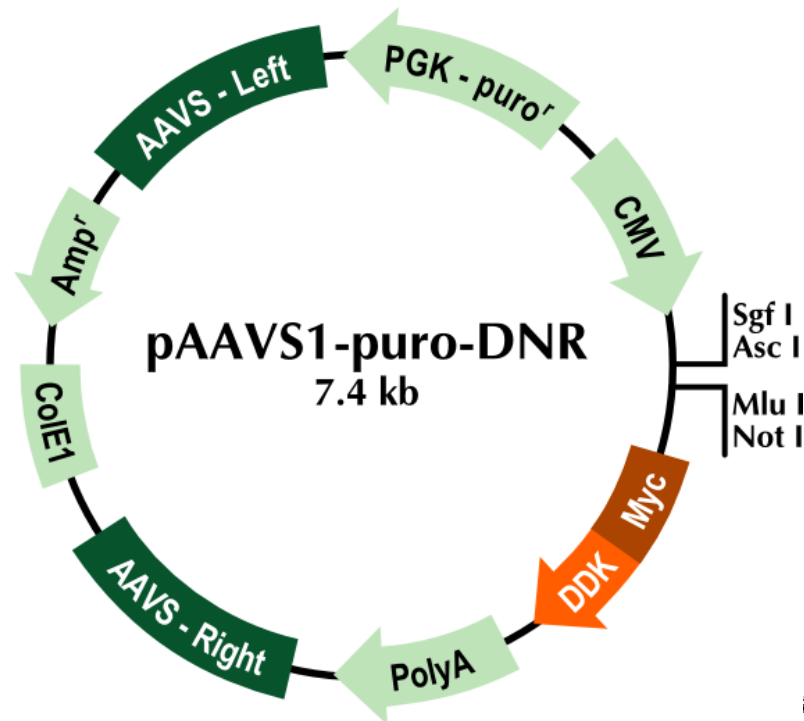


RFP was cloned in the AAVS1 donor vector to validate the system



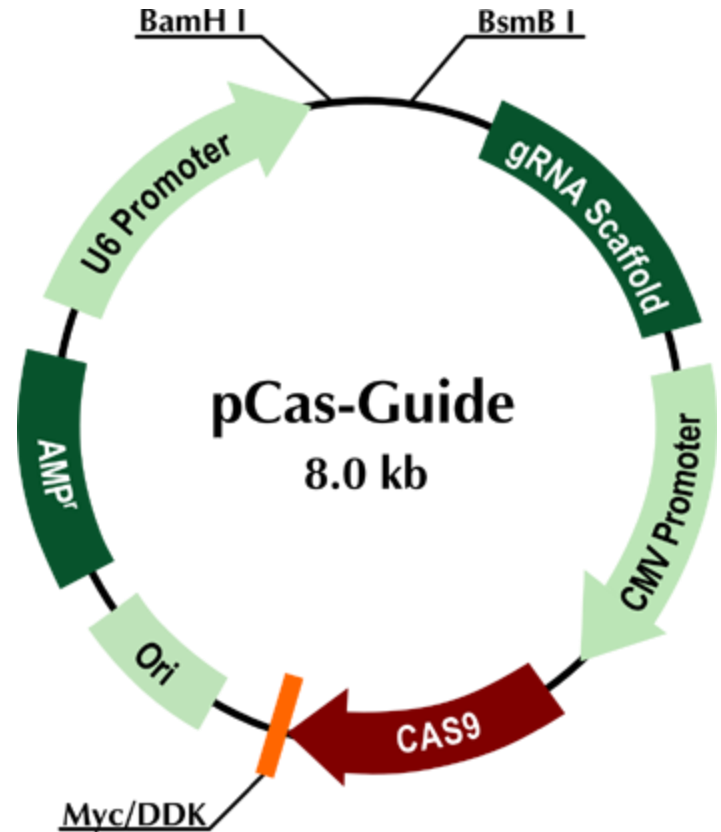
EcoR I Sat I Kpn I RBS ac Consensus Sgf I Asc I
 CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCGCGGATCGCCGGCGCGCCAGATCT

Mlu I Not I Myc.Tag
 CAAGCTTAACTAGCTAGCGGACCG ACG CGT ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG
 T R T R P L E Q K L I S E E

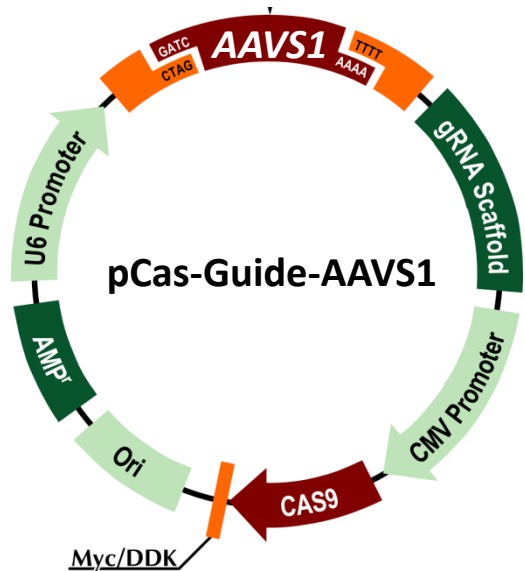
DDK.Tag Fse I
 GAT CTG GCA GCA AAT GAT ATC CTG GAT TAC AAG GAT GAC GAC GAT AAG GTT TAA ACGGCCGGCC
 D L A A N D I L D Y K D D D D K V Stop

Four AAVS1 target sequences were cloned in pCas-Guide vector:

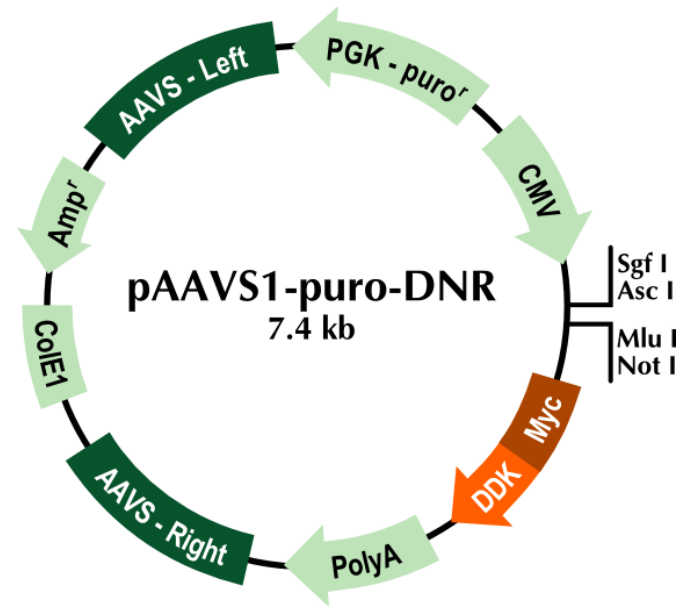
pCas-Guide-AAV1 T1	GTTAATGTGGCTCTGGTTCT
pCas-Guide-AAV1 T2	GAGAACCAGAGCCACATTAA
pCas-Guide-AAV1 T3	ACAGTGGGGCCACTAGGGAC
pCas-Guide-AAV1 T4	TGTCCCTAGTGGCCCCACTG



CRISPR/Cas Safe-harbor AAVS1 system



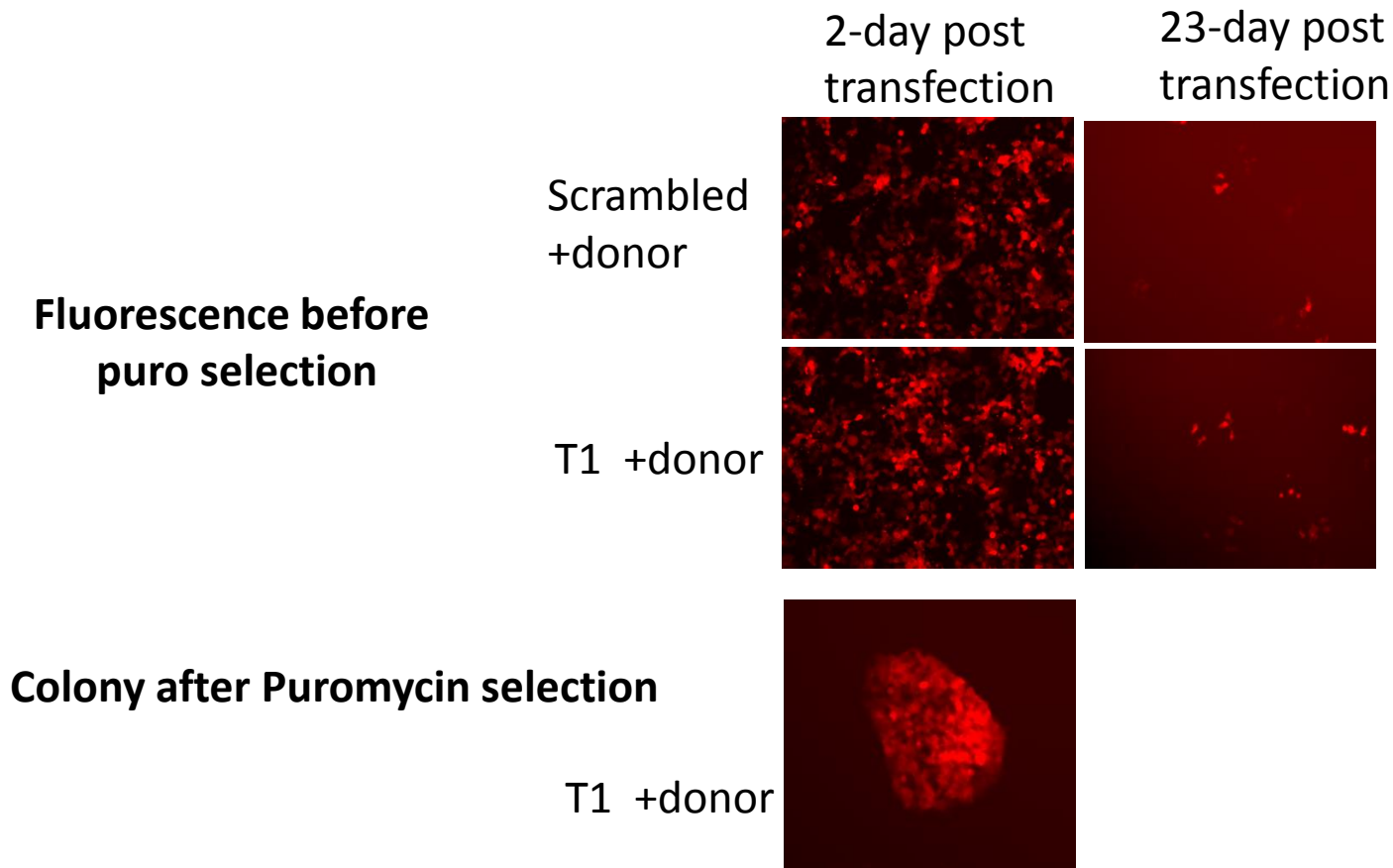
AAVS1 targeted by CRISPR/Cas



The gene with AAVS1 homologous seq as donor template DNA

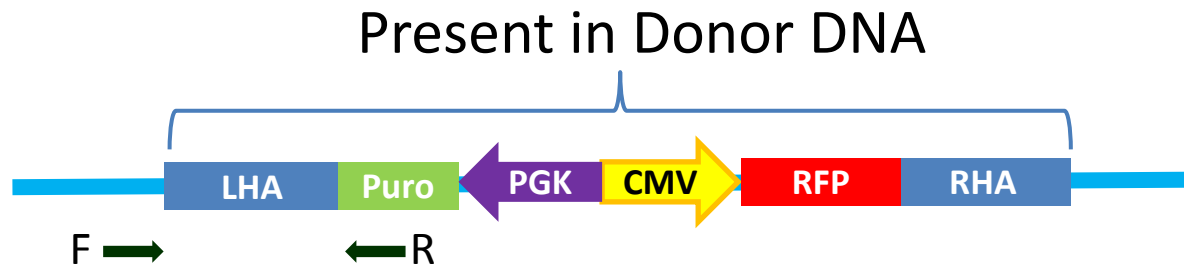
The gene will be integrated at AAVS1 in the genome

RFP Fluorescence after cotransfection of pCas-Guide-AAVS1 and pAAVS1-RFP-DNR



Genomic PCR primers to detect the RFP cassette integration at AAVS1 site

RFP cassette Inserted at AAVS1



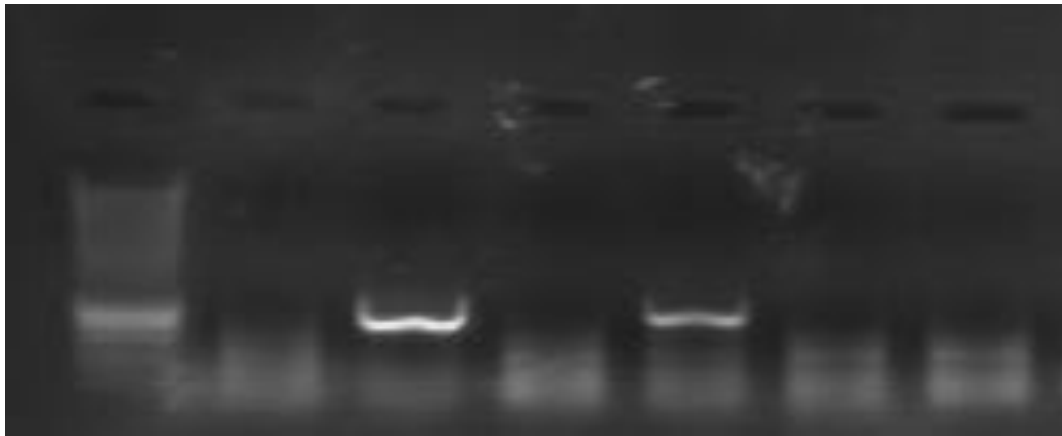
F: forward primer

R: reverse primer

Nested Genomic PCR Before Puro Selection

5' integration

1 2 3 4 5 6

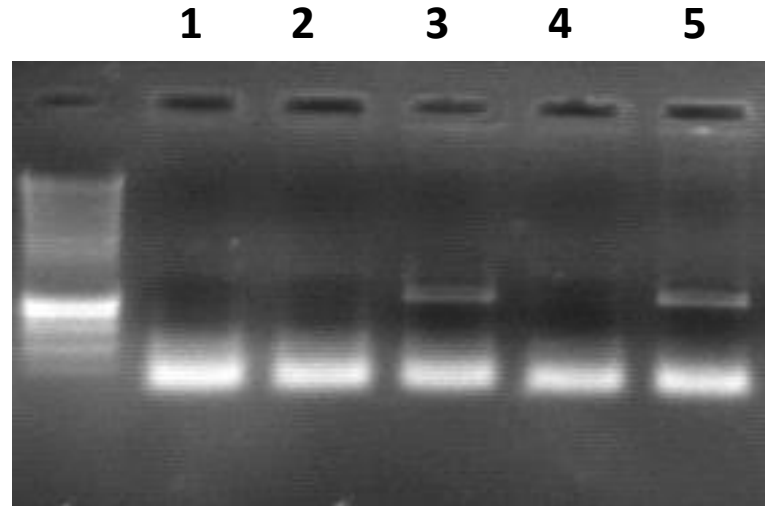


1. Scrambled + donor
2. pCas-Guide T1 + donor
3. pCas-Guide T2 + donor
4. pCas-Guide T3 + donor
5. pCas-Guide T4 + donor
6. HEK293T DNA

HEK293T cells were cotransfected with AAVS1 gRNA and RFP donor constructs. 5 days later, genomic DNA was extracted and nested genome PCR was performed to detect the integration of the RFP cassette.

Genomic PCR from 5 puro positive colonies

(from pCas-Guide-AAVS1 T1 + RFP donor transfected cells)



HEK293T cells were cotransfected with AAVS1 gRNA and RFP donor vectors; cells were passaged for around 20 days, then applied to puro selection.

5 single cell colonies were isolated for each transfection group.

Genomic PCR was performed using single cell colonies from pCas-Guide-AAVS1 T1 and RFP donor cotransfection. Two out 5 puro+ colonies amplified the correct PCR products.

AAVS1 Integration Was Confirmed By Sequencing

