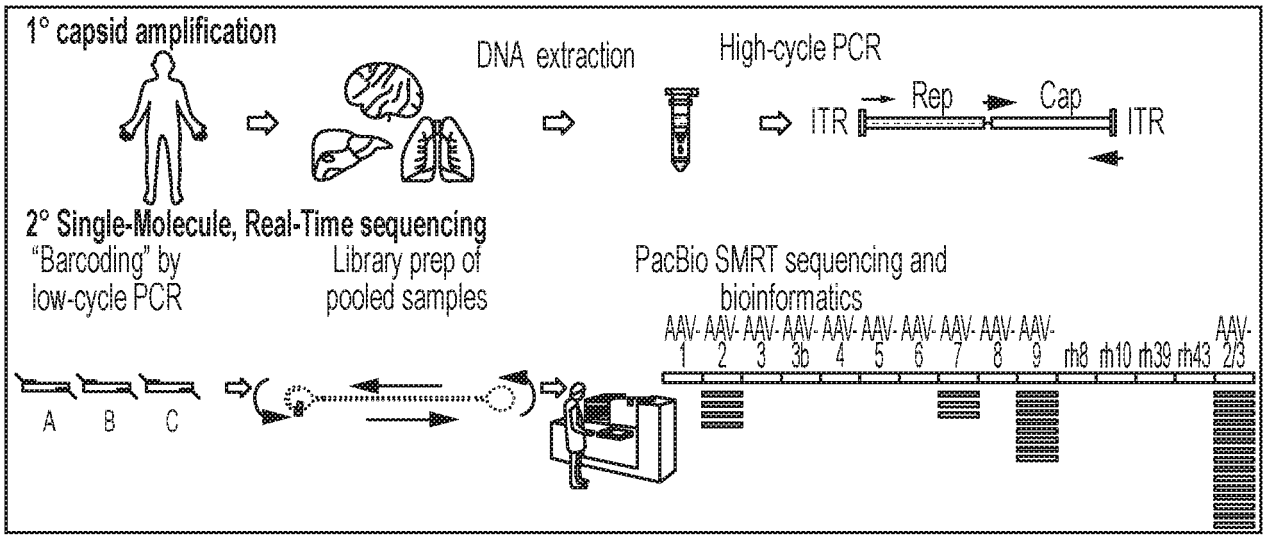

1° capsid amplification
DNA extraction
High-cycle PCR
ITR
Rep
Cap
ITR
2° Single-Molecule, Real-Time sequencing
"Barcoding" by low-cycle PCR
Library prep of pooled samples
PacBio SMRT sequencing and bioinformatics
AAV-1
AAV-2
AAV-3
AAV-3b
AAV-4
AAV-5
AAV-6
AAV-7
AAV-8
AAV-9
rh8
rh10
rh39
rh43
AAV-2/3
A
B
C

FIG. 1A

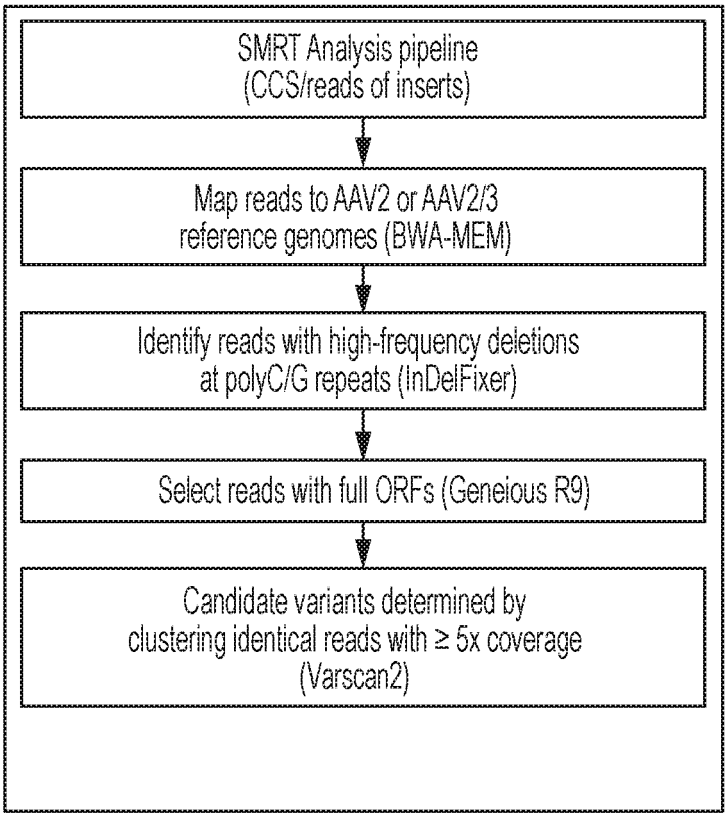

SMRT Analysis pipeline (CCS/reads of inserts)
Map reads to AAV2 or AAV2/3 reference genomes (BWA-MEM)
Identify reads with high-frequency deletions at polyC/G repeats (InDelFixer)
Select reads with full ORFs (Geneious R9)
Candidate variants determined by clustering identical reads with ≥ 5x coverage (Varscan2)

FIG. 1B

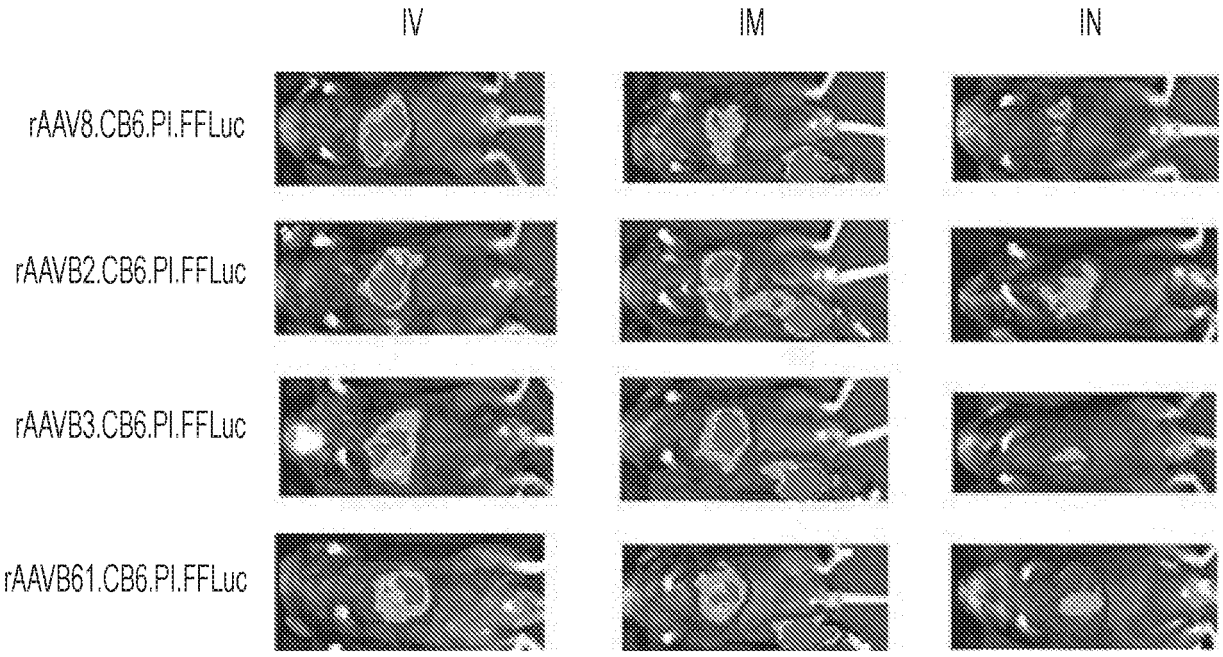

IV
IM
IN
rAAV8.CB6.PI.FFLuc
rAAVB2.CB6.PI.FFLuc
rAAVB3.CB6.PI.FFLuc
rAAVB61.CB6.PI.FFLuc

FIG. 2A

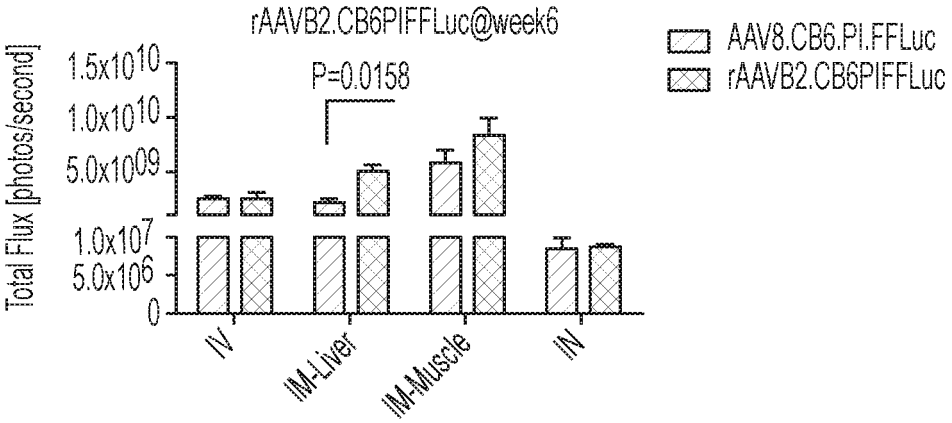


FIG. 2B

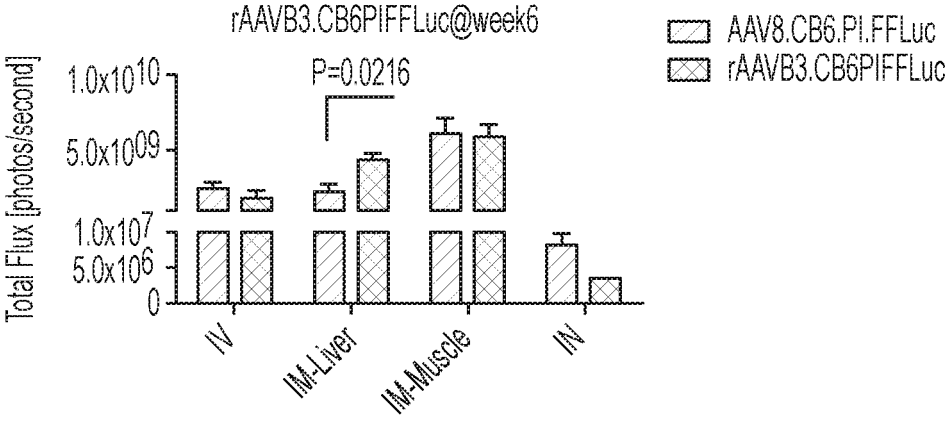


FIG. 2C

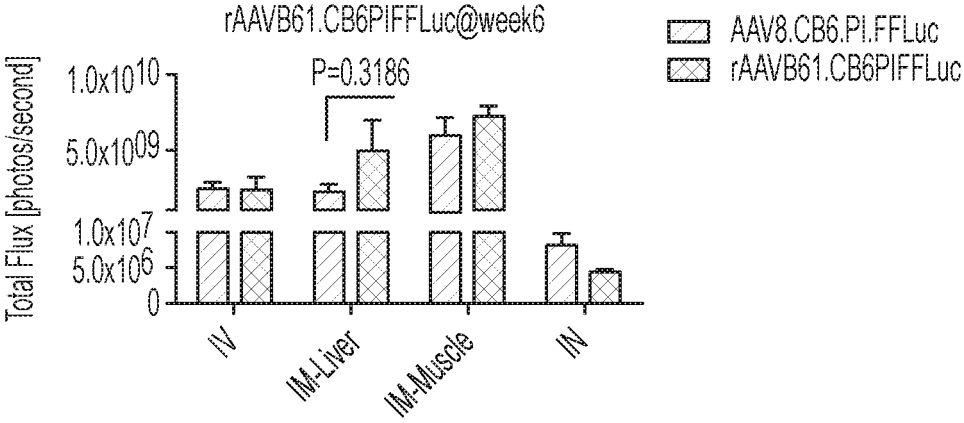


FIG. 2D

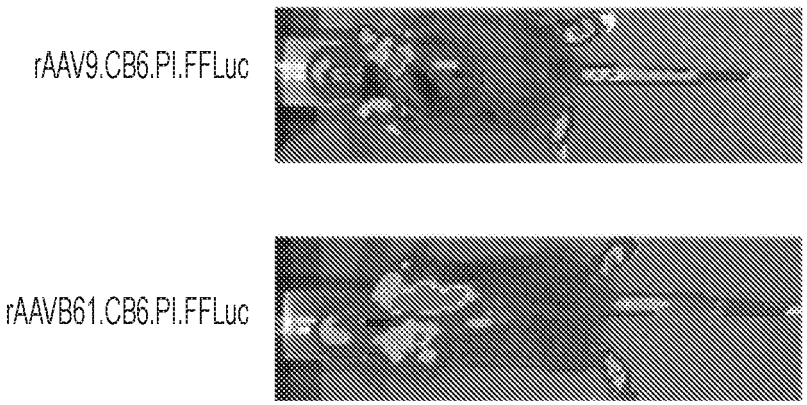

rAAV9.CB6.PI.FFLuc
rAAVB61.CB6.PI.FFLuc

FIG. 3A

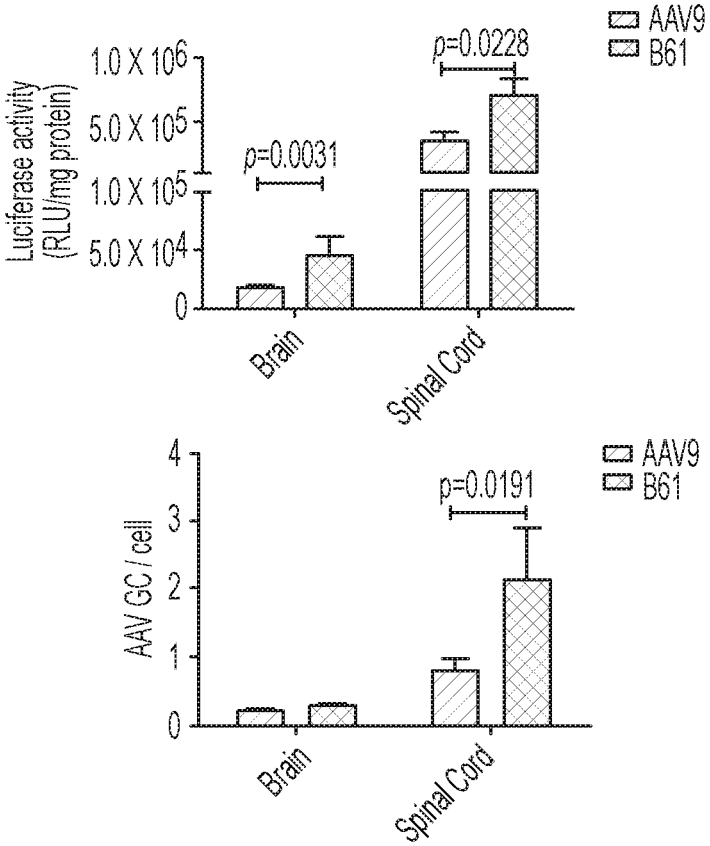

Luciferase activity (RLU/mg protein)
1.0 X 10^6
5.0 X 10^5
1.0 X 10^5
5.0 X 10^4
0
p=0.0031
p=0.0228
AAV9
B61
Brain
Spinal Cord
AAV GC / cell
4
3
2
1
0
p=0.0191
AAV9
B61
Brain
Spinal Cord

FIG. 3B

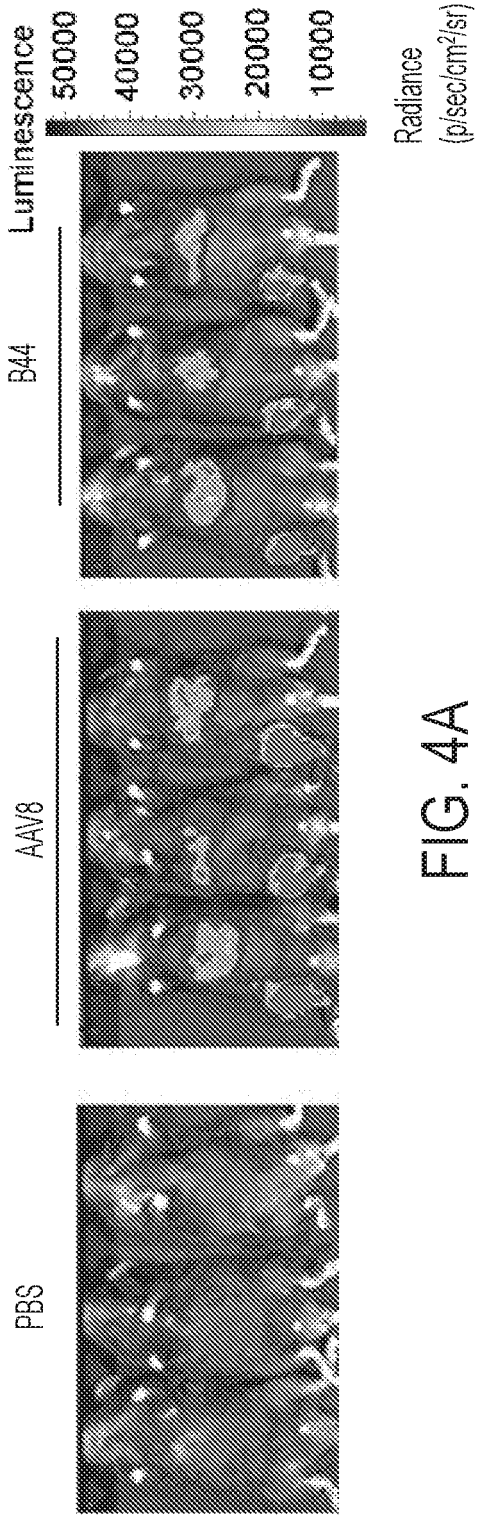

PBS
AAV8
B44
Luminescence
50000
40000
30000
20000
10000
Radiance
(p/sec/cm²/sr)

FIG. 4A

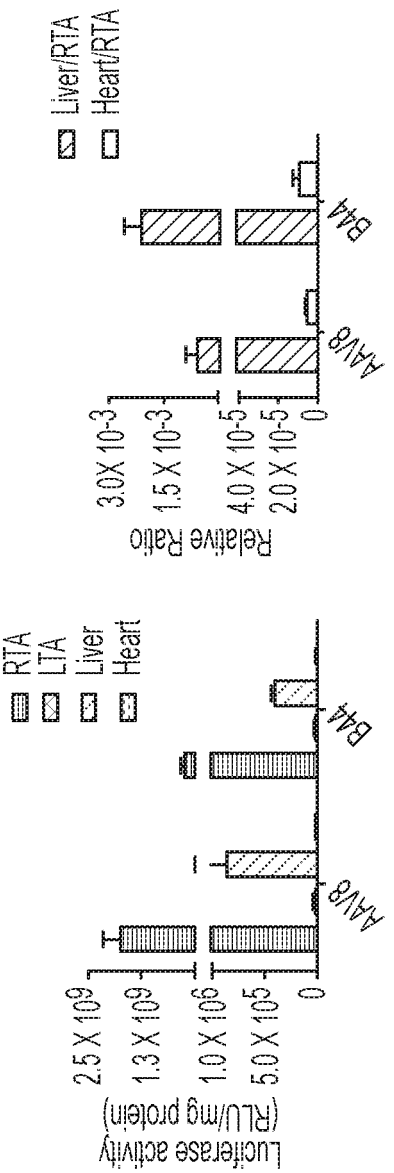

RTA
LTA
Liver
Heart
Luciferase activity
(RLU/mg protein)
2.5 X 10⁹
1.3 X 10⁹
1.0 X 10⁶
5.0 X 10⁵
0
AAV8
B44
Liver/RTA
Heart/RTA
Relative Ratio
3.0X 10⁻³
1.5 X 10⁻³
4.0 X 10⁻⁵
2.0 X 10⁻⁵
0
AAV8
B44

FIG. 4B

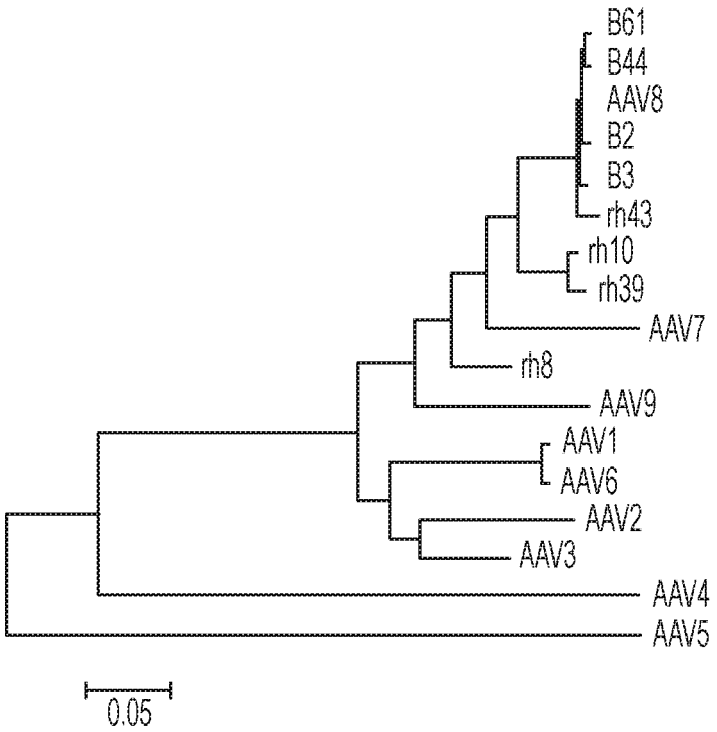

B61
B44
AAV8
B2
B3
rh43
rh10
rh39
AAV7
rh8
AAV9
AAV1
AAV6
AAV2
AAV3
AAV4
AAV5
0.05

FIG. 5

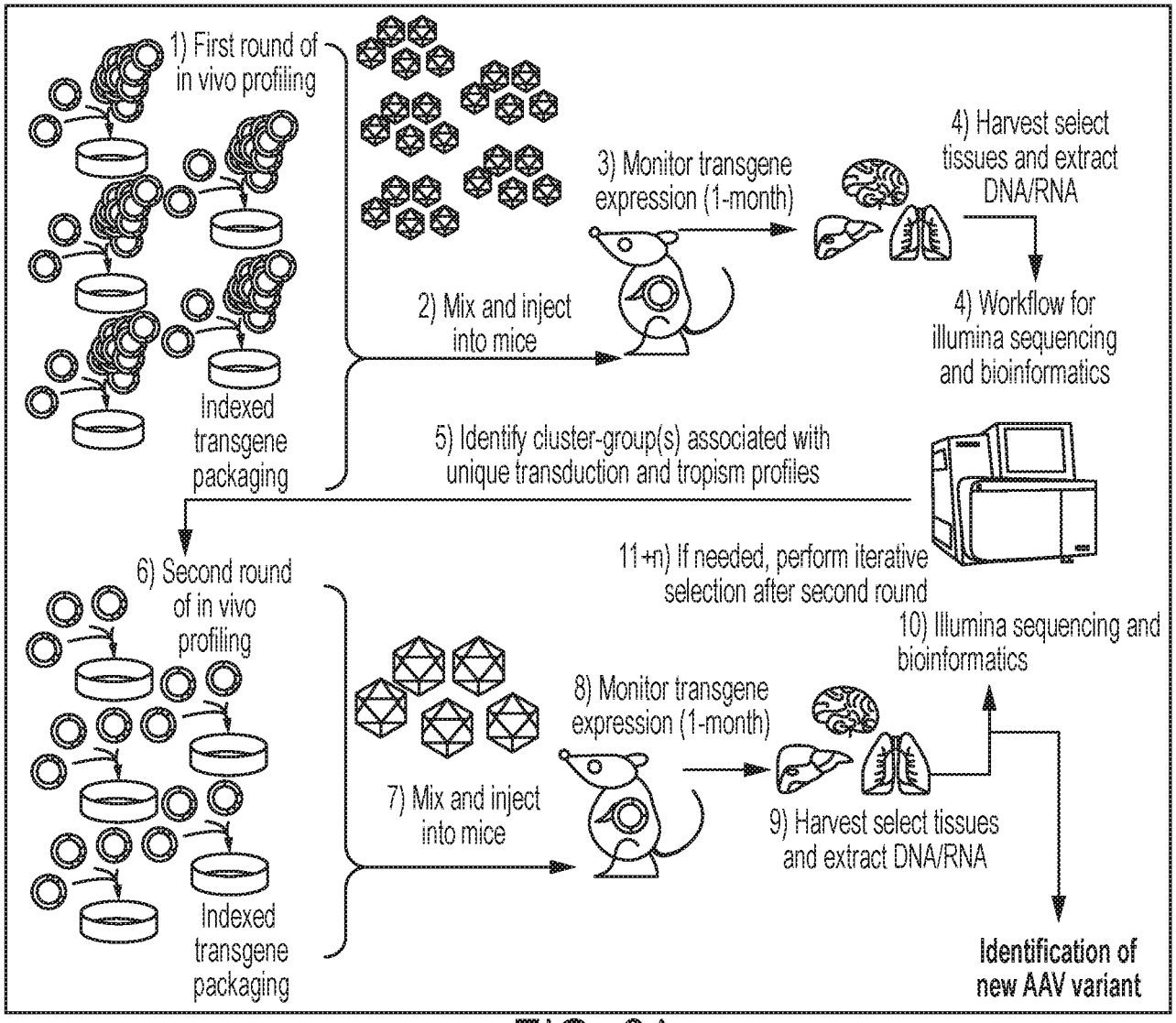

1) First round of in vivo profiling
Indexed transgene packaging
2) Mix and inject into mice
3) Monitor transgene expression (1-month)
4) Harvest select tissues and extract DNA/RNA
4) Workflow for illumina sequencing and bioinformatics
5) Identify cluster-group(s) associated with unique transduction and tropism profiles
6) Second round of in vivo profiling
Indexed transgene packaging
7) Mix and inject into mice
8) Monitor transgene expression (1-month)
9) Harvest select tissues and extract DNA/RNA
10) Illumina sequencing and bioinformatics
11+n) If needed, perform iterative selection after second round
Identification of new AAV variant

FIG. 6A

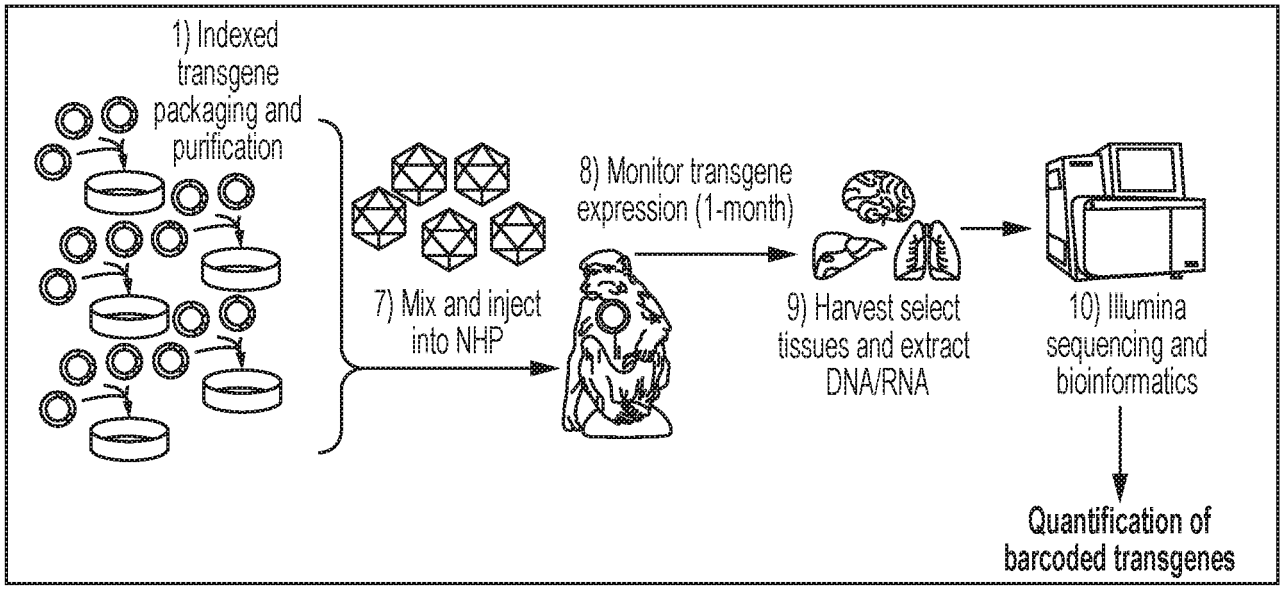

1) Indexed transgene packaging and purification
7) Mix and inject into NHP
8) Monitor transgene expression (1-month)
9) Harvest select tissues and extract DNA/RNA
10) Illumina sequencing and bioinformatics
Quantification of barcoded transgenes

FIG. 6B

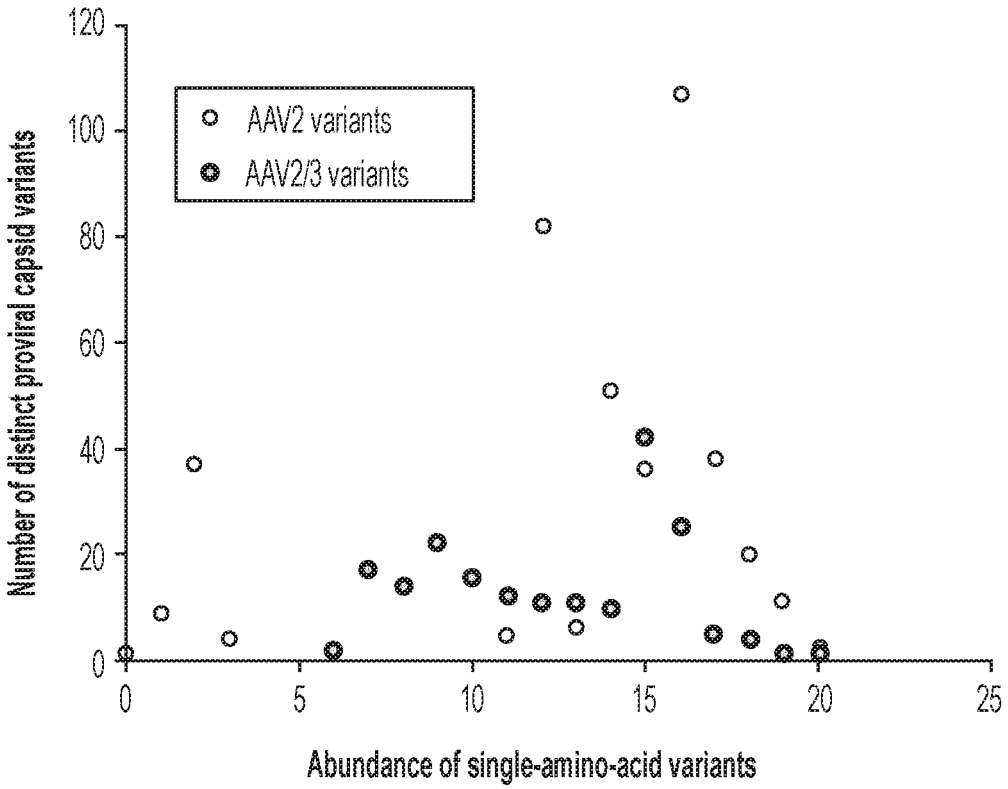

120
100
80
60
40
20
0
0
5
10
15
20
25
AAV2 variants
AAV2/3 variants
Number of distinct proviral capsid variants
Abundance of single-amino-acid variants

FIG. 7

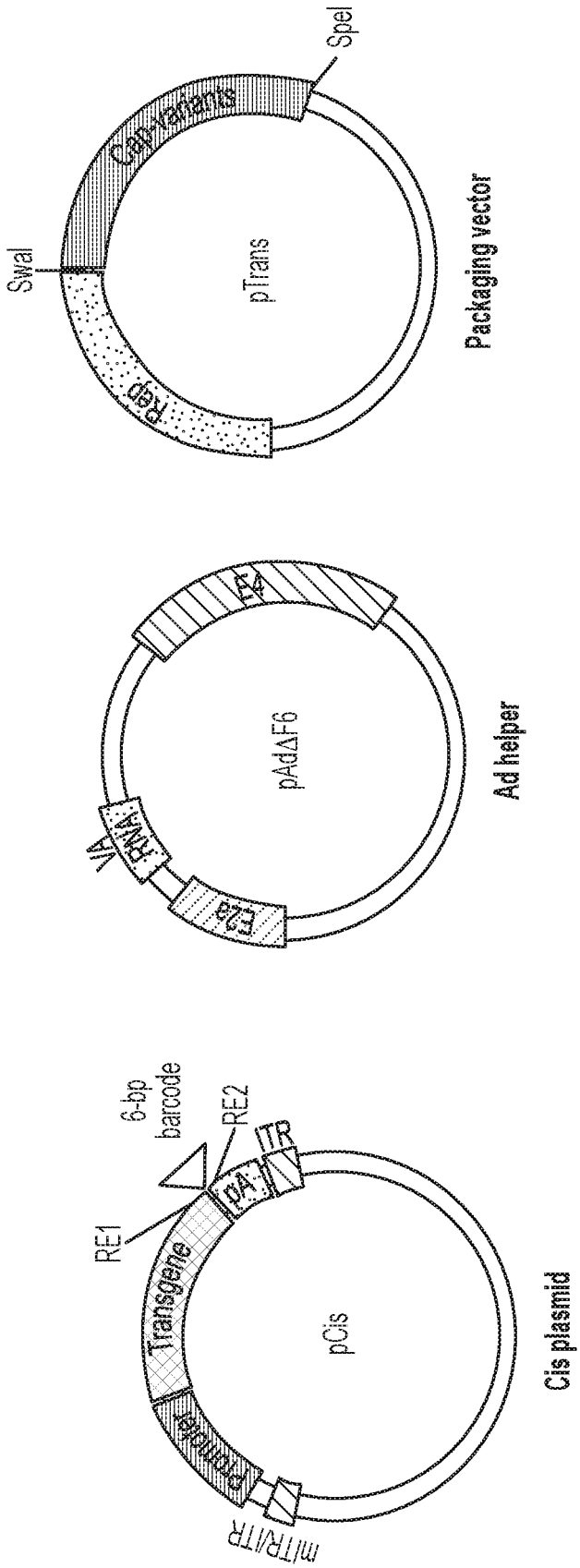

SpeI
CBA-variants
SwaI
pTrans
Rep
Packaging vector
E4
pAdΔF6
VA
RNA
E2a
Ad helper
6-bp
barcode
RE2
ITR
RE1
pA
Transgene
Promoter
pCis
ITR/ITR
Cis plasmid

FIG. 8

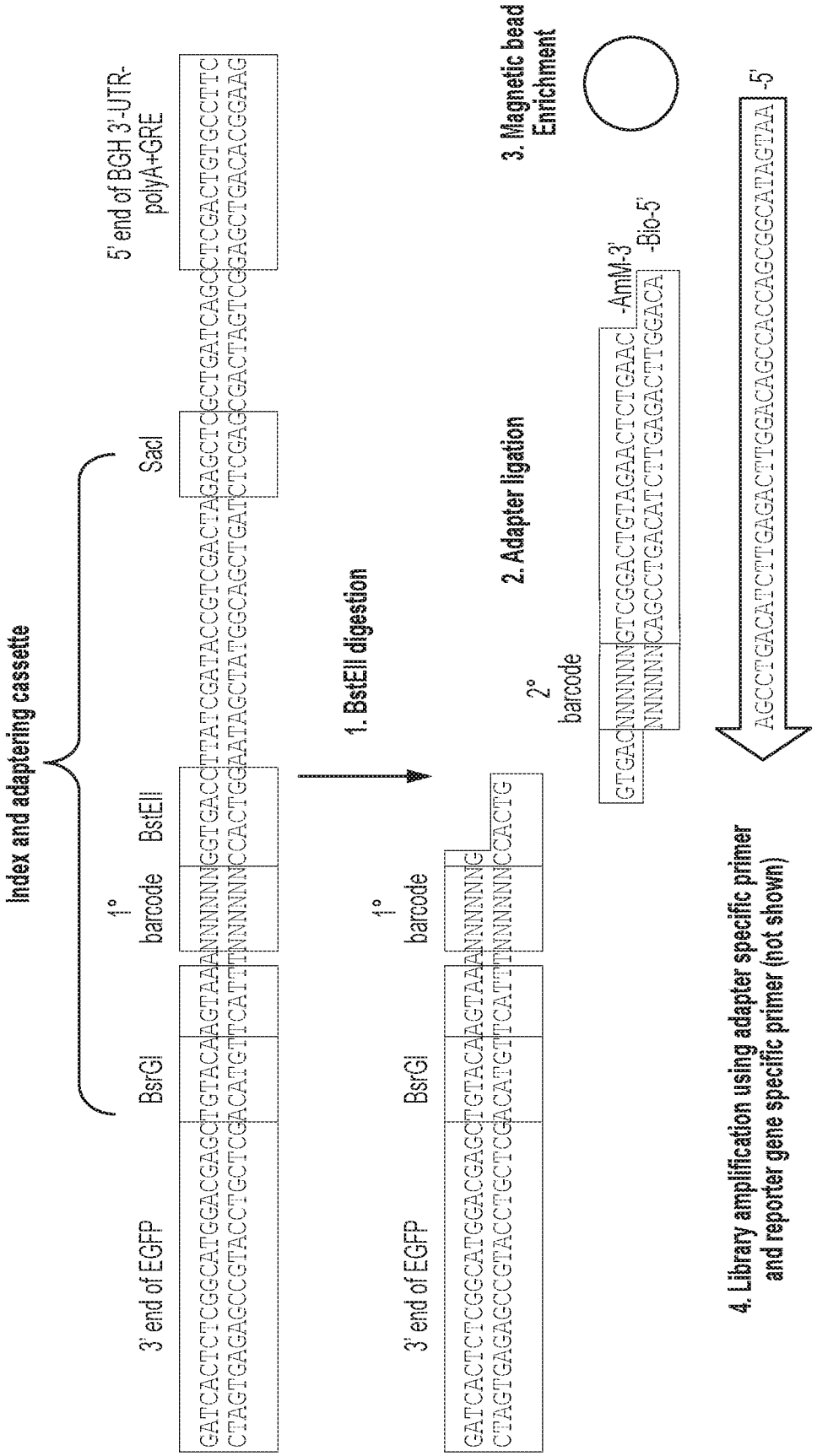

Index and adaptering cassette
3' end of EGFP
BsrGI
1° barcode
BstEII
SacI
5' end of BGH 3'-UTR-polyA+GRE
1. BstEII digestion
2. Adapter ligation
2° barcode
3. Magnetic bead Enrichment
-AmM-3'
-Bio-5'
4. Library amplification using adapter specific primer and reporter gene specific primer (not shown)
-5'

FIG. 9