Supplementary Materials

Protease-deleted Adenovirus as an alternative for replication-competent adenovirus vector

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Materials and methods for generation of adenovirus transfer vectors

The pDE3 was a gift from H. Lochmüller (Ludwig-Maximilians, Munich, Germany). It contains the right end of the Ad5 genome from the *Bam*HI site (nucleotide 21562) to the end of the genome, with an E3 deletion as found in pBHG11 (Bett *et al.*, 1994). The pE4 was constructed from pDE3 by removing the BamHI-XbaI fragment. The pE4 contains the right end of Ad5 genome from position 30751 to end of the genome.

The fragment of AvrII-ClaI in pE4 was replaced with two PCR products over the pE4 vector with two sets of primers; (1) AvrII-F; 5' gcc**cctagg**caaaatagcaccctcccgct 3' and SnaBI-NotI-R; 5' cgc**tacgtagcggccgc**acgtgggaaaacggaagtga 3' (2) SnaBI-F; 5' cgc**tacgta**acttcccattttaagaaaactac 3' and ClaI-FseI-R; 5' cc**atcgatggccggcc**catcaataatataccttattttg 3'. The restriction enzyme sites are bolded. The digested fragments were swapped with their corresponding fragment in the pE4 to generate pE4-FseI/NotI. Inserted SnaBI-NotI restriction site is used for insertion of transgene and FseI for vector linearization. The right arm of Adenovirus in the pE4-FseI/NotI vector was extended by replacing SphI/BsmI fragment (470 nt) with SphI/BsmI of pED3 (2161ng) that extended the right arm of Ad5 sequence. The resulting plasmid is named pE4-Ext-FseI/NotI.

The polyA rabbit globin was excised from pAdCMV5 (Massie et al., 1998a) by BlgII/BamHI digestion and was inserted in BamHI site of pE4-Ext-FseI/NotI. Then the ORF6 of E4 region (904 nt) was reinserted in BamHI site after amplification with BamHI-F-polyA; 5' cg**ggatcc**tctagaatgact 3' and BamHI-R-polyA; 5' gg**ggatcc**attacatgggggta 3' primers and digested with BamHI to generate pE4-ext-polyA. A PCR-based gene deletion strategy was used to delete E4 gene but not E4 promoter. Two sets of primers were used to amplify flanking sequences of E4 region. The first set of primers were; BamHI-F; 5' cgc**ggatcc**cgacacggcaccagctcaatca 3' and SnaBI-R; 5' cgc**tacgta**gcggccgcacgtgggaaaacggaagt 3'. The second set of primer were; BstXI-F; 5' tc**ccagaatattgg**aactttagaaatgg 3' and BamHI-R: 5' cgc**ggatcc**acgttgaaacataacacaaacgattc 3'. Two digested PCR fragments with SnaBI/BamHI and BamHI/BstXI were replaced with SnaBI/BstXI fragment of pE4-Ext-FseI/NotI to generate pE4-ΔE4/Orf6-polyA.

The expression cassette has a truncated form of CMV promoter (300 nt of CMV promoter) which express a fusion of Renilla Luciferase and GFP genes. The truncated form of CMV is a PCR product using two primers; SalI-PvuI-F 5' acgc**gtcgaccgatcg**cctggcattatgcccagtacat 3' and BamHI-PmeI-SpeI-R 5' cgc**ggatccgtttaaacactagt**cttggacctgggagtggacac 3'. The PCR product was digested with SalI/BamHI and insert in XhoI/BamHI digested pAdTR5F (Massie et al., 1998b) to generate pAdCMV5-300F. The Renilla Luciferase gene was amplified by RLuc-F-XbaI 5' ctag**tctaga**atgacttcgaaagtttatgatcc 3' and RLuc-R-NotI 5' ataagaat**gcggccgc**ttgttcatttttgagaactcgc 3'. The GFP gene and its polyA was amplified from pAdCMV5-GFPq (Massie et al., 1998a) by two primers of GFP-F-NotI 5' ataagaatgcg**gccgccgc**cgccaccatggctagcaaaggagaagaac 3' and PolyA-GFP-R-SmaI-PvuI 5' tcc**cccgggcgatcg**cgaccaaatgatttgccctccc 3'. The RLuc and GFP-polyA fragments were digested with NotI. After ligation the fusion gene was digested with XbaI/SmaI and ligated in pAdCMV5-300F digested previously with BglII, blunted and redigested with SpeI. The complete expression cassette was exceed from pAdCMV5-300-RLuc-GFPq by PvuI digestion and after being blunted, the fragment was inserted in blunted NotI digested pE4-Ext-FseI/NotI or pE4-ΔE4/Orf6-polyA to generate the final adenovirus transfer vector pE4-CMV-300-RLuc-GFPq and pΔE4-(Orf6+)-CMV-300-RLuc-GFPq.

References

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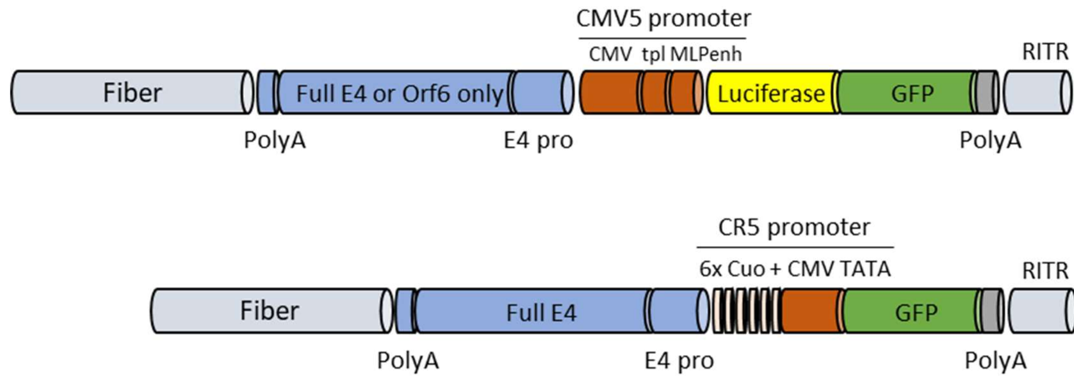

Fig 1S. Schematic representation of the expression cassettes at E4 region in different backbones of AdV. Only the left side of AdV from fiber until the right ITR are shown. The diagrams are not drawn to scale. The abbreviations are: tpl, tripartite leader; pro, promoter, MLPenh, Major late promoter enhancer; 6x Cuo, 6 copies of cumate operator sequence, GFP, green fluorescent protein.

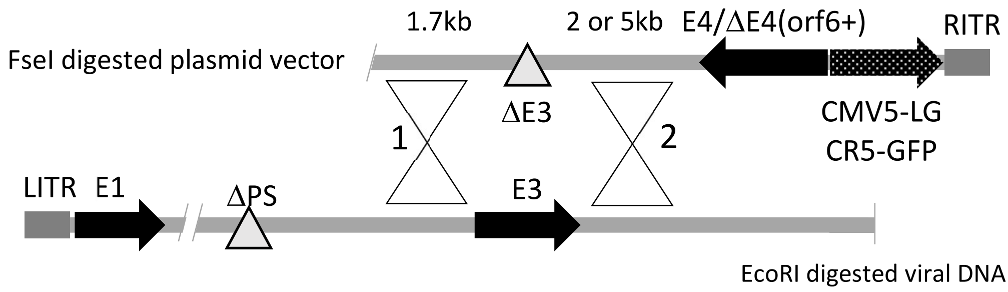

Fig 2S. Possible outcome of AdPS- sequence following homologous recombination in mammalian cells. Depending on the recombination starting position (1 or 2), the recombinant viruses are either E3- (position 1) or E3+ (position 2).

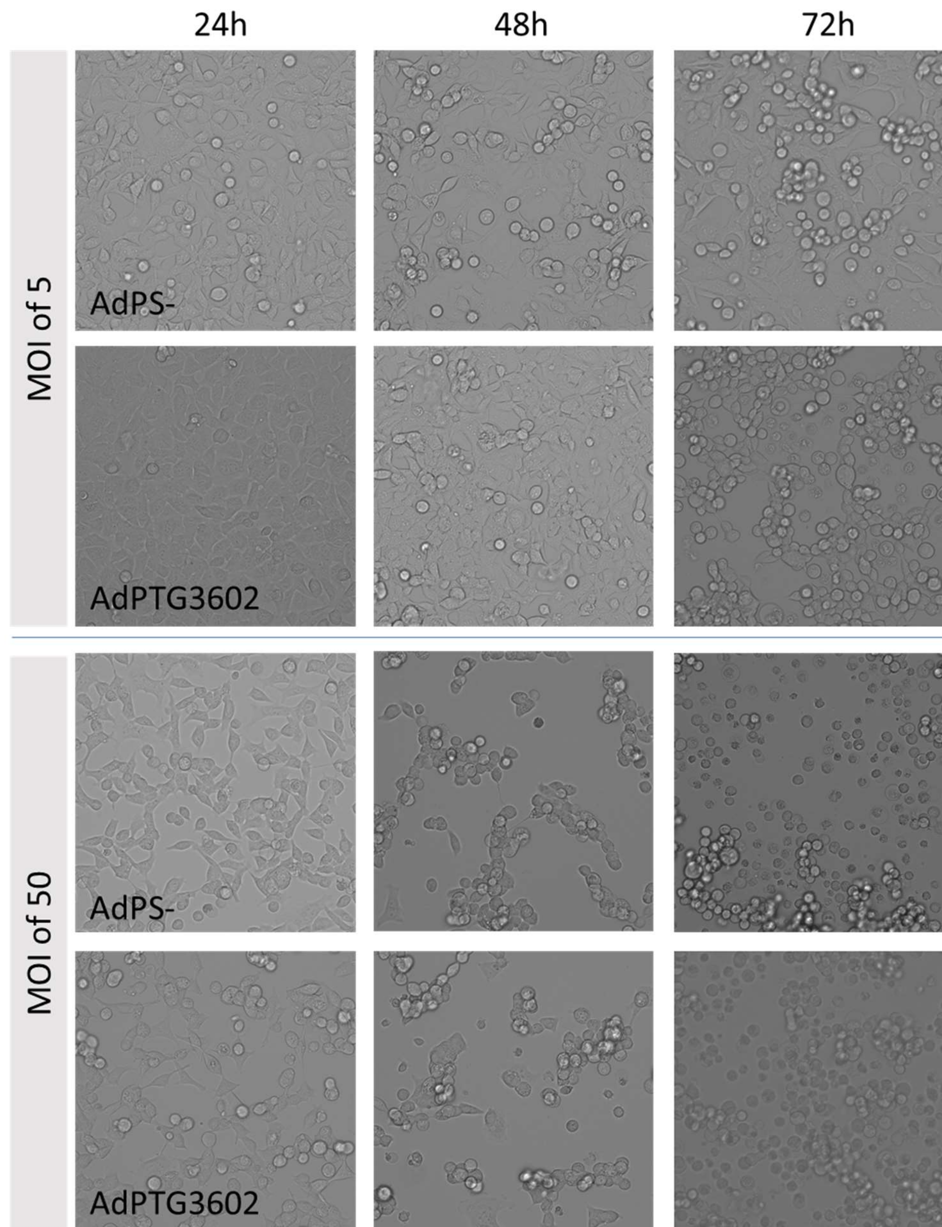

Fig 3S. CPE of SC-AdV vs. RC-AdV in infected HEK293A. The HEK293A cells were infected with AdPS- (SC-AdV) or AdPTG3602 (RC-AdV). The CPE was visualized each day under a brightfield microscope. Cells infected with SC-AdV show the same level of CPE as cells infected with RC-AdV.

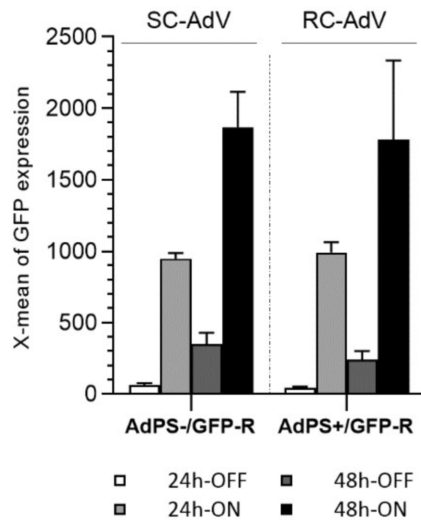

Fig 4S. GFP expression by AdVs regulated by the cumate inducible promoter (CR5) in HEK293A cells. GFP expression by AdVs regulated by the cumate inducible promoter (CR5) after co-infection with Ad-cTA (for ON condition) or absence of Ad-cTA (OFF condition) in HEK293A cells. Data are the average intensity of GFP in the cell population (X mean) ± SEM. Difference between ON and OFF condition for both viruses at 24 and 48 hpi are significance but difference between the AdPS-/GFP-R and AdPS+/GFP-R at each condition are not significant.

In the OFF condition, Ad-cTA was absent to measure the background level without transactivator. Knowing the background level is important to predict the potential effect of expression of toxic gene on cell growth and virus yield. During the production of an AdV carrying the gene for a toxic protein, expression of the transactivator should be prevented or kept to a minimum. Using a cell line like HEK293A or HEK293A-CymR-PS which does not express any transactivator is ideal to prevent production of the toxic protein. Only when the expression of GOI is needed *in vitro,* will the transactivator be added by co-infecting cells with Ad-cTA or by using another cell line which expresses the transactivator such as HeLa-cTA.