viruses

MDPI

Supplementary Materials

Expanding the Scope of Adenoviral Vectors by Utilizing Novel Tools for Recombination and Vector Rescue

Table S1. Plasmid List. List of plasmids used and generated for this study, including general features, size and GenBank Accession Number.

Plasmid Name	Size	Features	Accession
pGPS1.1	4814 bp	GPS-1 Genome Priming System transposon donor; kanamycin resistance (KanR); tetracycline resistance (TetR)	10666445
pKD46	6329 bp	red-recombineering plasmid; Arabia sugar dependent expression of lambda red phage exo, beta and gam protein; temperature sensitive; β-lactamase expression (AmpR)	10829079
pKSB2	6457 bp	Single-copy BACmid backbone; chloramphenicol resistance (CamR)	
pBWH-A12-RC	40563 bp	Genomic BACmid; carrying HAdV-A12 genome in reverse orientation; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442630
pBWH-B3	41781 bp	Genomic BACmid; carrying HAdV-B3 genome; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442581
pBWH-B35-RC	41232 bp	Genomic BACmid; carrying HAdV-B35 genome in reverse orientation; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442594
pBWH-C2-RC	42375 bp	Genomic BACmid; carrying HAdV-C2 genome in reverse orientation; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442595
pBWH-F41-RC	40626 bp	Genomic BACmid; carrying HAdV-F41 genome in reverse orientation; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442608
pBWH-SE25	42957 bp	Genomic BACmid; carrying SAdV-E25 genome; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR)	PP442609
pUC-B3-Ins0	2232 bp	HAdV-B3-targeted transfer vector, designated Insertion site 0; empty multi cloning site; β-lactamase expression (AmpR)	PP442615
pUC-B3-Ins1	2625 bp	HAdV-B3-targeted transfer vector, designated Insertion site 1; empty multi cloning site; β-lactamase expression (AmpR)	PP442616
pUC-B3-Ins2	3055 bp	HAdV-B3-targeted transfer vector, designated Insertion site 2; empty multi cloning site; β-lactamase expression (AmpR)	PP442617
pUC-B3-Ins3	2255 bp	HAdV-B3-targeted transfer vector, designated Insertion site 3; empty multi cloning site; β-lactamase expression (AmpR)	PP442618
pUC-B3-Ins4	2414 bp	HAdV-B3-targeted transfer vector, designated Insertion site 4; empty multi cloning site; β-lactamase expression (AmpR)	PP442619
pUC-E4-Ins0	2231 bp	HAdV-E4-targeted transfer vector, designated Insertion site 0; empty multi cloning site; β-lactamase expression (AmpR)	PP442625
pUC-E4-Ins1	2821 bp	HAdV-E4-targeted transfer vector, designated Insertion site 1; empty multi cloning site; β-lactamase expression (AmpR)	PP4462626
pUC-E4-Ins2	2386 bp	HAdV-E4-targeted transfer vector, designated Insertion site 2; empty multi cloning site; β-lactamase expression (AmpR)	PP442627
pUC-E4-Ins3	2779 bp	HAdV-E4-targeted transfer vector, designated Insertion site 3; empty multi cloning site; β-lactamase expression (AmpR)	PP442628
pUC-E4-Ins4	2134 bp	HAdV-E4-targeted transfer vector, designated Insertion site 4; empty multi cloning site; β-lactamase expression (AmpR)	PP442629

pUC-B3-I0-mCMV-GFPt2aGLuc	5014 bp	HAdV-B3-targeted transfer vector, designated Insertion site 0; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442610
pUC-B3-I1-mCMV-GFPt2aGLuc	5407 bp	HAdV-B3-targeted transfer vector, designated Insertion site 1; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442611
pUC-B3-I2-mCMV-GFPt2aGLuc	5837 bp	HAdV-B3-targeted transfer vector, designated Insertion site 2; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442612
pUC-B3-I3-mCMV-GFPt2aGLuc	5037 bp	HAdV-B3-targeted transfer vector, designated Insertion site 3; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442613
pUC-B3-I4-mCMV-GFPt2aGLuc	5196 bp	HAdV-B3-targeted transfer vector, designated Insertion site 4; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442614
pUC-E4-I0-mCMV-GFPt2aGLuc	5013 bp	HAdV-E4-targeted transfer vector, designated Insertion site 0; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442620
pUC-E4-I1-mCMV-GFPt2aGLuc	5603 bp	HAdV-E4-targeted transfer vector, designated Insertion site 1; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442621
pUC-E4-I2-mCMV-GFPt2aGLuc	5168 bp	HAdV-E4-targeted transfer vector, designated Insertion site 2; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442622
pUC-E4-I3-mCMV-GFPt2aGLuc	5561 bp	HAdV-E4-targeted transfer vector, designated Insertion site 3; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442623
pUC-E4-I4-mCMV-GFPt2aGLuc	4916 bp	HAdV-E4-targeted transfer vector, designated Insertion site 4; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase; β-lactamase expression (AmpR)	PP442624
pBWH-B3delE3-I0n-Kan	39090 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 0n primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442583
pBWH-B3delE3-I0n-mCMV-GFPt2aGLuc	40882 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 0n; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442584
pBWH-B3delE3-Ins0-Kan	39535 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 0 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442589
pBWH-B3delE3-I0-mCMV-GFPt2aGLuc	40925 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 0; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442582
pBWH-B3delE3-Ins1-Kan	39142 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 1 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442590
pBWH-B3delE3-I1-	40925 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from	PP442585

mCMV-GFPt2aGLuc		Insertion site 1; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	
pBWH-B3delE3-Ins2-Kan	38712 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 2 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442591
pBWH-B3delE3-I2-mCMV-GFPt2aGLuc	40925 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 2; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442586
pBWH-B3delE3-Ins3-Kan	39512 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 3 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442592
pBWH-B3delE3-I3-mCMV-GFPt2aGLuc	40925 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 3; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442587
pBWH-B3delE3-Ins4-Kan	39353 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; Insertion Site 4 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442593
pBWH-B3delE3-I4-mCMV-GFPt2aGLuc	40925 bp	Genomic BACmid; carrying HAdV-B3 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 4; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442588
pBWH-E4delE3-I0n-Kan	39073 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 0n primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442597
pBWH-E4delE3-I0n-mCMV-GFPt2aGLuc	40895 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 0n; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442598
pBWH-E4delE3-Ins0-Kan	39549 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 0 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442603
pBWH-E4delE3-I0-mCMV-GFPt2aGLuc	40938 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 0; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442596
pBWH-E4delE3-Ins1-Kan	38959 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 1 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442604
pBWH-E4delE3-I1-mCMV-GFPt2aGLuc	40938 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 1; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442599
pBWH-E4delE3-Ins2-Kan	39390 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 2 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442605
pBWH-E4delE3-I2-	40938 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from	PP442600

mCMV-GFPt2aGLuc		Insertion site 2; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	
pBWH-E4delE3-Ins3-Kan	39001 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 3 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442606
pBWH-E4delE3-I3-mCMV-GFPt2aGLuc	40938 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 3; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442601
pBWH-E4delE3-Ins4-Kan	39646 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; Insertion Site 4 primed for HFR via insertion of KanR; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442607
pBWH-E4delE3-I4-mCMV-GFPt2aGLuc	40938 bp	Genomic BACmid; carrying HAdV-E4 genome, E3-region deleted; constitutive expression of T2A-peptide-linked GFP and gaussia luciferase from Insertion site 4; ITRs flanked by ACT sequences; chloramphenicol resistance (CamR); kanamycin resistance (KanR)	PP442602

Table S1: Bacmid assembly primers**.** Primers generating ITR overlaps used for genomic bacmid assembly. ITR homologous sequences are underlined.

Virus	Primer name	Sequence
HAdV-A12	BWHA12for	AGTCCAGTATAAGGTATATTATTAGATAGGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
	BWHA12rev	AGTCCAGTATAAGGTATATTATTAGATAGGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC
HAdV-B3	BWHB03for	GTTGGCACCATTCCATCTATAAGGTATATTATATAGATAGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
	BWHB03rev	GTTGGCACCATTCCATCTATAAGGTATATTATATAGATAGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC
HAdV-B35	BWHB35for	ATTGGCACCATTCCATCTATAAGGTATATTATTGATGATGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
	BWHB35rev	ATTGGCACCATTCCATCTATAAGGTATATTATTGATGATGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC
HAdV-C2	BWHC02for	ATATTGGCTTCAATCCAAAATAAGGTATATTATGATGATGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC
	BWHC02rev	ATATTGGCTTCAATCCAAAATAAGGTATATTATGATGATGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
HAdV-F41	BWHF41for	TTCCAGCTTTAAGGTATATTATTGATGATGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
	BWHF41rev	TTCCAGCTTTAAGGTATATTATTGATGATGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC
SAdV-E25	BWHSE25for	TAACGCGCACAAAAAGTTTGAGGTATATTATTGATGATGGCCTCCGGGGTCCACTGCAATTACTTCTCGACCAATTCTCATGTTTGAC
	BWHSE25rev	TAACGCGCACAAAAAGTTTGAGGTATATTATTGAAGATGGCCTCCGGGGTCCACTGCAATTATAAACTCGACAGCGACACACTTGC

Table S3. Primers used for cloning of recombinant virus bacmids & transfer vectors.

Name	Sequence
B3-Ins0_For	AGTCATTTCGCGGTTATGCCAGG
B3-Ins0_Rev	ACCTCCTGTGGCAGGAAGCG
B3-Ins0-Kan_For	TTCTGAGTCATTTCGCGGTTATGCCAGGGTGGAGTATTTAAATCCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins0-Kan_Rev	AGATAACCTCCTGTGGCAGGAAGCGCAGGTGTCTCATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
B3-Ins0n-Kan_for	AACTGTTGAATGGTAGATTTATGTTTTTTTCTTGCGATTTAAATTCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins0n-Kan_rev	AAACGTATCCCTTGCTGAAGTGAGTTTGGCGGATCCATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
B3-Ins1_For	GAGTGGAAGCGCTTCTTTTG
B3-Ins1_Rev	CATGCACTCCGACCTTGCTA
B3-Ins1-Kan_For	GCCATGAGTGGAAGCGCTTCTTTTGAGGGGGGAGTATTTAAATCCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins1-Kan_Rev	AACACCATGCACTCCGACCTTGCTATGACTGGGTGATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
B3-Ins2_for	ACTCTTAAAGAGTAGCCCTTGCCC
B3-Ins2_rev	TGTGAACCCGTCAGGGGAAATGAA
B3-Ins2-Kan_For	CGCTGACTCTTAAAGAGTAGCCCTTGCCCGCGCTCATTTAAATACGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins2-Kan_Rev	CTTTGTGTGAACCCGTCAGGGGAAATGAAACCAGGATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
B3-Ins3_For	AACTACTCAGGCAACCCTCA
B3-Ins3_rev	TGAAAGCGCTTTAAAAGACTAT
B3-Ins3-Kan_For	CCAGAAACTACTCAGGCAACCCTCATAACCTCCCCATTTAAATCCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins3-Kan_Rev	GACTGTGAAAGCGCTTTAAAAGACTATCCCGATGCATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
B3-Ins4_For	AAAAGGCACAGGAGAATAAAAAAT
B3-Ins4_Rev	ATGGAATGGTGCCAACATGT
B3-Ins4-Kan_For	AAAGTAAAAGGCACAGGAGAATAAAAAATATAATTATTTAAATCCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
B3-Ins4-Kan_Rev	TATAGATGGAATGGTGCCAACATGTAAATGAGGTAATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins0_For	AGTAATTTAGTGTTTATGACAGGGAG
E4-Ins0_Rev	CTCATCGGGCAGGTCTCTTA
E4-Ins0-Kan_For	ATCTGAGTAATTTAGTGTTTATGACAGGGAGGAGTATTTAAATTCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
E4-Ins0-Kan_Rev	ATTTCCTCATCGGGCAGGTCTCTTAGGTGCCTCATATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins0n-Kan_For	AGGCAAAGAGTAGGGTGACTGGGTGATGACTGGTTTATTTAAATTCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
E4-Ins0n-Kan_Rev	GCTGGTAATCCCTGCTGAAATGGGTTTCTTGACTCCATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins1_For	TGGATGTGACGGAGGACCTG
E4-Ins1_Rev	CACGCCCTGCGTCCCTGCTA
E4-Ins1-Kan_For	GTGTGTGGATGTGACGGAGGACCTGCGACCCGATCATTTAAATGCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA

E4-Ins1-Kan_Rev	AACACCACGCCCTGCGTCCCTGCTATGACTGGGTGATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins2_For	TAAAGATTAAAGAGACGATGATTTTGAATTGATCAATAAA
E4-Ins2_Rev	CCTCCCCCAGCTTGAGGGTA
E4-Ins2-Kan_For	ATCCAGTAAAAAAAATAAAGATTAAAGAGACGATGATTTAAATGCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
E4-Ins2-Kan_Rev	CGTCAAGGTCCACCCCCTCCCCCAGCTTGAGGGTAATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins3_for	TGATGACACCACCAGTGCATACTC
E4-Ins3_rev	GGCAGTGTGTTCTGGAGGGG
E4-Ins3-Kan_For	GGTACTGATGACACCACCAGTGCATACTCAATGTCATTTAAATCCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
E4-Ins3-Kan_Rev	ACATGGGCAGTGTGTTCTGGAGGGGACGCCACCTGATTTAAATAAATGTGGGCG-GACAAAATAGTTGG
E4-Ins4_For	TGCACTTCCTCAAACGCCCA
E4-Ins4_Rev	TGTGAGTTAATATGCAAATAAGGCG
E4-Ins4-Kan_For	ATACGTGCACTTCCTCAAACGCCCAAACTGGCGTCATTTAAATTCGTGTGGGCG-GACAATAAAGTCTTAAACTGAA
E4-Ins4-Kan_Rev	TTTTGTGTGAGTTAATATGCAAATAAGGCGTGAAAATTTAAATAAATGTGGGCG-GACAAAATAGTTGGGAACTGGGAG
H3_B3-I0n_P1rev	GGGTACCGAGCTCGAATTCACTGGCCGTCGAACACTCAG-CAGCTGACATAATTTTATTACATGAC
H3_B3-I0n_P2rev	CTTGCTGAAGTGAGTTTGGCGGATC
H3_E4-I0n_P1rev	GGGTACCGAGCTCGAATTCACTGGCCGTCGACCACGCAC-TTAAAGTCAACACATATTTTATTGCA
H3_E4-I0n_P2rev	CCTGCTGAAATGGGTTTCTTGACTCCAT
H5_B3-I0n_P1for	TGGTAGATTTATGTTTTTTTCTTGCGATTTTTTGTAGGTC
H5_B3-I0n-P2for	GGGGATCCTCTAGAGTCGACCTGCAGGCATTTATTACTTCTTGGGTGGGGACTT-GGATATATAA
H5_E4-I0n_P1for	TAGGGTGACTGGGTGATGACTGGTTTATT
H5_E4-I0n_P2for	GGGGATCCTCTAGAGTCGACCTGCAGGCATTTATGACTCAGGGGAGGGGACTTTG
Ins_CMV-GLuc_Rev	ATGCCTGCAGGTCGACTCTAGAGGATCCCCGAGTCGACAAGCTTGAATTCG
Ins_mCMV-E4-1_for	GAGCTCGGTACCCGGGGATCCTCTAGAGTCGTACTGAGTCATTAGGGACTTTCCAA
Ins_mCMV-E4-1_rev	GATTACGCCAAGCTTGCATGCCTGCAGGTCGAGTCGACAAGCTTGAATTCG
Ins_mCMV-for	CGACGGCCAGTGAATTCGAGCTCGGTACCCGTACTGAGTCATTAGGGACTTTCCAA
PUC-AV_For	AATTCTAGAGCTCGTCAGGTGGCAC
PUC-AV_Rev	TGCATCGCAGGAAAGAACATGTGAGC

Table S4. Overview of synthetic DNA used to construct sub-cloning vectors for application in this study.

Insert name	Sequence
B3-Ins1	TTTTGCTCACATGTTCTTTCCTGCGATGCAGAGTGGAAGCGCTTCTTTTGAGGGGGGAG- TATTTAGCCCTTATCTGACGGGCAGGCTCCCACCATGGGCAGGAGTTCGTCAGAATGTCATGGGA TCCACTGTGGATGGGAGACCCGTCCAGCCCGCCAATTCCTCAACGCTGACCTATGCCACTTT- GAGTTCGTCACCATTGGATGCAGCTGCAGCCGCCGCCGCTACTGCTGCCGCCAACACCATCCTTG GAATGGGCTATTATGGAAGCATCGTTGCCAATTCCAGTTCCTCTAA- TAACCCTTCAACCCTGGCTGAGGACAAGCTACTTGTTCTCTTGGCGCAGCTCGAGGCCTTAACCC AACGCTTAGGCGAACTGTCTAAGCAGGTGGCCCAGTTGCGTGAGCAAACTGAGTCTGCTGTT- GCCACAGCAAAGTCTAAATAAAGATCTCAAATCAATAAATAAAGAAATACTTGTAAAACGACG GCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAA- GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGGATATAAAACAAATGAATGTTTATTTGATTTTT CGCGCGCGGTATGCCCTGGACCATCGGTCTCGATCATTGAGAACGCGGTGGATCTTTTCCAG- TACCCTGTAAAGGTGGGATTGAATGTTTAGATACATGGGCATTAGTCCGTCTCGGGGGTGGAGAT AGCTCCATTGAAGAGCCTCTTGCTCCGGGGTAGTGTTATAAATCACCCAG- TCATAGCAAGGTCGGAGTGCATGAATTCTAGAGCTCGTCAGGTGGCACTTTTC
B3-Ins2	TTTTGCTCACATGTTCTTTCCTGCGATGCAACTCTTAAAGAGTAGCCCTT- GCCCGCGCTCATTTTGAAAACGGCGGGAATCACGTCACCCTTGGCACCTGTCCTTTGCCCTTGTC ATGAGTAAAGAGATTCCCACGCCTTACATGTGGAGCTATCAGCCCCAAATGGGGTTGGCAG- CAGGCGCTTCCCAGGACTACTCCACCCGCATGAATTGGCTTAGCGCCGGGCCCTCAATGATATC ACGGGTTAATGATATACGAGCTTATCGAAACCAGTTACTCCTAGAACAGTCAGCTCTCAC- CACCACACCCCGTCAACACCTTAATCCCCGAAATTGGCCCGCCACCCTGGTGTACCAGGAAAAT CCCGCTCCCACCACCGTACTACTTCCTCGAGACGCCCAGGCCGAAGTTCAGATGACTAAC- GCAGGTGTACAGCTGGCGGGCGGTTCCGCCCTATGTCGTCACCGACCTCAACAGAGTATAAAAC GCCTGGTGATTAGAGGCCGAGGTATCCAGCTCAACGACGAGTCGGTTAGCTCTTCGCTT- GGTCTGCGACCAGACGGAGTCTTCCAAATCGCCGGCTGTGGGAGATCTTCCTTCACTCCTCGTCA GGCTGTGCTGACTTTGGAGAGTTCGTCCTCGCAGCCCCGCTCGGGCGGCATT- GGAACTCTCCAGTTTGTGGAGGAGTTTACTCCCTCTGTCTACTTCAACCCCTTCTCCGGCTCTCCT GGCCAGTACCCGGACGAGTTCATACCAAACTTCGACGCAATCAGCGAGTCAGTGGATGGC- TATGATTGACGCGTCATTGCCCCTACCTTACCCAATCAAAATATTAATAAAGACACTGTAAAACG ACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAA- GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTACTTGAAATCAGCAATACAGTCTTTGTCAAA ACTTTCTACCAGCAGCACCTCACCCTCTTCCCAACTCTGGTACTCTAAACGTCGGAGGGTGG- CATACTTTCTCCACACTTTGAAAGGGATGTCAAATTTTATTTCCTCTTCTTTGCCCACAATCTTCAT TTCTTTATCCCCAGATGGCCAAGCGAGCTCGGCTAAGCACTTCCTTCAACCCGGTG- TACCCTTATGAAGATGAAAGCAGCTCACAACACCCATTTATAAATCCTGGTTTCATTTCCCCTGA CGGGTTCACAAATTCTAGAGCTCGTCAGGTGGCACTTTTC
B3-Ins3	TTTTGCTCACATGTTCTTTCCTGCGATGCAAACTACTCAGGCAACCCTCATAAC- CTCCCCATTTACCTTTTCCTATATTAGAGAAGATGACTGACAACAAAAATAAAGTTCAGTAAAAC GACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTG- CAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGACATTTTTTATTGAAATTCCTT TTACAGTATTCGAGTAGTTATTTTGCCTCCCCCTTCCCATTTAACAGAATACAC- CAATCTCTCCCCACGCACAGCTTTAAACATTTGGATACCATTAGAGATAGACATAGTTTTAGATT CCACATTCCAAACAGTTTCAGAGCGAGCCAATCTGGGGTCAG- TAATACATAAAAATGCATCGGGATAGTCTTTTAAAGCGCTTTCAAATTCTAGAGCTCGTCAGGTG GCACTTTTC

B3-Ins4	TTTTGCTCACATGTTCTTTCCTGCGATGCAAAAAGGCACAGGAGAATAAAAAA-TATAATTATTTCTCTGCTGCTGTTCAGGCAACGTTGCTCCCGGTCCCTCTAAATAGACATACAAAGCCTCATCAGCCATGGCTTACCAGGCAAAGTACAGCGGGCGCACAAAGCACAAGCTCTAAA-GAAGCTCTAAAAACACTCTCCAACCTCTCCACAATATATACACAAGCCCTAAACTGACGTAATGGGAGTAAAGTGAAAAAAAAATACCGCCAAGCCCAACACACACCCCGAAACTGCGTCAGCAG-GAAAAAGTACAGTTTCACTTCCGCATTCCCAACAAGCGTAACTTCCTCTTTCTCATGGTACGTCACATCCGATTAACTTGCGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGG-GATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGAACGTCATTTTCCCACGGTCGCGCCGCCCCTTTTAGCCGTTAACCCCGCAGCCAATCAC-CACACAGCGCGCACTTTTTTAAATTACCTCATTTACATGTTGGCACCATTCCATAATTCTAGAGCTCGTCAGGTGGCACTTTTC
E4-Ins1	TTTTGCTCACATGTTCTTTCCTGCGATGCATGGATGTGACGGAGGAC-CTGCGACCCGATCATTTGGTGTTGTCCTGCACCGGGACGGAGTTCGGCTCCAGTGGGGAAGAATCTGACTAGAGTGAGTAGTGTTTTGGGGAGGGAGAGGACCTGCATAAGGGGCAGAATGAT-TAAAATCTGTGCTTTTCTGTGTGTTGCAGCAGCATGAGCGGAAACGGCTCCTTTGAGGGAGGGGTATTCAGCCCTTATCTGACGGGGCGTCTCCCCTCCTGGGCGGGAGTGCGTCAAAATGTGATGG-GATCCACGGTGGACGGCCGGCCCGTACAGCCCGCGAACTCTTCAACCCTGACCTATGCAACCCTGAGCTCCTCGTCGGTGGACGCAGCTGCCGCCG-CAGCTGCTGCTTCTGCCGCCAGCGCCGTGCGCGGAATGGCCATGGGCGCCGGCTATTACGGCACTCTGGTGGCCAACTCGAGTTCCACTAATAATCCCGCCAGCCTGAACGAGGAGAA-GCTGCTGCTGTTGATGGCCCAGCTCGAGGCCTTGACCCAGCGCCTGGGCGAGCTGACCCAGCAGGTGGCTCAGCTGCAGGAGCAGACGCGGGCCGCGGTTGCCACGGTGAAATCCAAA-TAAAAAATGAATCAATAAATAAACGGAGACGGGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCG-TAATCATGGTCATAGCTGTTTCCTGTTGTTGATTTTAAAAATCAGAGTCTGAATCTTTATTTGATTTTTCGCGCACGGTAGGCCCTGGACCACCGGCCTCGATCATTGAGCACCCGGTG-GATCTTTTCCAAGACCCGGTAGAGGTGGGATTGGATATTGAGGTACATGGGCATGAGCCCGTCCCGGGGGTGAAGGTAGCTCCATTGCAGGGCCTCGTGCTCGGGGGTGGTGTT-GTAAATCACCCAGTCATAGCAGGGACGCAGGGCGTGAATTCTAGAGCTCGTCAGGTGGCACTTTTC
E4-Ins2	TTTTGCTCACATGTTCTTTCCTGCGATGCAAAAAAATAAAGATTAAAGAGACGATGATTTT-GAATTGATCAATAAAGAATGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGCAC-TTACTTGAAATCTGAAACCAGGTCTCTGTCCATGTTTTCTGTCAGCAGCACTTCGCTCCCCTCTTCCCAGCTCTGGTACTGCAGGCCCCGGCGGGCTGCAAACTTCCTCCACACTCTGAAGGG-GATGTCAAATTCCTCCTGTCCCTCAATCTTCATTTTTTATTTCTATTAGATGTCCAAAAAGCGCGCGCGGGTGGATGATGGCTTCGACCCCGTGTATCCCTACGATGCAGACAACGCACCGAC-CGTGCCCTTCATCAACCCTCCCTTCGTCTCTTCAGATGGATTCCAAGAAAAGCCCCTGGGGGTGTTGTCCCTTAGGCTGGCCGACCCTGTCACCACCAAGAATGGGGAAATTACCCTCAAGCTGGGG-GAGGGGGTGGACCTAATTCTAGAGCTCGTCAGGTGGCACTTTTC

E4-Ins3	TTTTGCTCACATGTTCTTTCCTGCGATGCATGATGACACCACCAGTGCAT- ACTCAATGTCATTTTCATACACCTGGACTAACGGAAGCTATATCGGAGCAACATTTGGAGCTAAC TCATACACCTTCTCCTACATAGCCCAACAATAATCCCACCCTGCATGCCAACCCAC- CTTTTCCCTCTATTTATAAATGGAAACTGAAACAAAAATAAAGTTCGTAAAACGACGGCCAGTG AATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCG- TAATCATGGTCATAGCTGTTTCCTGAAGTGTTTTATTGATTCAACAGTTTTTCACAGGATTCGAGT AGTTATTTTCCCTCCACCCTCCCATCTCATGGAATACACTATCCTCTCCCCAC- GCACAGCCTTAAACATCTGAATGCTATTGGTAATGGACATGGTTTTGATCTCCACATTCCACACA GTTTCAGAGCGAGACAGTCTCGGGTCGGTCAAGGAGATGAAACCCTCCGGGCACTCCTG- CATCTGCACCTCACAGTTCAACAGCTGAGGGCTGTCCTCGGTGATTGGAATCACAGTTATCTGGA ATAAGAGCGATGAGAATCATAATCCGCAAACGGGATCGGGCGGTTGTGGCG- CATCAGGCCCCGCAGCAGTCGCTGTCTGCGCCGCTCCGTCAAGCTGCTACTCAAGGGGTCCGGG TCCAGGGACTCCCTGCGCATGATGCCAATGGCCCTGAGCATCAGTCGCCTGGTAC- GGCGGGCGCAGCAGCGGATGCGGATCTCACTCAGGTCGGAGCAGTACGTGCAGCACAGCACCA CCAAGTTGTTCAACAGTCCATAGTTCAACGTGCTCCAGCCAAAACTCATTTGTGGAAC- TATGCTGCCCACATGTCCATCGTACCAGATCCTGATGTAAATCAGGTGGCGTCCCCTCCAGAACA CACTGCCAATTCTAGAGCTCGTCAGGTGGCACTTTTC
E4-Ins4	TTTTGCTCACATGTTCTTTCCTGCGATGCATGCACTTCCTCAAAC- GCCCAAACTGGCGTCATTTCCGGTTTCCCACGCTACGTCACCTCTCAACGACTTTCAAATTCCGTC GACCGTTAAACACATCAGTTAGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGG- GATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGC CCCGCCCCTAACGAACGCCGCTGTCACAGCCAATCAGCGCGCCCCATCCCCAAATTTTCAC- GCCTTATTTGCATATTAACTCACAAATTCTAGAGCTCGTCAGGTGGCACTTTTC

Table S5. Genomic insertion sites, demonstrated by 30 nt sequences directly adjacent to transgene insertion.

5' homologous region	Site	3' homologous region
GTAATAAAATTATGTCAGCTGCTGAGTGTT	**B3-I0n**	TTATTACTTCTTGGGTGGGGACTTGGATAT
GACTTTGACCGTTTACGTGGAGGTTTCGAT	**B3-I0**	TACCGTGTTTTTCACCTAAATTTCCGCGTA
GATCTCAAATCAATAAATAAAGAAATACTT	**B3-I1**	GATATAAAACAAATGAATGTTTATTTGATT
TTACCCAATCAAAATATTAATAAAGACACT	**B3-I2**	TACTTGAAATCAGCAATACAGTCTTTGTCA
GAAGATGACTGACAACAAAAATAAAGTTCA	**B3-I3**	ACATTTTTTATTGAAATTCCTTTTACAGTA
CTCATGGTACGTCACATCCGATTAACTTGC	**B3-I4**	AACGTCATTTTCCCACGGTCGCGCCGCCCC
TAAAATATGTGTTGACTTTAAGTGCGTGGT	**E4-I0n**	TTATGACTCAGGGGAGGGGACTTTGGGTAT
GACTTTGACCGTTTACGTGGGGGTTTCGAT	**E4-I0**	TACCGTGTTTTTCACCTAAAGTTCCGCGTA
AAAAATGAATCAATAAATAAACGGAGACGG	**E4-I1**	TTGTTGATTTTAAAAATCAGAGTCTGAATC
ACGATGATTTTGAATTGATCAATAAAGAAT	**E4-I2**	CACTTACTTGAAATCTGAAACCAGGTCTCT
TAAATGGAAACTGAAACAAAAATAAAGTTC	**E4-I3**	AAGTGTTTTATTGATTCAACAGTTTTTCAC
AATTCCGTCGACCGTTAAACACATCAGTTA	**E4-I4**	CCCCGCCCCTAACGAACGCCGCTGTCACAG

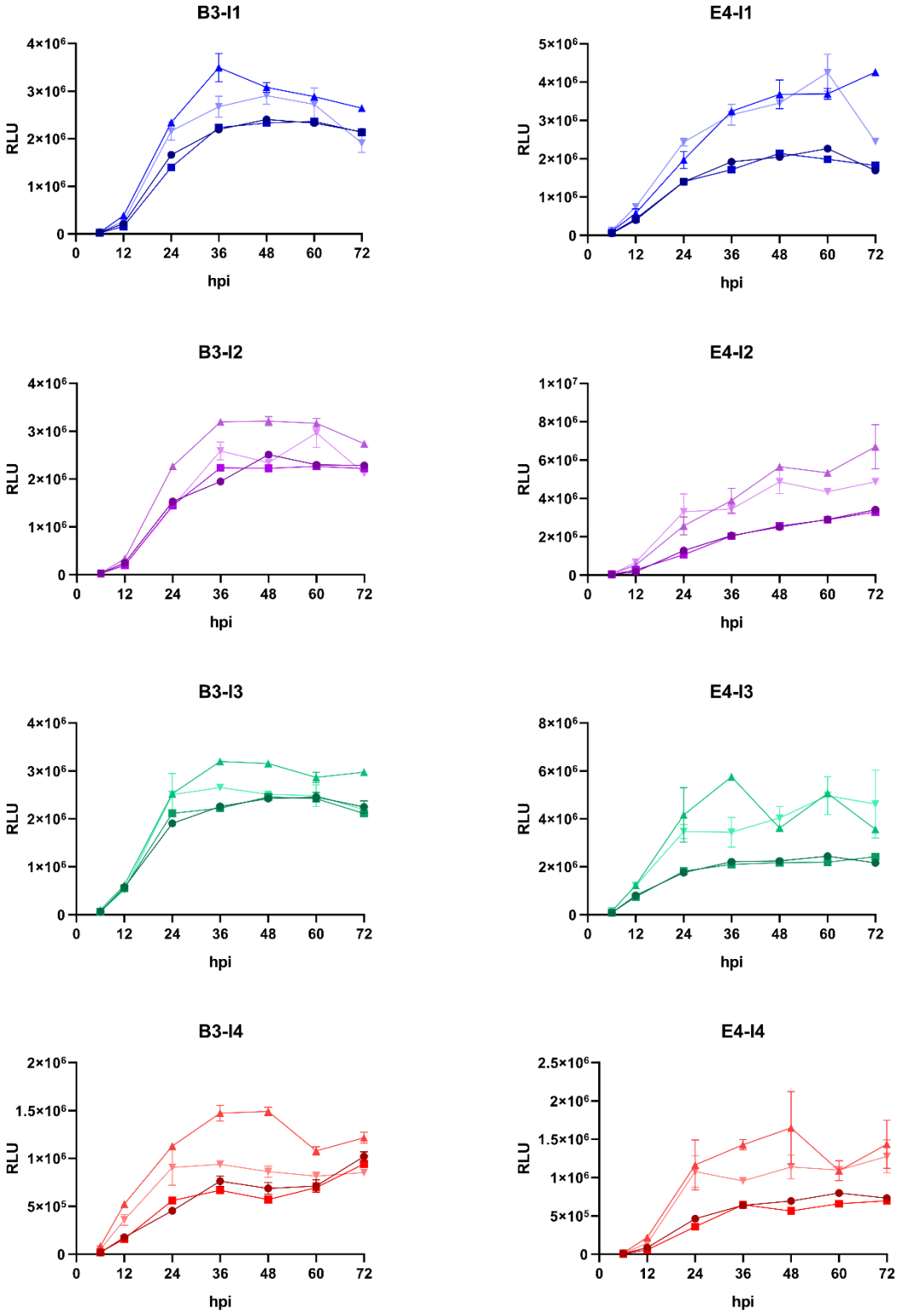

Figure S1. Expression profiles of insertion sites. Expression of transgene over time as determined by Luciferase assay for HAdV-B3 and HAdV-E4 based vectors. Colour and connecting lines indicate single experiments.