEQ ID NO: 125	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 125		
PWFNKSSL		8
SEQ ID NO: 126	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 126		
QDMNSQRS		8
SEQ ID NO: 127	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 127		
QGASNSQL		8
SEQ ID NO: 128	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	

-continued

SEQUENCE: 128	organism = synthetic construct	
QQNGTRPS		8
SEQ ID NO: 129	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 129		
QRSAYPTS		8
SEQ ID NO: 130	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 130		
QRTPSITP		8
SEQ ID NO: 131	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 131		
QWMKEQAG		8
SEQ ID NO: 132	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 132		
RDGRHPSE		8
SEQ ID NO: 133	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 133		
RGTVTVEQ		8
SEQ ID NO: 134	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 134		
RPANHSTA		8
SEQ ID NO: 135	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	

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	mol_type = protein organism = synthetic construct	
SEQUENCE: 135 RQGDADTL		8
SEQ ID NO: 136 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 136 SATISLQV		8
SEQ ID NO: 137 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 137 SHTNLRDT		8
SEQ ID NO: 138 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 138 SRMGETPQ		8
SEQ ID NO: 139 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 139 SSGYLTAN		8
SEQ ID NO: 140 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 140 TGNSPEQA		8
SEQ ID NO: 141 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE source	1..8 1..8 mol_type = protein organism = synthetic construct	
SEQUENCE: 141 THSQGRLA		8
SEQ ID NO: 142 FEATURE REGION	moltype = AA length = 8 Location/Qualifiers 1..8 note = peptide	
PEPTIDE	1..8	

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source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 142		
TPIVGSNV		8
SEQ ID NO: 143	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 143		
TPPKSPSM		8
SEQ ID NO: 144	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 144		
TRMDERSP		8
SEQ ID NO: 145	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 145		
TTATTSIT		8
SEQ ID NO: 146	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 146		
VVQGEQKR		8
SEQ ID NO: 147	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 147		
WNDRSGER		8
SEQ ID NO: 148	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 148		
WSQDAVKG		8
SEQ ID NO: 149	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	

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PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 149		
WTGGGSGT		8
SEQ ID NO: 150	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 150		
AGAAYTPA		8
SEQ ID NO: 151	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 151		
APSVSREK		8
SEQ ID NO: 152	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 152		
DYMHKTGL		8
SEQ ID NO: 153	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 153		
GGMNETTR		8
SEQ ID NO: 154	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 154		
GGSAFVTG		8
SEQ ID NO: 155	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 155		
GNSHTGSS		8
SEQ ID NO: 156	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	

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PEPTIDE	note = peptide	
1..8		
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 156		
NSARTQLS		8
SEQ ID NO: 157	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 157		
PLTILNRH		8
SEQ ID NO: 158	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 158		
QGTRTNPP		8
SEQ ID NO: 159	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 159		
QSSAMPRN		8
SEQ ID NO: 160	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 160		
SHNSQPVA		8
SEQ ID NO: 161	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 161		
TAQGAAFR		8
SEQ ID NO: 162	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 162		
TPGLNNAR		8
SEQ ID NO: 163	moltype = AA length = 8	
FEATURE	Location/Qualifiers	

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REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 163		
TSLGTPEA		8
SEQ ID NO: 164	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 164		
TTNLAKNS		8
SEQ ID NO: 165	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 165		
WSPDAVEG		8
SEQ ID NO: 166	moltype = AA length = 7	
FEATURE	Location/Qualifiers	
REGION	1..7	
	note = peptide	
PEPTIDE	1..7	
source	1..7	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 166		
WTGGRHL		7
SEQ ID NO: 167	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 167		
AVAGDRLL		8
SEQ ID NO: 168	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 168		
DLLTRSVS		8
SEQ ID NO: 169	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 169		
EWKTQLAL		8
SEQ ID NO: 170	moltype = AA length = 8	

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FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 170		
GNINVVPH		8
SEQ ID NO: 171	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 171		
GSPAASSW		8
SEQ ID NO: 172	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 172		
KHSLTLES		8
SEQ ID NO: 173	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 173		
KPVSTDTF		8
SEQ ID NO: 174	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 174		
LDRSGSTG		8
SEQ ID NO: 175	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 175		
LGAQNHVV		8
SEQ ID NO: 176	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 176		
LMATDYGP		8

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SEQ ID NO: 177	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 177		
LRATDYGP		8
SEQ ID NO: 178	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 178		
MERTEPLG		8
SEQ ID NO: 179	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 179		
NDGLRLHL		8
SEQ ID NO: 180	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 180		
NLSAHSXA		8
SEQ ID NO: 181	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 181		
NLSAHSXD		8
SEQ ID NO: 182	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 182		
RALDLVTR		8
SEQ ID NO: 183	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 183		
SAGMARNS		8

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SEQ ID NO: 184	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 184		
SGQRVGSA		8
SEQ ID NO: 185	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 185		
SGQRVGSD		8
SEQ ID NO: 186	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 186		
TGRPEQPK		8
SEQ ID NO: 187	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 187		
THSPIKLP		8
SEQ ID NO: 188	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 188		
TQFSQAQR		8
SEQ ID NO: 189	moltype = AA length = 8	
FEATURE	Location/Qualifiers	
REGION	1..8	
	note = peptide	
PEPTIDE	1..8	
source	1..8	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 189		
VGDSANLR		8
SEQ ID NO: 190	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 190		
cgagccaacc		10
SEQ ID NO: 191	moltype = DNA length = 27	

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FEATURE	Location/Qualifiers					
source	1..27					
	mol_type = other DNA					
	organism = adeno-associated virus					
SEQUENCE: 191						
caaaccctgg	ccgtgccctt	caaggca 27				
SEQ ID NO: 192	moltype = DNA length = 2211					
FEATURE	Location/Qualifiers					
source	1..2211					
	mol_type = other DNA					
	organism = adeno-associated virus					
SEQUENCE: 192						
atggctgccg	atggttatct	tccagattgg	ctcgaggaca	accttagtga	aggaattcgc	60
gagtgggtggg	ctttgaaacc	tggagcccct	caacccaagg	caaatcaaca	acatcaagac	120
aaacgctcgag	gtcttgtgct	tccgggttac	aaataccttg	gacccggcaa	cggactcgac	180
aaagggggagc	cgggtcaacgc	agcagacgcg	gcggccctcg	agcacgacaa	ggcctacgac	240
cagcagctca	aggccggaga	caacccgtac	ctcaagtaca	accacgccga	cgccgagttc	300
caggagcggc	tcaaagaaga	tacgtctttt	gggggcaacc	tcgggcgagc	agtcttccag	360
gccaaaaaga	ggcttcttga	acctcttggt	ctggttgagg	aagcggctaa	gacggctcct	420
ggaaagaaga	ggcctgtaga	gcagtctcct	caggaaccgg	actcctccgc	gggtattggc	480
aaaatcgggtg	cacagcccgc	taaaaagaga	ctcaatttcg	gtcagactgg	cgacacagag	540
tcagtcccag	accctcaacc	aatcggagaa	cctcccgcag	ccccctcagg	tgtgggatct	600
cttacaatgg	cttcaggtgg	tggcgcacca	gtggcagaca	ataacgaagg	tgccgatgga	660
gtgggtagtt	cctcgggaaa	ttggcattgc	gattcccaat	ggctggggga	cagagtcatc	720
accaccagca	cccgaacctg	ggccctgccc	acctacaaca	atcacctcta	caagcaaatc	780
tccaacagca	catctggagg	atcttcaaat	gacaacgcct	acttcggcta	cagcaccctc	840
tgggggtatt	ttgacttcaa	cagattccac	tgccacttct	caccacgtga	ctggcagcga	900
ctcatcaaca	acaactgggg	attccggcct	aagcgactca	acttcaagct	cttcaacatt	960
cagggtcaaa	aggttacgga	caacaatgga	gtcaagacca	tcgccataa	ccttaccagc	1020
acgggtccagg	tcttcacgga	ctcagactat	cagctcccgt	acgtgctcgg	gtcgggtcac	1080
gagggctgcc	tcccgcggtt	cccagcggac	gttttcatga	ttcctcagta	cgggtatctg	1140
acgcttaatg	atggaagcca	ggccgtgggt	cgttcgtcct	tttactgcct	ggaatatttc	1200
ccgtcgcaaa	tgctaagaac	gggtaacaac	ttccagttca	gctacgagtt	tgagaacgta	1260
cctttccata	gcagctacgc	tcacagccaa	agcctggacc	gactaatgaa	tccactcatc	1320
gaccaatact	tgtactatct	ctcaaagact	attaacggtt	ctggacagaa	tcaacaaacg	1380
ctaaaattca	gtgtggccgg	accagcaaac	atggctgtcc	agggaagaaa	ctacatacct	1440
ggaccagct	accgacaaca	acgtgtctca	accactgtga	ctcaaaacaa	caacagcgaa	1500
tttgcttgge	ctggagcttc	ttcttgggct	ctcaatggac	gtaatagctt	gatgaatcct	1560
ggacctgcta	tggccagcca	caaagaagga	gaggaccgtt	tctttccttt	gtctggatct	1620
ttaatttttg	gcaaacgaag	aactggaaga	gacaacgtgg	atgcggacaa	agtcatgata	1680
accaacgaag	aagaaattaa	aactactaac	ccggtagcaa	cggagtctta	tggacaagtg	1740
gccacaaacc	accagagtgc	ccaagcacag	gcgcagaccg	gctgggttca	aaaccaagga	1800
atacttccgg	gtatggtttg	gcaggacaga	gatgtgtacc	tgcaaggacc	catttggggc	1860
aaaaattcctc	acacggacgg	caactttcac	ccttctccgc	tgatgggagg	gtttggaatg	1920
aagcaccctgc	ctcctcagat	cctcatcaaa	aacacacctg	tacctgcgga	tcctccaacg	1980
gccttcaaca	aggacaagct	gaactctttc	atcacccagt	attctactgg	ccaagtcagc	2040
gttgagatcg	agtggaagct	gcagaaggaa	aacagcaagc	gctggaacct	ggagatccag	2100
tacacttcca	actattacaa	gtctaataat	gttgaatttg	ctgttaatac	tgaagggtga	2160
tatagtgaac	ccgcgcccat	tggcaccaga	tacctgactc	gtaatctgta	a	2211
SEQ ID NO: 193	moltype = DNA length = 2211					
FEATURE	Location/Qualifiers					
source	1..2211					
	mol_type = other DNA					
	organism = adeno-associated virus					
SEQUENCE: 193						
atggctgctg	acggttatct	tccagattgg	ctcgaggaca	acctttctga	aggcattcgt	60
gagtgggtggg	ctctgaaacc	tggagtccct	caacccaaag	cgaaccaaca	acaccaggac	120
aaaccgtcggg	gtcttgtgct	tccgggttac	aaatacctcg	gacccggtaa	cggactcgac	180
aaaggagagc	cgggtcaacga	ggcggacgcg	gcagccctcg	aacacgacaa	agcttacgac	240
cagcagctca	aggccgggtga	caacccgtac	ctcaagtaca	accacgccga	cgccgagttt	300
caggagcgtc	ttcaagaaga	tacgtctttt	gggggcaacc	ttggcagagc	agtcttccag	360
gccaaaaaga	ggatccttga	gcctcttggt	ctggttgagg	aagcagctaa	aacggctcct	420
ggaaagaaga	ggcctgtaga	tcagtctcct	caggaaccgg	actcatcatc	tgggtgttggc	480
aaaatcgggca	aacagcctgc	cagaaaaaga	ctaaatttcg	gtcagactgg	cgactcagag	540
tcagtcccag	accctcaacc	tctcggagaa	ccaccagcag	ccccacaag	tttgggatct	600
aatacaatgg	cttcaggcgg	tggcgcacca	atggcagaca	ataacgaggg	tgccgatgga	660
gtgggttaatt	cctcaggaaa	ttggcattgc	gattcccaat	ggctggggca	cagagtcatc	720
accaccagca	ccagaacctg	ggccctgccc	acttacaaca	accatctcta	caagcaaatc	780
tccagccaat	caggagcttc	aaacgacaac	cactactttg	gctacagcac	cccttggggg	840
tattttgact	ttaacagatt	ccactgccac	ttctcaccac	gtgactggca	gcgactcatt	900
aaacaacaact	ggggattccg	gcccagaaaa	ctcagcttca	agctcttcaa	catccaagtt	960
aaaagaggtca	cgcagaacga	tggcacgacg	actattgcca	ataaccttac	cagcacgggt	1020
caagtgttta	cggactcgga	gtatcagctc	ccgtacgtgc	tcgggtcggc	gcaccaaggc	1080
tgtctcccgc	cgtttccagc	ggacgtcttc	atggctccctc	agtatggata	cctcaccctg	1140

```
SEQ ID NO: 199      moltype = DNA  length = 10
FEATURE
source              1..10
                    mol_type = other DNA
                    organism = adeno-associated virus
```

-continued

SEQUENCE: 199		
cttaccagca		10
SEQ ID NO: 200	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 200		
tttaccttca		10
SEQ ID NO: 201	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 201		
tataccttcg		10
SEQ ID NO: 202	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 202		
attacctgaa		10
SEQ ID NO: 203	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 203		
gctacctgga		10
SEQ ID NO: 204	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 204		
tttacctgga		10
SEQ ID NO: 205	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 205		
attacctggc		10
SEQ ID NO: 206	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 206		
cttacctggc		10
SEQ ID NO: 207	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 207		
tgtacctgca		10
SEQ ID NO: 208	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 208		
gttaccttac		10
SEQ ID NO: 209	moltype = DNA length = 10	

-continued

FEATURE	Location/Qualifiers	
source	1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 209 gatacctgac		10
SEQ ID NO: 210 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 210 cttaccggtc		10
SEQ ID NO: 211 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 211 ctcacacgaa		10
SEQ ID NO: 212 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 212 agcgtctgca		10
SEQ ID NO: 213 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 213 ggctcctgga		10
SEQ ID NO: 214 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 214 gcccgcataaa		10
SEQ ID NO: 215 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 215 tcaccctgaa		10
SEQ ID NO: 216 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 216 actgcctgga		10
SEQ ID NO: 217 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	
SEQUENCE: 217 actacctgaa		10
SEQ ID NO: 218 FEATURE source	moltype = DNA length = 10 Location/Qualifiers 1..10 mol_type = other DNA organism = adeno-associated virus	

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SEQUENCE: 218		
cgtttctaaa		10
SEQ ID NO: 219	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 219		
aaacactgca		10
SEQ ID NO: 220	moltype = DNA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 220		
gggagctgca		10
SEQ ID NO: 221	moltype = AA length = 736	
FEATURE	Location/Qualifiers	
source	1..736	
	mol_type = protein	
	organism = adeno-associated virus	
SEQUENCE: 221		
MAADGYLPDW LEDNLSEGIR	EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD	60
KGEPVNAADA AALEHDKAYD	QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFQ	120
AKKRILLEPLG LVEEAAKTAP	GKKRPVEQSP QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE	180
SVPDPQPIGE PPAAPSGVGS	LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI	240
TTSTRTWALP TYNNHLYKQI	SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR	300
LINNNWGFRP KRLNFKLFNI	QVKEVTDNNG VKTIANNLTS TVQVFTDSY QLPYVLGSAH	360
EGCLPPFPAD VFMIPQYGYL	TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV	420
PFHSSYAHSQ SLDRLMNPLI	DQYLYYLSKT INSGSQNQQT LKFSVAGPSN MAVQGRNYIP	480
GPSYRQQRVS TTVTQNNNSE	FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS	540
LIFGKQGTGR DNVDADKVM	TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNGQ	600
ILPGMVWQDR DVYLQGPPIWA	KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT	660
AFNKDKLNSF ITQYSTGQVS	VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFVNTTEGV	720
YSEPRPIGTR YLTRNL		736
SEQ ID NO: 222	moltype = AA length = 736	
FEATURE	Location/Qualifiers	
REGION	1..736	
	note = synthesized	
PEPTIDE	1..736	
source	1..736	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 222		
MAADGYLPDW LEDNLSEGIR	EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD	60
KGEPVNAADA AALEHDKAYD	QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFQ	120
AKKRILLEPLG LVEEAAKTAP	GKKRPVEQSP QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE	180
SVPDPQPIGE PPAAPSGVGS	LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI	240
TTSTRTWALP TYNNHLYKQI	SNSTSGGSSN DAAYFGYSTP WGYFDFNRFH CHFSPRDWQR	300
LINNNWGFRP KRLNFKLFNI	QVKEVTDNNG VKTIANNLTS TVQVFTDSY QLPYVLGSAH	360
EGCLPPFPAD VFMIPQYGYL	TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV	420
PFHSSYAHSQ SLDRLMNPLI	DQYLYYLSKT INSGSQNQQT LKFSVAGPSN MAVQGRNYIP	480
GPSYRQQRVS TTVTQNNNSE	FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS	540
LIFGKQGTGR DNVDADKVM	TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNGQ	600
ILPGMVWQDR DVYLQGPPIWA	KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT	660
AFNKDKLNSF ITQYSTGQVS	VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFVNTTEGV	720
YSEPRPIGTR YLTRNL		736
SEQ ID NO: 223	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 223		
gcaagaaagg ccagcagccc gccagaaaga gactcaattt c		41
SEQ ID NO: 224	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 224		

-continued

gcaagaaagg ccagcagccc gccggaaaga gactcaattt c	41
SEQ ID NO: 225	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 225	
gcaagaaagg ccagcagccc gctagaaaga gactgaactt t	41
SEQ ID NO: 226	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 226	
gcaagaaggg ccagcagccc gccagaaaga gactcaattt c	41
SEQ ID NO: 227	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 227	
gcaagaaagg ccagcggccc gctaaaaaga gactgaactt t	41
SEQ ID NO: 228	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 228	
gcaagaaagg ccagcagccc gctaaaaaga gactgaactt t	41
SEQ ID NO: 229	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 229	
gcaagaaagg ccagcagccc gctaaaaaga gactgagctt t	41
SEQ ID NO: 230	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 230	
gcaagaaagg ccaccagccc gcgagaaaga gactgaactt t	41
SEQ ID NO: 231	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 231	
gcaagacagg ccagcagccc gcgaaaaaga gactcaactt t	41
SEQ ID NO: 232	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 232	
gcaagaaagg ccagcagccc gctaaaaaga agtcaactt t	41
SEQ ID NO: 233	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 233	
gcaagaaagg ccagcagccc gctaaaaaga gactcaactt t	41
SEQ ID NO: 234	moltype = DNA length = 41
FEATURE	Location/Qualifiers

-continued

source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 234		
gcaagaaagg ccagcagccc gcgaaaaga gactcaactt t		41
SEQ ID NO: 235	moltype = DNA length = 40	
FEATURE	Location/Qualifiers	
source	1..40	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 235		
gcaagtcagg ccagcagccc gcgaaaaga gactgaattt		40
SEQ ID NO: 236	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 236		
gcaagtcagg ccggcagccc gcgaaaaga gactgaattt t		41
SEQ ID NO: 237	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 237		
gcaagaaagg ccaacagccc gccagaaaaa gactcaattt t		41
SEQ ID NO: 238	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 238		
gcaagacagg ccagcagccc gctaaaaaga gactcaattt t		41
SEQ ID NO: 239	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 239		
gcaagaacgg ccagccgccc gctaaaaaga agtcaactt t		41
SEQ ID NO: 240	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 240		
gcaaaaaagg ccagcagccc gctaaaaaga agtcaattt t		41
SEQ ID NO: 241	moltype = DNA length = 40	
FEATURE	Location/Qualifiers	
source	1..40	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 241		
gaaaggcggg ccagcagcct gcaagaaaaa gatgaatttt		40
SEQ ID NO: 242	moltype = DNA length = 40	
FEATURE	Location/Qualifiers	
source	1..40	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 242		
gaaaagcggg caaccagcct gcaagaaaaa gatgaatttc		40
SEQ ID NO: 243	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 243		

-continued

gaaaagcggg ccagcagcct gcaagaaaga gattgaattt c	41
SEQ ID NO: 244	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 244	
gaaaagcggg caaccagcct gcaagaaaga gattgaattt c	41
SEQ ID NO: 245	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 245	
gaaaagcggg ccagcagcct gcgagaaaga gattgaattt t	41
SEQ ID NO: 246	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 246	
gaaaagcggg ccagcagcct gcaagaaaaa gattgaattt c	41
SEQ ID NO: 247	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 247	
gaaagtcggg caaccagcct gcaagaaaga gattgaattt c	41
SEQ ID NO: 248	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 248	
gaaaagcggg ccagcagcct gcaagaaaga gattgaattt t	41
SEQ ID NO: 249	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 249	
gaaaagcggg caaccagcct gcaagaaaaa gattgaattt t	41
SEQ ID NO: 250	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 250	
gaaaagcggg ccagcagcct gcgagaaaaa gattgaattt t	41
SEQ ID NO: 251	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 251	
gaaaagcggg ccagcagcct gcaagaaaaa gattgaattt t	41
SEQ ID NO: 252	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 252	
gaaaagcggg ccagcagcct gcaagaaaaa gattaaattt t	41
SEQ ID NO: 253	moltype = DNA length = 41
FEATURE	Location/Qualifiers

-continued

source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 253		
gaaaagcggg ccagcggcct gcaagaaaaa gattaaat	t	41
SEQ ID NO: 254	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 254		
gaaaagcggg ccacagcct gcgagaaaga gattgaat	t	41
SEQ ID NO: 255	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 255		
gaaaggcggg ccacagcct gcgagaaaga gattgaat	t	41
SEQ ID NO: 256	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 256		
gaaaagcggg ccagcagcct gcaagaaaaa gactgaat	t c	41
SEQ ID NO: 257	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 257		
gcaaatcggg caaacagcct gccagaaaaa gactaaat	t c	41
SEQ ID NO: 258	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 258		
gcaaatcagg ccagcagccc gctagaaaaa gactgaat	t	41
SEQ ID NO: 259	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 259		
gcaaatcagg ccagcagccc gctaagaaaa gactcaat	t	41
SEQ ID NO: 260	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 260		
gcaaatcggg tgcacagccc gctaaaaaga gactcaat	t c	41
SEQ ID NO: 261	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 261		
gcaaatcggg ttcacagccc gctaaaaaga aactcaat	t c	41
SEQ ID NO: 262	moltype = DNA length = 41	
FEATURE	Location/Qualifiers	
source	1..41	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 262		

-continued

gcaaaaaagg caaacaacca gccaaaaaga gactcaactt t	41
SEQ ID NO: 263	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 263	
gcaaaaaagg caaacaacca gccagaaaga ggctcaactt t	41
SEQ ID NO: 264	moltype = DNA length = 60
FEATURE	Location/Qualifiers
source	1..60
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 264	
gccaaagaaaa agcaaaaaga cggcgaacca gccgactctg ctagaaggac actcgacttt	60
SEQ ID NO: 265	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 265	
gcaaaaaagg caagcagccg gctaaaaaga agctcgtttt c	41
SEQ ID NO: 266	moltype = DNA length = 41
FEATURE	Location/Qualifiers
source	1..41
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 266	
gcaagaaagg caaacagcct gccagaaaga gactcaactt t	41
SEQ ID NO: 267	moltype = DNA length = 38
FEATURE	Location/Qualifiers
source	1..38
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 267	
ccaagaaaaa caagaagcct cgcaaggaaa gaccttcc	38
SEQ ID NO: 268	moltype = DNA length = 27
FEATURE	Location/Qualifiers
source	1..27
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 268	
aagaggactc caagccttcc acctcgt	27
SEQ ID NO: 269	moltype = DNA length = 26
FEATURE	Location/Qualifiers
source	1..26
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 269	
aagaggactc caagccttcc actcgt	26
SEQ ID NO: 270	moltype = DNA length = 35
FEATURE	Location/Qualifiers
source	1..35
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 270	
ttaagaagcc taaacctacc gaggaagtca gtgcg	35
SEQ ID NO: 271	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 271	
tgtttactct gagcctcgcc ccattggcac tcgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 272	moltype = DNA length = 55
FEATURE	Location/Qualifiers

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source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 272
tgtttactct gagcctcgcc ccattggcac tcgttaccct acccgtaatc tgtaa      55

SEQ ID NO: 273        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 273
tgtttactct gagcctcgcc ctattggcac tcgttaccct acccgtaatc tgtaa      55

SEQ ID NO: 274        moltype = DNA length = 54
FEATURE              Location/Qualifiers
source                1..54
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 274
tgtttattct gagcctcgcc cattgggtact cgttacctca cccgtaatct gtaa      54

SEQ ID NO: 275        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 275
aacttattct gagcctcgcc ccattgggtac tcgttaccct acccgtaatc tgtaa      55

SEQ ID NO: 276        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 276
aacttattct gagcctcgcc ccattgggtac tcgttaccct acccgtaatc tgtaa      55

SEQ ID NO: 277        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 277
tacatattca gagcctcgcc ccattgggtac tcgttatctg acacgtaatc tgtaa      55

SEQ ID NO: 278        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 278
aacttattct gagcctcgcc ccattgggtac tcgttatctg acacgtaatc tgtaa      55

SEQ ID NO: 279        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 279
tacttattca gagcctcgcc ccattggcac tcgttatctc acccgtaatc tgtaa      55

SEQ ID NO: 280        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 280
tacttattca gagcctcgcc ccattggcac ccgttaccct acccgtaacc tgtaa      55

SEQ ID NO: 281        moltype = DNA length = 54
FEATURE              Location/Qualifiers
source                1..54
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 281

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tgtgtattca gagcctcgcc cattggcacc agatacctga ctcgtaatct gtaa	54
SEQ ID NO: 282	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 282	
tacttattca gagcctcgcc ccattggcac ccgttacctc acccgtagcc tgtaa	55
SEQ ID NO: 283	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 283	
cacttattct gagcctcgcc ccacggcac ccgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 284	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 284	
tgtatatagc gaaccccgcc ccattggcac tcgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 285	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 285	
tgtatatagt gaaccccgcc ccattggcac tcgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 286	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 286	
cgtgtactct gaaccccgcc ccattggcac ccgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 287	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 287	
tgtttactct gagcctcgcc ccattggcac tcgttacctc acccgtaatt tgtaa	55
SEQ ID NO: 288	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 288	
ggtttatagc gagcctcgcc ccattggcac ccgttacctc acccgcaacc tgtaa	55
SEQ ID NO: 289	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 289	
ggtttatact gagcctcgcc ccattggcac ccgttacctc acccgtaacc tgtaa	55
SEQ ID NO: 290	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 290	
tgtttatact gagcctcgcc ccattggcac tcgttacctc acccgtaatc tgtaa	55
SEQ ID NO: 291	moltype = DNA length = 55
FEATURE	Location/Qualifiers

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source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 291
tgtttataact gagcctcgcc ccattggcac tcgttacctc ccccgtaatc tgtaa      55

SEQ ID NO: 292        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 292
actttataact gagcctcgcc ccattggcac ccgttacctt acccgcccc tgtaa      55

SEQ ID NO: 293        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 293
actttataact gagcctcgcc ccattggcac ccgttacctc acccgcccc tgtaa      55

SEQ ID NO: 294        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 294
cgtgtattca gagcctcgcc ccattggcac cagatacctg actcgtaatc tgtaa      55

SEQ ID NO: 295        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 295
cgtgtattca gagcctcgcc ccattggcac cagatacctg actcgtaatt tgtaa      55

SEQ ID NO: 296        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 296
gggtgtattca gagcctcgcc ctattggcac cagatacctg actcgtaatc tgtaa      55

SEQ ID NO: 297        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 297
tgtgtattca gagcctcgcc ccattggcac caggtacctg actcgtaatc tgtaa      55

SEQ ID NO: 298        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 298
tgtgtattca gagcctcgcc ccattggcac cagatacctg actcgtaatc tgtaa      55

SEQ ID NO: 299        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 299
tgtgtattca gagcctcgcc ccattggcgc cagatacctg actcgtaatc tgtaa      55

SEQ ID NO: 300        moltype = DNA   length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 300

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tgtgtattca gagcctcgcc ccattggcac cagatacccg actcgtaatc tgtaa	55
SEQ ID NO: 301	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 301	
tgtgtattca gagcctcgcc ccattggcac cacatacctg actcgtaatc tgtaa	55
SEQ ID NO: 302	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 302	
cgtgtactca gagcctcgcc ccattggcac cagatacctg actcgtaatc tgtaa	55
SEQ ID NO: 303	moltype = DNA length = 56
FEATURE	Location/Qualifiers
source	1..56
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 303	
tgtgtattca gagcctcgcc ccattggcac cagatacctg actcgtaatc tgtaat	56
SEQ ID NO: 304	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 304	
tgtgtattca gagccttgcc ccattggcac cagatacctg actcgtaatc tgtaa	55
SEQ ID NO: 305	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 305	
tgtttatagt gaacctcgcc ctattggaac ccggtatctc acacgaaact tgtga	55
SEQ ID NO: 306	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 306	
tgtttatagt gaacctcgcc ctattggaac ccggtatctc acacgaaact tgtaa	55
SEQ ID NO: 307	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 307	
tgtttattct gaaccccgcc ctattggcac tcgttacctt acccggaact tgtaa	55
SEQ ID NO: 308	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 308	
tgtatatagt gaaccccgcc ccattggcac cagatacctg actcgtaatc tgtaa	55
SEQ ID NO: 309	moltype = DNA length = 55
FEATURE	Location/Qualifiers
source	1..55
	mol_type = other DNA
	organism = adeno-associated virus
SEQUENCE: 309	
gaagtataca gagccgctgg ttattggctc tcgttatttg actaatcatt tgtaa	55
SEQ ID NO: 310	moltype = DNA length = 55
FEATURE	Location/Qualifiers

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source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 310
caactaccac gaactccggg ctattgggtc ccgtttcctc acccaccact tgtaa      55

SEQ ID NO: 311        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 311
gaaatacact gagcctaggg ctatcggtac ccgctacctc acccaccacc tgtaa      55

SEQ ID NO: 312        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 312
agcctacaaa gagcccaggg ccattggatc ccgatacctc accaaccacc tctag      55

SEQ ID NO: 313        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 313
atcctattcc gaacctcgtc ccatcggtac ccgttacctt accaaacctc tgtaa      55

SEQ ID NO: 314        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 314
ggaatacaga accaccagac ctatcggaac ccgatacctt acccgacccc tttaa      55

SEQ ID NO: 315        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 315
cgaatacaga accaccagag ccatcggaac ccgatacctc acccgacccc tttaa      55

SEQ ID NO: 316        moltype = DNA length = 55
FEATURE              Location/Qualifiers
source                1..55
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 316
tg gatatgta gaagatagat tgattggaac cagatatcta actcaaaatc tgtaa      55

SEQ ID NO: 317        moltype = DNA length = 106
FEATURE              Location/Qualifiers
source                1..106
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 317
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctcttgaaa gaagagaccg  60
gtagagccgt caccacagcg ttccccgac tcctccacgg gcatcg                106

SEQ ID NO: 318        moltype = DNA length = 105
FEATURE              Location/Qualifiers
source                1..105
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 318
ctcgaacctc tcggtctggt tgaggaagct gctaagacgg ctcttgaaa gaagagaccg  60
gtagacccgtc acctcagcgt tcccccgact cctccacggg catcg                105

SEQ ID NO: 319        moltype = DNA length = 106
FEATURE              Location/Qualifiers
source                1..106
                      mol_type = other DNA

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                                organism = adeno-associated virus
SEQUENCE: 319
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctctgcaaa gaagagaccg 60
gtagagccgt cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 320      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 320
ctcgaacctc tcggtctggc tgaggaagct gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 321      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 321
ctcgaacctc tcggtctggt tgaggaagct gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 322      moltype = DNA  length = 107
FEATURE            Location/Qualifiers
source             1..107
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 322
ctcgaacctc tcggtctggt tgaggaagct gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctccacg ggcacg 107

SEQ ID NO: 323      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 323
ctcgaacctc tcggtccggt tgaggaagct gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 324      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 324
ctcgaacctc tcggtctggt tgaggaagct gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctcccggg gcatcg 106

SEQ ID NO: 325      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 325
ctcgaacctc tcggtctggt tgaggaagcg gctaagacgg ctctggaaa gaagagaccg 60
gtagaacctg cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 326      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 326
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctctggaaa gaagagaccg 60
gtagagccat cacctcagcg ttcccccgac tcctccacgg gcatcg 106

SEQ ID NO: 327      moltype = DNA  length = 106
FEATURE            Location/Qualifiers
source             1..106
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 327
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctctggaaa gaagagaccg 60
gtagagccat caccacagcg ttctccagac tcctctacgg gcatcg 106

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SEQ ID NO: 328 moltype = DNA length = 91
 FEATURE Location/Qualifiers
 source 1..91
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 328
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagagaccc 60
 atagaatccc ccgactcctc cacgggcatc g 91

SEQ ID NO: 329 moltype = DNA length = 106
 FEATURE Location/Qualifiers
 source 1..106
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 329
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagagaccg 60
 gtagagccat caccacagcg ttctccagac tccactacgg gcatcg 106

SEQ ID NO: 330 moltype = DNA length = 88
 FEATURE Location/Qualifiers
 source 1..88
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 330
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagcggcca 60
 gtagaacggg actccagctc gggcatcg 88

SEQ ID NO: 331 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 331
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagagacca 60
 gtagagcagt caccacaaga accagactcc tctcgggca tcg 103

SEQ ID NO: 332 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 332
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagagaccg 60
 gtagagcagt cgccacaaga gccagactcc tctcgggca tcg 103

SEQ ID NO: 333 moltype = DNA length = 91
 FEATURE Location/Qualifiers
 source 1..91
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 333
 ctcgaaacctc tcggtctggt tgaggaagtc gctaagacgg ctcttgaaa gaagagaccc 60
 atagaatccc ccgactcctc cacgggcatc g 91

SEQ ID NO: 334 moltype = DNA length = 91
 FEATURE Location/Qualifiers
 source 1..91
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 334
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg cttccgaaa gaagagaccc 60
 atagaatccc ccgactcctc cacgggcatc g 91

SEQ ID NO: 335 moltype = DNA length = 91
 FEATURE Location/Qualifiers
 source 1..91
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 335
 ctcgaaacctc tcggtctggt tgaggaaggg gctaagacgg ctcttgaaa gaagagaccc 60
 ataggctctc cagactcctc cacgggcatc g 91

SEQ ID NO: 336 moltype = DNA length = 91
 FEATURE Location/Qualifiers
 source 1..91

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mol_type = other DNA
organism = adeno-associated virus
SEQUENCE: 336
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctcttgaaa gaagagaccc 60
atagactctc cagactctc cacgggcatc g 91

SEQ ID NO: 337      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 337
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctcttgaaa gaaacgtccg 60
gtagagcagt cgccacaaga gccagactcc tctcgggca tcg 103

SEQ ID NO: 338      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 338
ctcgaacctc tcggtctggt tgaggaaggc gctaagacgg ctctccaaa gaaacgtccg 60
gtagagcagt cgccacaaga gccagactcc cctcgggca tcg 103

SEQ ID NO: 339      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 339
ctcgaacctc tcggtctggt tgaggaaggc gctgagacgg ctcttgaaa gaaacgtccg 60
gtagagcagt cgccacaagg gccagactcc tctcgggca tcg 103

SEQ ID NO: 340      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 340
ctcgaacctt ttggtctggt tgaggaaggc gctaagacgg ctcttgaaa gaaacgtccg 60
gtagagcagt cgccacaaga gccagactcc tctcgggca ttg 103

SEQ ID NO: 341      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 341
cttgaacctc tgggcctggt tgaggaacct gttaagacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtcca gccagactcc tctcgggaa ccg 103

SEQ ID NO: 342      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 342
cttgaacctc tgggcctggt tggggaacct gttaagacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactcc tctcgggaa ccg 103

SEQ ID NO: 343      moltype = DNA length = 102
FEATURE            Location/Qualifiers
source              1..102
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 343
cttgaacctc tgggcctggt tggggaacct gtcaagacgg ctccaggaaa aagaggccgg 60
tagagcactc tctgtggag ccagactcct cctcgggaac cg 102

SEQ ID NO: 344      moltype = DNA length = 103
FEATURE            Location/Qualifiers
source              1..103
                    mol_type = other DNA
                    organism = adeno-associated virus
SEQUENCE: 344
cttgaacctc tgggcctggt tgaggaacct gttaagacgg ctccgggaaa aaagaggccg 60

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gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 345 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 345
 cttgaacctc tgggcctggt tgaggaaact gttaagacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 346 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 346
 cttgaacctc tgggcctggt tgaggagcct gttaagacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 347 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 347
 cttgaacctc tgggcctggt tgaggaaact gttaagacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 348 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 348
 cttgaacctc tgggcctggt tgaggagcct gttaaaacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 349 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 349
 ctggaacctc tgggcctggt tgaggagcct gttaagacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagattcc tcctcgggaa ctg 103

SEQ ID NO: 350 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 350
 ctggaacctc tgagcctggt tgaggagcct gttaagacgg ctccgggaaa aaagaggccg 60
 gtagagcaact ctcttcgcca gccagattcc tcctcgggaa ctg 103

SEQ ID NO: 351 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 351
 cttgaacctc tgggcctggt tgaggaaact gttaaggcgg ctccgggaga aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 352 moltype = DNA length = 103
 FEATURE Location/Qualifiers
 source 1..103
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 352
 cttgaacctc tgggcctggt tgaggaaact gttaagacgg ctccgggaga aaagaggccg 60
 gtagagcaact ctcttcgcca gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 353 moltype = DNA length = 103
 FEATURE Location/Qualifiers

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source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 353
cttgaacctc tgggcttggt tggggagcct gttaaaacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 354        moltype = DNA length = 102
FEATURE              Location/Qualifiers
source                1..102
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 354
cttgaacctc tgggcttggt tgaggagcct gttaaaacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactcc tcctcggaac cg 102

SEQ ID NO: 355        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 355
cttgaacctc tgggcttggt tgaggagcct gttaaaacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 356        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 356
cttgaacctc tgggcttggt tgaggagcct gttaagacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgcgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 357        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 357
cttgaacctc tgggcttggt tgaggagcct gttaaaacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactgg tggcgcccaa ccg 103

SEQ ID NO: 358        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 358
cttgaacctc tgggcttggt tgaggagcct gttaaaacgg ctccgggaaa aaagagaccg 60
gtagagcact ctctgcgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 359        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 359
cttgaacctc tgggcttggt tgaggagcct gttaagacgg ctccgggaaa aaagaggccg 60
gtagagcact ctctgtgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 360        moltype = DNA length = 105
FEATURE              Location/Qualifiers
source                1..105
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 360
cttgaacctc tgcggcctgg tttgaggaaa cctgttaaga cggctccggg aaaaaagagg 60
ccggtagagc actctcctgt tagccagact cctcctcggg aaccg 105

SEQ ID NO: 361        moltype = DNA length = 103
FEATURE              Location/Qualifiers
source                1..103
                      mol_type = other DNA
                      organism = adeno-associated virus

SEQUENCE: 361

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cttgaacctc tgggcctggt tgaggaacct gtaaaacgg ctccgggaaa aaagaggccg 60
gtagagcacc ctctgtgga gccagactcc tcctcgggaa ccg 103

SEQ ID NO: 362      moltype = DNA  length = 103
FEATURE            Location/Qualifiers
source             1..103
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 362
cttgagcctc ttggtctggt tgaggaagca gtaaaacgg ctctgggaaa gaagggggct 60
gtagatcagt ctctcagga accggactca tcctctggtg ttg 103

SEQ ID NO: 363      moltype = DNA  length = 103
FEATURE            Location/Qualifiers
source             1..103
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 363
cttgagcctc ttggtctggt tgaggaagca gtaaaacgg ctctgggaaa gaagaggcct 60
gtagatcagt ctctcagga accggactca tcctctggtg ttg 103

SEQ ID NO: 364      moltype = DNA  length = 103
FEATURE            Location/Qualifiers
source             1..103
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 364
cttgagcctc tgggtctggt tgaggaagcg gtaagacgg ctctgggaaa aaagagacct 60
gtagagcaat ctccagcaga accggactcc tcctcgggca tcg 103

SEQ ID NO: 365      moltype = DNA  length = 103
FEATURE            Location/Qualifiers
source             1..103
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 365
ctcgagcctc ttggtctggt tgaggaagct gtaagacgg ctctgggaaa aaagagacct 60
atagagcagt ctctgcaga accggactct tcctcgggca tcg 103

SEQ ID NO: 366      moltype = DNA  length = 103
FEATURE            Location/Qualifiers
source             1..103
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 366
cttgaacctc ttggtctggt tgaggaagcg gtaagacgg ctctgggaaa gaagaggcct 60
gtagagcagt ctctcagga accggactcc tcgcgggta ttg 103

SEQ ID NO: 367      moltype = DNA  length = 100
FEATURE            Location/Qualifiers
source             1..100
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 367
ctcgaacctc tgggcctggt tgaagaaggt gtaagacgg ctctgggaaa gaagagaccg 60
ttagagtcac cacaagagcc cgactcctcc tcaggaatcg 100

SEQ ID NO: 368      moltype = DNA  length = 100
FEATURE            Location/Qualifiers
source             1..100
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 368
ctcgaaccac tgggcctggt tgaagaaggt gtaagacgg ctctgggaaa gaagagaccg 60
ttagagtcac cacaagagcc cgactcctcc tcaggaatcg 100

SEQ ID NO: 369      moltype = DNA  length = 100
FEATURE            Location/Qualifiers
source             1..100
                   mol_type = other DNA
                   organism = adeno-associated virus

SEQUENCE: 369
ctcgaacctc tgggcctggt tgaagaaggt gtaaaacgg ctctgggaaa gaagagaccg 60
ttagagtcac cacaagagcc cgactcctcc tcgggcatcg 100

SEQ ID NO: 370      moltype = DNA  length = 111

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FEATURE	Location/Qualifiers	
source	1..111	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 370		
ctcgagcctc	tgggtctggt	tgaagagggc gttaaaacgg ctcttgaaa gaaacgccc 60
ttagaaaaga	ctccaaatcg	gccgaccaac ccgactctg ggaaggcccc g 111
SEQ ID NO: 371	moltype = DNA length = 102	
FEATURE	Location/Qualifiers	
source	1..102	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 371		
cttgaacctc	ttggtctggt	tgagcaagcg ggtgagacgg ctcttgaaa gaagagaccg 60
ttgattgata	ccccagcag	cccgactcct ccacgggtat cg 102
SEQ ID NO: 372	moltype = DNA length = 106	
FEATURE	Location/Qualifiers	
source	1..106	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 372		
ctcgaacctc	ttggcctggt	tgagacgccg gataaaacgg cgctgcggc aaaaaagagg 60
cctctagagc	agagtctca	agagccagac tcctcgagcg gagttg 106
SEQ ID NO: 373	moltype = DNA length = 103	
FEATURE	Location/Qualifiers	
source	1..103	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 373		
ctcgaacctc	ttggtctggt	ggaagactca aagacggctc cgaccggaga caagcggaaa 60
ggcgaagacg	aacctcggtt	gcccgcact tcttcacaga ctc 103
SEQ ID NO: 374	moltype = DNA length = 97	
FEATURE	Location/Qualifiers	
source	1..97	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 374		
ctcgaacctt	ttggcctggt	tgaagagggt gctaagacgg cccctaccgg aaagcggata 60
gacgaccact	ttccaaaaag	aaagaaggct cggaccg 97
SEQ ID NO: 375	moltype = DNA length = 88	
FEATURE	Location/Qualifiers	
source	1..88	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 375		
ttagagccat	ttggcctagt	agaagatcct gtcaacacgg cacctgcaaa aaaaaatata 60
gggaagctta	ctgaccatta	cccggtag 88
SEQ ID NO: 376	moltype = DNA length = 57	
FEATURE	Location/Qualifiers	
source	1..57	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 376		
cagcgtagat	cgtgtgggag	ctgcagaagg agaacagcaa gcgcaggaac ccagaat 57
SEQ ID NO: 377	moltype = DNA length = 60	
FEATURE	Location/Qualifiers	
source	1..60	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 377		
cagcgtggag	atcgagtggg	agctgcagaa ggagaacagc aagcgctgga acccagagat 60
SEQ ID NO: 378	moltype = DNA length = 60	
FEATURE	Location/Qualifiers	
source	1..60	
	mol_type = other DNA	
	organism = adeno-associated virus	
SEQUENCE: 378		
cagcgtggag	atcgagtggg	agctgcagaa ggagaacagc aagcgctgga gccacagat 60

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SEQ ID NO: 379                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 379
cagcgtggag atcgagtggg agctgcagaa ggagaacagc aagtgcctgga acccagagat 60

SEQ ID NO: 380                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 380
cagcgtggaa atcgagtggg agctgcagaa ggaaaacagc aagcgcctggg acccggagat 60

SEQ ID NO: 381                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 381
cagcgtggaa atcgagtggg agctgcagaa ggagaacagc aaacgcctgga acccagagat 60

SEQ ID NO: 382                moltype = DNA   length = 61
FEATURE                      Location/Qualifiers
source                      1..61
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 382
ccagcgtgga aatcgagtgg gagctgcaga aggagaacag caaacgttgg aacccagaga 60
t                                                    61

SEQ ID NO: 383                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 383
cagcgtggag atcgagtggg agctgcagaa ggagaacagc aagcgatgga acccagaaat 60

SEQ ID NO: 384                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 384
cagcgtggaa atcgaatggg agctgcagaa agaaaacagc aagcgcctgga acccagagat 60

SEQ ID NO: 385                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 385
cagcgtggaa attgaatggg agctgcagaa agagaacagc aagcgcctgga acccagagat 60

SEQ ID NO: 386                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 386
cagcgtggaa attgaatggg agctgcagaa agaaaacagc aaacgcctgga acccagagat 60

SEQ ID NO: 387                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers
source                      1..60
                             mol_type = other DNA
                             organism = adeno-associated virus

SEQUENCE: 387
cagcgtggag attgagtggg agctgcagaa ggagaacagc aagcgcctgga atcccagagat 60

SEQ ID NO: 388                moltype = DNA   length = 60
FEATURE                      Location/Qualifiers

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source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 388
cagcgtggaa attgaatggg agctgcagaa ggaaaacagc aagcgctgga accccgagat 60

SEQ ID NO: 389 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 389
cagcgtggaa attgaatggg agctacagaa ggaaaacagc aagcgctgga accccgagat 60

SEQ ID NO: 390 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 390
cagcgtggaa atcgagtggg agctgcagaa agaaaacagc aagcgctgga atccagagat 60

SEQ ID NO: 391 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 391
cagcgtggaa atcgagtggg agctgcagaa agaaaacagc aaacgctgga atccagagat 60

SEQ ID NO: 392 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 392
cagcgtggag atcgagtggg aactgcagaa agaaaacagc aaacactgga atccagagat 60

SEQ ID NO: 393 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 393
cagcgtggag atcgagtggg aactgcagaa agagaacagc aaacgctgga atccagagat 60

SEQ ID NO: 394 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 394
cagcgtggag atcgagtggg aactgcagaa agaaaacagc aaacgctgga atccagagat 60

SEQ ID NO: 395 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 395
cagcgtggaa atcgagtggg agctgcagaa agaaaacagc aagcgctgga acccagaaat 60

SEQ ID NO: 396 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 396
gagtgtggaa attgaatggg agctgcagaa agaaaacagc aagcgctgga atcccgaagt 60

SEQ ID NO: 397 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 397

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gagcgtggag attgaatggg agctgcagaa agaaaacagc aaacgctgga atcccgaagt 60

SEQ ID NO: 398 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 398
cagcgtggag atcgagtggg agctgcagaa ggaaaacagc aaacgctgga atcccgaat 60

SEQ ID NO: 399 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 399
cagcgtggag atcgagtggg agctgcagaa ggagaacagc aaacgctgga atcccgaat 60

SEQ ID NO: 400 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 400
cagcgtggag atcgagtggg agctgcagaa ggagaacagc aaacgctgga atcccagat 60

SEQ ID NO: 401 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 401
cagcgtggag atcgagtggg agctacagaa ggagaacagc aaacgctgga atcccagat 60

SEQ ID NO: 402 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 402
cagcgtggag atcgagtggg agctgcagaa ggagaacagc aaacgctgga accccgagat 60

SEQ ID NO: 403 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 403
cagcgtggag atcgagtggg agctgcagaa ggaggacagc aaacgctgga accccgagat 60

SEQ ID NO: 404 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 404
cagcgtagag atcgagtggg agctgcagaa ggagaacagc aaacgctgga acccgagat 59

SEQ ID NO: 405 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 405
cagcgtggag atcgagtggg agctgcagaa agagaacagc aaacgctgga atcccgaat 60

SEQ ID NO: 406 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 406
cagcgtggag attgagtggg agctgcagaa ggagaacagc aaacgctgga accccgagat 60

SEQ ID NO: 407 moltype = DNA length = 60
FEATURE Location/Qualifiers

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source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 407
cagcgtggag attgagtggg agctgcggaa ggagaacagc aaacgctgga accccgagat 60

SEQ ID NO: 408 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 408
cagcgtggaa attgagtggg agctacagaa agaaaacagc aaacgttgga atccagagat 60

SEQ ID NO: 409 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 409
cagcgtggaa atagagtggg agctgcagaa agaaaacagc aaacgctgga acccagaaat 60

SEQ ID NO: 410 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 410
cagcgtggag atcgagtggg agctgcagaa ggaaaacagc aagcgtgga acccggagat 60

SEQ ID NO: 411 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 411
cagcgtggag atcgagtggg agctgcagaa ggaaaacagc aagcgtggaa cccggagat 59

SEQ ID NO: 412 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 412
cagcgtggag attgagtggg agctgcagaa ggaaaacagc aagcgtgga acccggagat 60

SEQ ID NO: 413 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 413
cgctgttcag attgaatggg aaatcgaaaa ggaacgctcc aaacgctgga atcctgaagt 60

SEQ ID NO: 414 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 414
cgctgttcag attgaatggg aaatcgaaaa ggaacgctcc aaacgccgga atcctgaagt 60

SEQ ID NO: 415 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 415
tgccgttcag atcgactggg aaattcagaa ggagcattcc aaacgctgga atcccgaagt 60

SEQ ID NO: 416 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 416

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gtcgggtgcag attgactggg agatccagaa ggagcgggtcc aaacgctgga accccgaggt 60

SEQ ID NO: 417 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 417
ggctgtcaaa atagaatggg aaatccagaa ggagcgggtcc aagagatgga acccagaggt 60

SEQ ID NO: 418 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 418
cacggtcgaa atcttttggg aactcaagaa ggaaacctcc aagcgctgga accccgaaat 60

SEQ ID NO: 419 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 419
cacggtggag atggagtggg agctcaagaa ggaaaactcc aagaggtgga acccagagat 60

SEQ ID NO: 420 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 420
cacggtggag atggaatggg agctcaaaaa ggaaaactcc aagaggtgga acccagagat 60

SEQ ID NO: 421 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 421
tacagtagag atggtgtggg agctgagaaa agagaattca aagagatgga acccagaaat 60

SEQ ID NO: 422 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 422
ggagccttac ctggcatggt ctggcagaac cgggacgtgt acctgcaggg tcctatttg 59

SEQ ID NO: 423 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 423
ggagccttac ctggcatggt ctggcagaac cgggacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 424 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 424
ggagccttac ctggtatggt ctggcagaac cgggacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 425 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 425
ggagccttac ctggcatggt ctggcagaac cgagacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 426 moltype = DNA length = 59
FEATURE Location/Qualifiers

-continued

source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 426
ggggccttac ctggtatggt ctggcaaaac cgggacgtgt acctgcaggg ccccatctg 59

SEQ ID NO: 427 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 427
ggagccttac ctggcatggt ctggcagaac cgggacgtgt acctgcaggg tccatctg 59

SEQ ID NO: 428 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 428
ggggccttac cgggtatggt ctggcagaac cgggacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 429 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 429
ggagttattc ctggtatggt ctggcagaac cgggacgtgt acctgcaggg ccctatttg 59

SEQ ID NO: 430 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 430
gggggtgattc cgggcatggt gtggcagaat agagacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 431 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 431
ggggctctgc cgggcatggt ctggcagaac cgggacgtgt acctgcaggg tcccatctg 59

SEQ ID NO: 432 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 432
ggggctctgc cgggcatggt ctggcagaac cgggacgtgt gcctgcaggg tcccatctg 59

SEQ ID NO: 433 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 433
ggagcactgc ctggcatggt ctggcagaac cgggacgtgt atctgcaggg tcccatctg 59

SEQ ID NO: 434 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 434
ggagcattac ctggcatggt gtggcaagat agagacgtgt acctgcaggg tccatttg 59

SEQ ID NO: 435 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 435

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ggagcattac ctggcatggt gtggcaaggt agagacgtgt acctgcaggg tcccatttg 59

SEQ ID NO: 436 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 436
ggagcattac ctggcatggt gtggcaaggt agagacgtgt acctgcaggg tcccatttg 59

SEQ ID NO: 437 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 437
ggcggtcttc caggcatggt ctggcaggac agagatgtgt accttcaggg gcccatctg 59

SEQ ID NO: 438 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 438
ggcggtcttc caggcatggt ctggcaggac agagacgtgt acctgcaggg gcccatctg 59

SEQ ID NO: 439 moltype = DNA length = 58
FEATURE Location/Qualifiers
source 1..58
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 439
ggcggtcttc caggcatggt gtggcaggac agagacgtgt acctgcaggg gccatctg 58

SEQ ID NO: 440 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 440
ggcggtcttc caggcatggt ctggcaggac agagacgtgt acctgcaggg gcctatctg 59

SEQ ID NO: 441 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 441
ggcggtcttc caggcatggt ctggcaggac agagacgtgc acctgcaggg gcctatctg 59

SEQ ID NO: 442 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 442
ggcggtcttc caggcatggt ctggcaagac agagacgtgt acctgcaggg gcctatttg 59

SEQ ID NO: 443 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 443
ggcggtcttc caggcatggt cgggcaagac agagacgtgt acctgcaggg gcctacttg 59

SEQ ID NO: 444 moltype = DNA length = 58
FEATURE Location/Qualifiers
source 1..58
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 444
ggcggtcttc caggcatggt ctggcaggac agagacgtgt acctgcgggg cccatctg 58

SEQ ID NO: 445 moltype = DNA length = 59
FEATURE Location/Qualifiers

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source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 445
ggagcgttac ctggtatggt gtggcaggat cgagacgtgt acctgcaggg acccatttg 59

SEQ ID NO: 446 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 446
ggagcgttac ctggtatggt gtggcaggat cgagacgtgt acctgcaggg acccatttg 59

SEQ ID NO: 447 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 447
ggagcgttac ctggtatggt gtggcaggat cgagacgtgt acctgcaggg acccatttg 59

SEQ ID NO: 448 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 448
ggagcattac ctggtatggt gtggcaggat cgagacgtgt acctgcaggg acccatttg 59

SEQ ID NO: 449 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 449
ggagcattac ctggtatggt gtggcaggat cgagacgtgt acctgcaggg acccatttg 59

SEQ ID NO: 450 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 450
ggggccttac ctggtatggt gtggcaagat cgtgacgtgt accttcaagg acctatctg 59

SEQ ID NO: 451 moltype = DNA length = 58
FEATURE Location/Qualifiers
source 1..58
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 451
ggagcgttac ctggtatggt gtggcaggat cgagacgtgt actgcaggga cccatttg 58

SEQ ID NO: 452 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 452
ggaatcttac ctggaatggt gtggcaggac cgcatgtct atcttcaagg tcccatttg 59

SEQ ID NO: 453 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 453
ggaatacttc cgggtatggt ttggcaggac agagatgtgt acctgcaagg acccatttg 59

SEQ ID NO: 454 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
mol_type = other DNA
organism = adeno-associated virus

SEQUENCE: 454

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ggagtgccttc ctggcatggt gtggcaaac agagacattt actaccaagg gccaatgtg 59

SEQ ID NO: 455 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 455
ggagtgccttc ctggcatggt gtggcaaac agagacattt actaccaagg gccaatgtg 59

SEQ ID NO: 456 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 456
ggaattgttc ccggaatggt ctggcaaac agagacattt actaccagg ccctatttg 59

SEQ ID NO: 457 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 457
ggagccgtgc ctggaatggt gtggcaaac agagacattt actaccagg tcccatttg 59

SEQ ID NO: 458 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 458
ggcgtgtacc cgggaatggt gtggcaggac agagacattt actaccaagg gcccatgtg 59

SEQ ID NO: 459 moltype = DNA length = 60
FEATURE Location/Qualifiers
source 1..60
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 459
ggggcgcttc ccgggatggt gtggcaaac agagacattt accctacagg gaccatgtg 60

SEQ ID NO: 460 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 460
gaaatcggtc ccggcagcgt gtggatggag agggacgtgt acctccaagg acccatctg 59

SEQ ID NO: 461 moltype = DNA length = 58
FEATURE Location/Qualifiers
source 1..58
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 461
gaagtgccttc ctggcagcgt atggatggag agggacgtgt acctccagga cccatctg 58

SEQ ID NO: 462 moltype = DNA length = 59
FEATURE Location/Qualifiers
source 1..59
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 462
ggagctttac caggaatggt ttggcagaac agggatatat atctgcagg acctattgg 59

SEQ ID NO: 463 moltype = DNA length = 10
FEATURE Location/Qualifiers
source 1..10
 mol_type = other DNA
 organism = adeno-associated virus

SEQUENCE: 463
gggagctaca

1. A nucleic acid molecule comprising a Parvoviridae cap gene sequence in an antisense orientation, wherein the Parvoviridae cap gene sequence in the antisense orientation comprises a messenger ribonucleic acid (mRNA) splicing suppression mutation.

2. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule further comprises a regulatory sequence.

3. The nucleic acid molecule of claim 2, wherein the regulatory sequence drives expression of the Parvoviridae cap gene sequence in the antisense orientation.

4. The nucleic acid molecule of claim 3, wherein the regulatory sequence is a promoter sequence.

5. The nucleic acid molecule of claim 4, wherein the promoter is a cell type-specific promoter, a tissue-specific promoter, or a ubiquitous promoter.

6. The nucleic acid molecule of claim 1, wherein the Parvoviridae cap gene sequence is derived from an adeno-associated virus (AAV) genome comprising an AAV cap gene sequence.

7. The nucleic acid molecule of claim 6, wherein the AAV cap gene sequence is a wild-type AAV cap gene sequence.

8. The nucleic acid molecule of claim 6, wherein the AAV cap gene sequence is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, or other natural AAV isolate cap gene sequence.

9. The nucleic acid molecule of claim 6, wherein the AAV cap gene sequence is an engineered AAV cap gene sequence.

10. The nucleic acid molecule of claim 6, wherein the AAV cap gene sequence is one of a library of diverse AAV cap gene sequences.

11. The nucleic acid molecule of claim 1, wherein the messenger ribonucleic acid (mRNA) splicing suppression mutation comprises a mutation within an exon-intron junction, wherein the exon-intron junction comprises a sequence as set forth in any one of SEQ ID NOs: 199-220 or 463.

12. The nucleic acid molecule of claim 1, wherein the Parvoviridae cap gene sequence in the antisense orientation

comprises two or more messenger ribonucleic acid (mRNA) splicing suppression mutations.

13. A nucleic acid molecule comprising:

(a) a first nucleic acid sequence comprising a first regulatory sequence and a Parvoviridae cap gene sequence in a sense orientation;

(b) a second nucleic acid sequence comprising a second regulatory sequence and the Parvoviridae cap gene sequence in an antisense orientation, wherein the Parvoviridae cap gene sequence in the antisense orientation comprises a messenger ribonucleic acid (mRNA) splicing suppression mutation.

14. The nucleic acid molecule of claim 13, wherein the second regulatory sequence drives expression of an antisense transcript.

15. The nucleic acid molecule of claim 13, wherein the second regulatory sequence is a cell type-specific promoter, a tissue-specific promoter, or a ubiquitous promoter.

16. The nucleic acid molecule of claim of claim 13, wherein the first regulatory sequence drives expression of the Parvoviridae cap gene sequence in the sense orientation.

17. The nucleic acid molecule of claim 13, wherein the Parvoviridae cap gene sequence is derived from an adeno-associated virus (AAV) genome comprising an AAV cap gene sequence.

18. The nucleic acid molecule of claim 17, wherein the AAV cap gene sequence is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, or other natural AAV isolate cap gene sequence.

19. The nucleic acid molecule of claim 17, wherein the AAV cap gene sequence is an engineered AAV cap gene sequence.

20. The nucleic acid molecule of claim 17, wherein the AAV cap gene sequence is one of a library of diverse AAV cap gene sequences.

21. (canceled)

22. (canceled)

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