Trans-spliced Cas9 allows cleavage of *HBB* and *CCR5* genes in human cells using compact expression cassettes

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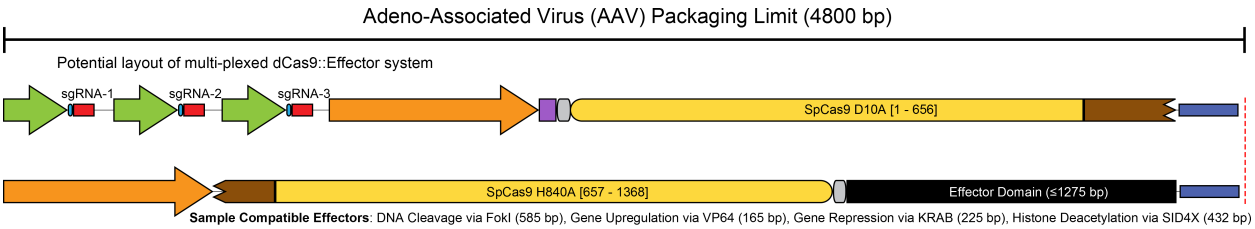
1. Supplementary Methods

Supplementary Method M1. PCR primers for T7E1 Assays

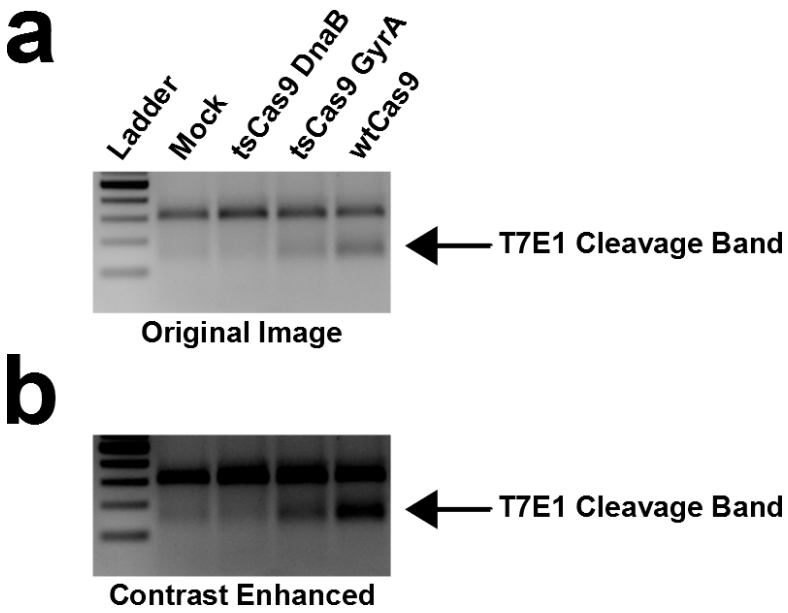
Gene	Forward Primer	Reverse Primer
HBB	CTGGAGACGCAGGAAGAGATCC	GCAATCATTCGTCTGTTTCCCATTC
CCR5	AGTCGACACTGCACAGGGTGGAACAAGATGG	GCATAGATCGACCACCCCAAAGGTGACCGT

All DNA sequences are written 5'→3'. All oligos were ordered from Eurofins MWG Operon. The CCR5 primers have barcode sequences on the 5' ends because they were originally designed for multiplexed DNA sequencing; this has no effect on the T7E1 assay.

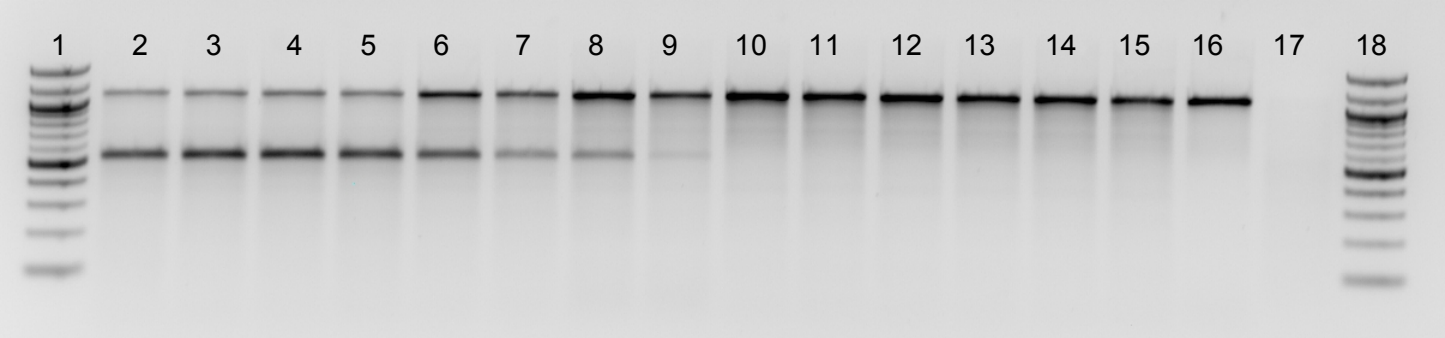
2. Supplementary Figures



Supplementary Figure S1. Potential layout of future multi-plexed trans-splicing system using synergistic sgRNAs and an effector fused to dCas9 to allow gene regulation or epigenetic modification.



Supplementary Figure S2. Initial test of DnaB and GyrA intein systems. To initially determine the feasibility of using the different intein systems, DnaB and GyrA trans-splicing Cas9 systems were transfected in triplicate into HEK-293T cells along with wild-type Cas9. All plasmids contained the guide strand targeted to *HBB* (See **Figure 3a, Supplementary Method M1**). Shown here is a representative gel (a) from the T7E1 assay and a version of the image with enhanced contrast to highlight the cleavage band (b). Because the DnaB system did not induce gene modification at a rate appreciably above the background signal in mock-treated cells, it was not considered further. We recognize the unwanted presence of a faint band appearing in the mock treated cells; this initial test was performed with an earlier set of PCR primers and a new set of primers (used in all other experiments) resolved this issue.



Supplementary Figure S3. Representative T7E1 gel of nuclease activity. T7E1 reactions were loaded into 2% agarose gels cast with ethidium bromide. This gel analyzed one replicate of the dosing experiment of the R-3 guide strand targeting *HBB*. The PCR amplicon is ~1100 bp and the two T7E1 cleavage products both co-localize at ~550 bp. Activity was observed for cells transfected with the wtSpCas9 plasmid and with both components of tsSpCas9, but no activity was observed (as expected) for control reactions containing only pUC, only one of the tsSpCas9 components, or Cas9 systems guided to *CCR5* and not to *HBB*.

Gel Lanes (from left to right):

1 – 100 bp ladder

2 – wtSpCas9 R-3 Full Dose

3 – wtSpCas9 R-3 ½ Dose

4 – wtSpCas9 R-3 ¼ Dose

5 – wtSpCas9 R-3 1/8 Dose

6 – tsSpCas9 R-3 Full Dose

7 – tsSpCas9 R-3 ½ Dose

8 – tsSpCas9 R-3 ¼ Dose

9 – tsSpCas9 R-3 1/8 Dose

10 – N-terminal tsSpCas9 R-3 only

11 – C-terminal tsSpCas9 R-3 only

12 – N-terminal tsSpCas9 R-30 (*CCR5*) only

13 – C-terminal tsSpCas9 R-30 (*CCR5*) only

14 – wtSpCas9 R-30 (*CCR5*) Full Dose

15 – tsSpCas9 R-30 (*CCR5*) Full Dose

16 – pUC only

17 – No template PCR control

18 – 100 bp ladder

3. Supplementary Data

Supplementary Data D1. Amino acid sequences of trans-splicing components

Intein domains underlined.

>tsCas9-C

MGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGFVSHNTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF
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>tsCas9-Nick

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Supplementary Data D2. Plasmid sequences of trans-splicing components

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