

SUPPLEMENTARY MATERIAL FOR:

Re-engineering adenovirus vector systems to enable high-throughput analyses of gene function

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Rendering AdZ vectors self-excising

In order to make the vector self-excising, I-*SceI* sites were inserted in between the *PacI* sites and the termini of the Ad5 genome in pAL840 by recombineering. The *sacB/lacZ/amp^r* cassette was amplified using primers SacB-R-ITR and SacB-FL (Supplementary Table 3) and inserted into the left end of the genome by recombineering. The oligonucleotides L-ITR+Sce and L-ITR+Sce(R) (Supplementary Table 3) were annealed and used to remove the *sacB/lacZ/amp^r* cassette from the genome by recombineering, leaving a I-*SceI* site just outside the left end of the genome. The same was done at the right end of the genome, using primers

SacB-R-ITR and SacB-FR to amplify the *sacB/lacZ/amp^r* cassette and annealed oligonucleotides R-ITR+Sce and R-ITR+Sce(R) to remove the cassette, leaving behind a second I-*SceI* site at the right of the genome and generating plasmid pAL898. Thus, the Ad5 genome can be excised from the BAC vector using I-*SceI*.

The I-*SceI* ORF was amplified from pCAG-ISceI using primers SceF (GGCCGCTAGCGCCG CCACT ATGGGATCAAG, *NheI* site underlined) and SceR (GGCCGGTACCTTATTTTC AGGAAAGTTTCGGAG, *KpnI* site underlined) and inserted into *KpnI/NheI* digested pREP10 (Invitrogen) to generate pAL914 expressing I-*SceI* from the RSV promoter and SV40 polyadenylation signal.

The I-*SceI* expression cassette from pAL914 was then inserted into pAL898 by recombineering. *galK* was amplified from pGalK using primers GalKF-BAC and GalKR-BAC (Supplementary Table 3) and recombineered into pAL898. The I-*SceI* cassette was then amplified using RSV-F-BAC and SV40-R-BAC and recombineered in place of *galK* to generate pAL923. After this, tet operators were inserted into the CMV promoter, generating pAL937. The *sacB/lacZ/amp^r* cassette was then re-inserted into the self-excising vector, along with the same N and C terminal strep-2, strep-3, and GFP tags as described above, generating vectors as detailed.

Table 1. Primers Used to Recombineer Various Tags and Tet Operators into AdZ Vectors

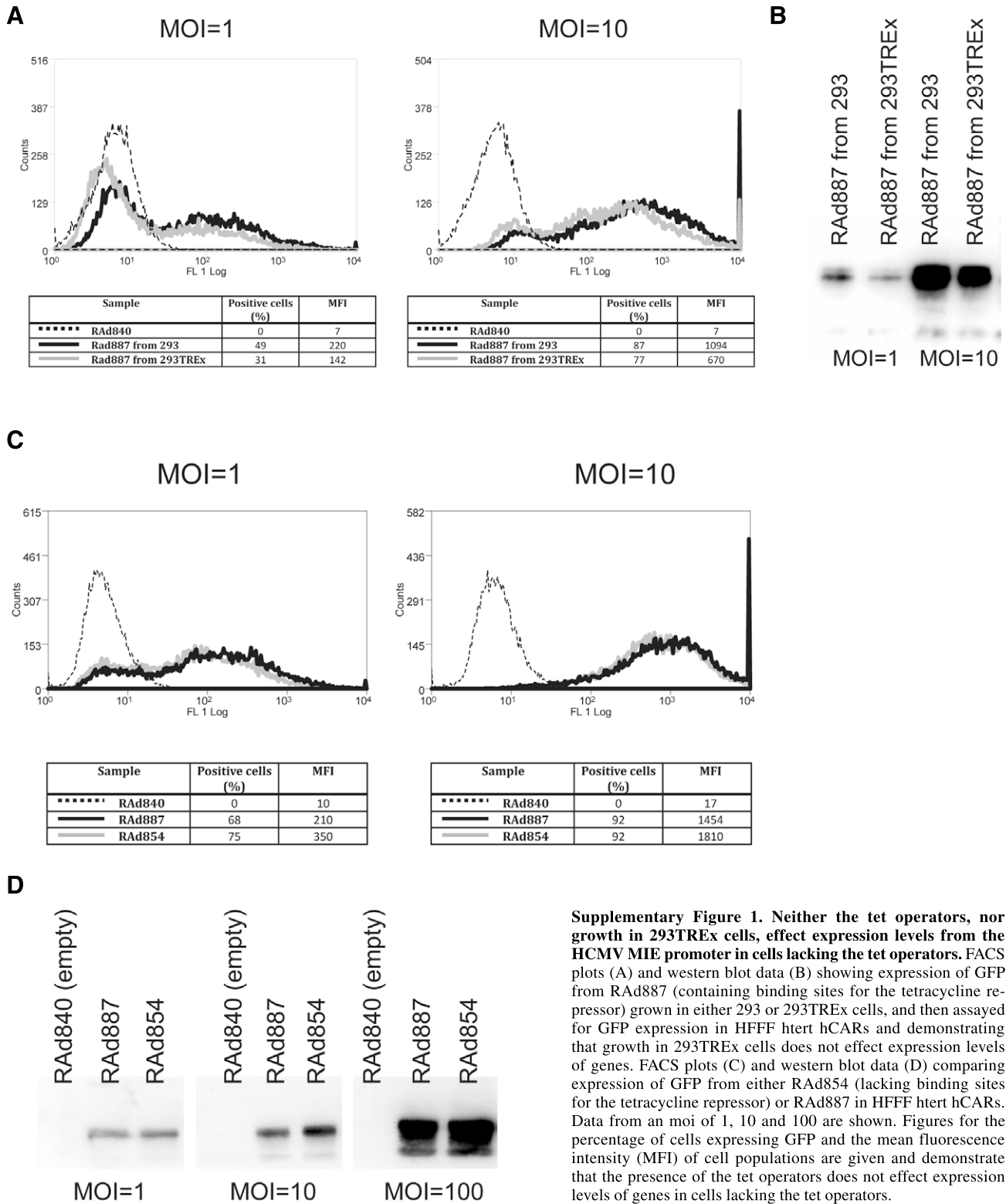
Primer Name	Primer sequence (arms of homology underlined; tags italicized)
SacBF-Strep-CMV	<u>GACACCGGGACCGATCCAGCCTGGATCC</u> <i>GAGGGCAA</i> <u>AAAAATGGCTAGCTGGAGCCACCCGCAGTTTCGAAAAAGGCGCCCCTGTGACGGAAGATCACTTCG</u>
SacBR-pA	<u>AGGATTACAGAGTATAACATAGAGTATAATATAGAGTATAACAATAGTGACGTGGGATCCCTGAGGTTCTTATGGCTCTTG</u>
SacBF-CMV	<u>ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTGGATCCCTGTGACGGAAGATCACTTCG</u>
SacBR-Strep-pA	<u>ATAACATAGAGTATAATATAGAGTATAACAATAGTGACGTGGGATCC</u> <i>TATTTTTCGAACTGCGGGTGGCTCCAAGCGCTCTGAGGTTCTTATGGCTCTTG</i>
eGFPF-CMV	<u>ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTGGATCCGCCACCATGGTGAGCAAGG</u>
eGFPR-pA	<u>TAGGATTACAGAGTATAACATAGAGTATAATATAGAGTATAACAATAGTGACGTGGGATCC</u> <i>TACTTGTACAGCTCGTCCATGC</i>
SacBF-eGFP	<u>GTCTTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGCCTGTGACGGAAGATCACTTCG</u>
SacBR-eGFP	<u>GTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATCTGAGGTTCTTATGGCTCTTG</u>
Strep3F	<i>AGCGCTTGGAGTCATCCACAATTTGAGAAGGGTGGAGGCTCCGGAGGTGGATCGGGCGGGGGCTCGTGGAGCCA</i> <i>CCCGCAGTTTCGAAAAATAA</i>
Strep3R	<i>TTATTTTTCGAACTGCGGGTGGCTCCACGAGCCCCCGCCGATCCACCTCCGGAGCCTCCACCTTCTCAAATTGTGGATGACTCCAAGCGCT</i>
Strep3F-CMV	<u>GACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTGGATCC</u> <i>AGCGCTTGGAGTCA</i> <i>TCCACA</i>
Strep3R-pA	<u>TAGGATTACAGAGTATAACATAGAGTATAATATAGAGTATAACAATAGTGACGTGGGATCC</u> <i>TATTTTTCGAACTGCGGGTG</i>
SacBR-Strep3	<u>CCACCTCCGGAGCCTCCACCTTCTCAAATTGTGGATGACTCCAAGCGCTCTGAGGTTCTTATGGCTCTTG</u>
SacBF-tet-CMV	<u>CCATTGACGCAAATGGGCGGTAGGGGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCCTGTGACGGAAGATCACTTCG</u>
SacBR-tet-pA	<u>TCTATGGAGGTCAAACAGCGTGGATGGCGTCTCCAGGCGATCTGACGGTTCACTAAACTCTCTATCACTGATAGGGAGCTGAGGTTCTTATGGCTCTTG</u>
TetF	<i>TACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTCCCTATCAGTGATAGAGAGTTTAGTGA</i> <i>ACCGTCAGATCGCCTGGAGAC</i>
TetR	<i>GTCTCCAGGCGATCTGACGGTTCATAA</i> <u>ACTCTCTATCACTGATAGGGAGATCTCTATCACTGATAGGGAGAGCTCTG</u> <i>CTTATATAGACCTCCCACCGTA</i>

Table 2. Primers Used to Clone HCMV UL120 and HHV-7 IE1 into AdZ Vectors

Primer Names	Primer sequence (arms of homology underlined; tags italicized)
UL120F	<u>AACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCC</u> <u>AGCCTGGATCCGCGACGTCGAGAGGGTAACT</u>
UL120R	<u>CCACCTCCGGAGCCTCCACCCTTCTCAAATTGTGGATGACTCCAAGCGCTCCTCTTGACGCCTAAACG</u>
HHV-7F	<u>ACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCA</u> <u>GCCTGGATCCTTGTTATTGTAGGTGTGGCTA</u>
HHV-7R	<u>CCACCTCCGGAGCCTCCACCCTTCTCAAATTGTGGATGACTCCAAGCGCTATAGATTTGAGCATTTTCCAA</u>

Table 3. Primers Used to Clone I-SceI Sites and I-SceI Expression Cassette into AdZ Vectors

Name	Primer sequence (arms of homology underlined; restriction sites italicized)
SacB-R-ITR	<u>CTCATTATCATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATGTAGGGATAACTGAGGTTCTTATGG</u> <u>CTCTTG</u>
SacB-FL	<u>CGAATTCGAGCTCGGTACCCGGGGATCCTTCGAAATTTAAATTTAATTAATTACCTGCCTGTGACGGAAG</u> <u>ATCACTTCG</u>
SacB-FR	<u>ATGCCTGCAGGTCGACTCTAGAGGATCCTTCGAAATTTAAATTTAATTAATTACCTGCCTGTGACGGAAG</u> <u>ATCACTTCG</u>
R-ITR+Sce	<u>ATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATGTAGGGATAACAGGGTAATTAATTAATTTAAATTT</u> <u>CGAAGGATCCTCTAGAGTCGACCT</u>
R-ITR+Sce(R)	<u>AGGTCGACTCTAGAGGATCCTTCGAAATTTAAATTTAATTAATTACCTGTTATCCCTACATCATCAATAATATA</u> <u>CCTTATTTTGGATTGAAGCCAATAT</u>
L-ITR+Sce	<u>AGCTCGGTACCCGGGGATCCTTCGAAATTTAAATTTAATTAATTACCTGTTATCCCTACATCATCAATAATAT</u> <u>ACCTTATTTTGGATTGAAGCCAATAT</u>
L-ITR+Sce(R)	<u>ATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATGTAGGGATAACAGGGTAATTAATTAATTTAAATTT</u> <u>TGAAGGATCCCCGGGTACCGAGCT</u>
GalKF-BAC	<u>CTCTAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTACCTAAATCCTGTTGACAATTAAT</u> <u>CATCGGCA</u>
GalKR-BAC	<u>AGCGGATGAATGGCAGAAATTCGATGATAAGCTGTCAAACATGAGAATTGGTCGACGGCCCGGGCGGCC</u> <u>GCAAGGGGTTCTCAGCACTGTCTGCTCCTT</u>
RSV-F-BAC	<u>TCTAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTACCTAAATATGGTGCCTCTCAG</u> <u>TACAATCT</u>
SV40-R-BAC	<u>TGGCAGAAATTCGATGATAAGCTGTCAAACATGAGAATTGGTCGACGGCCCGGGCGGCCGCAAGGGGT</u> <u>TCGGCAGCCGGATCATAATCAG</u>



Supplementary Figure 1. Neither the tet operators, nor growth in 293TREx cells, effect expression levels from the HCMV MIE promoter in cells lacking the tet operators. FACS plots (A) and western blot data (B) showing expression of GFP from RAd887 (containing binding sites for the tetracycline repressor) grown in either 293 or 293TREx cells, and then assayed for GFP expression in HFFF htert hCARs and demonstrating that growth in 293TREx cells does not effect expression levels of genes. FACS plots (C) and western blot data (D) comparing expression of GFP from either RAd854 (lacking binding sites for the tetracycline repressor) or RAd887 in HFFF htert hCARs. Data from an moi of 1, 10 and 100 are shown. Figures for the percentage of cells expressing GFP and the mean fluorescence intensity (MFI) of cell populations are given and demonstrate that the presence of the tet operators does not effect expression levels of genes in cells lacking the tet operators.