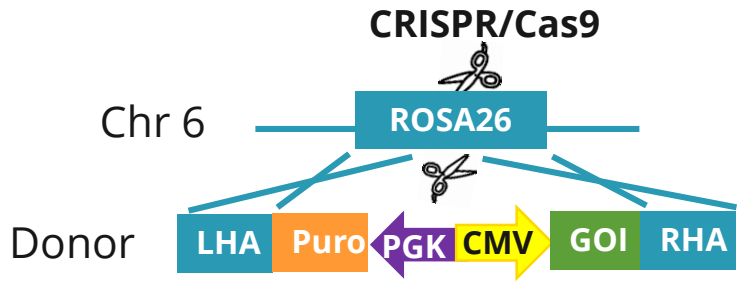
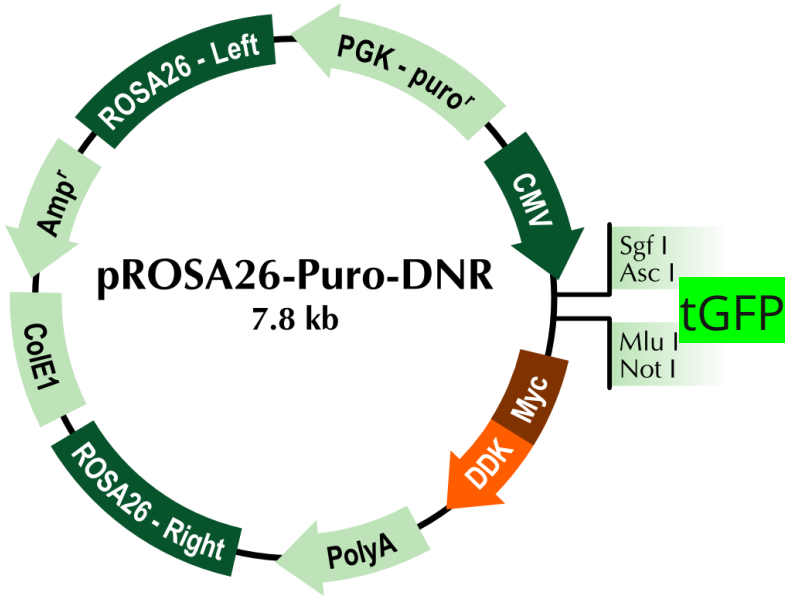
Transgene Knockin at ROSA26 Locus using CRISPR



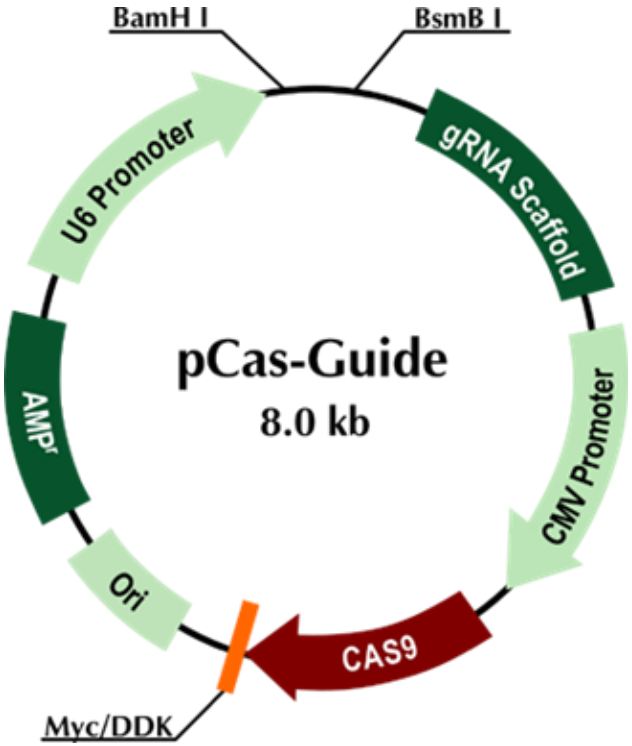
1. gRNA/CRISPR cuts ROSA26 locus in the Mouse genome
2. The gene of interest (GOI) cassette will be knocked in via homologous arms (LHA, left homologous arm and RHA, right homologous arm)

tGFP Was Used to Validate ROSA26 Transgene Knockin via CRISPR



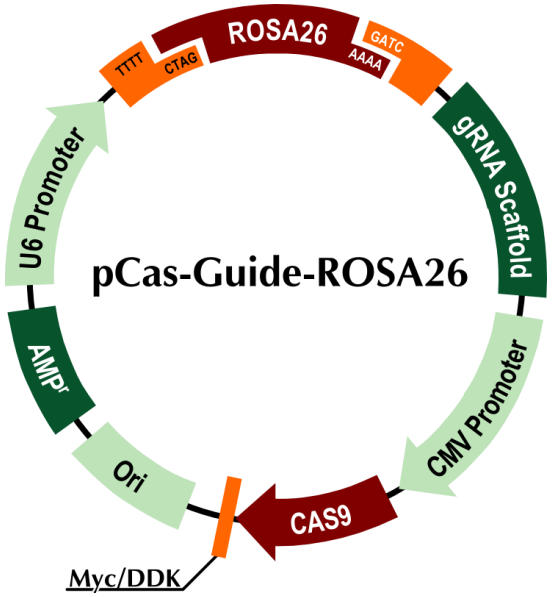
tGFP was cloned into ROSA26 donor vector via Sgf I / Mlu I

Two ROSA26 Target Sequences Were Cloned in pCas-Guide Vector

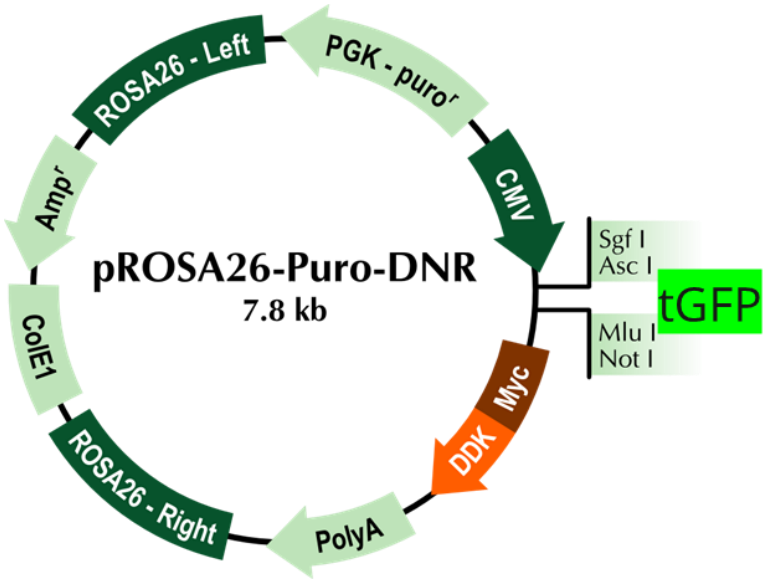


ROSA26 G1	TTATTGCTTGTGATCCGCCT
ROSA26 G2	TACTACAGTATGAAATTACA

How ROSA26 Transgene Insertion Works



ROSA26 targeted by CRISP/Cas9



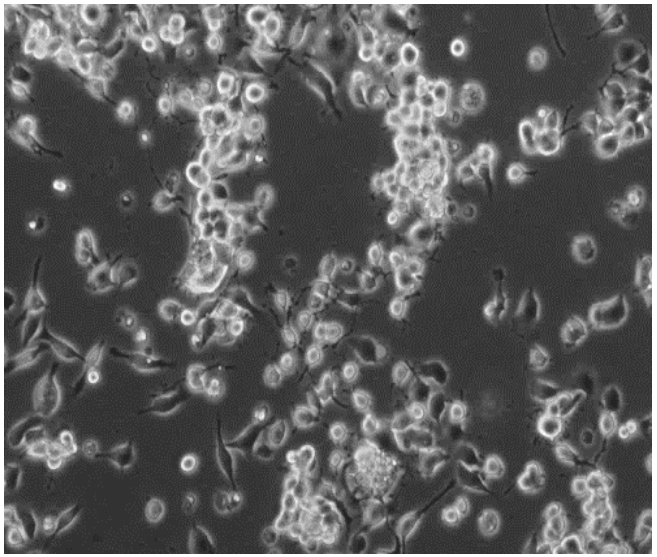
target gene cloned ROSA26 Donor vector

Co-transfection

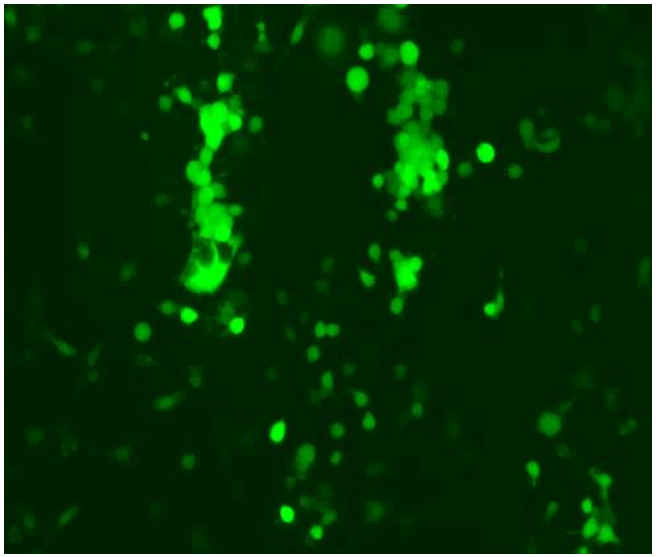
The transgene will be integrated in ROSA26 in the genome

GFP Fluorescence After Puromycin Selection

Phase contrast

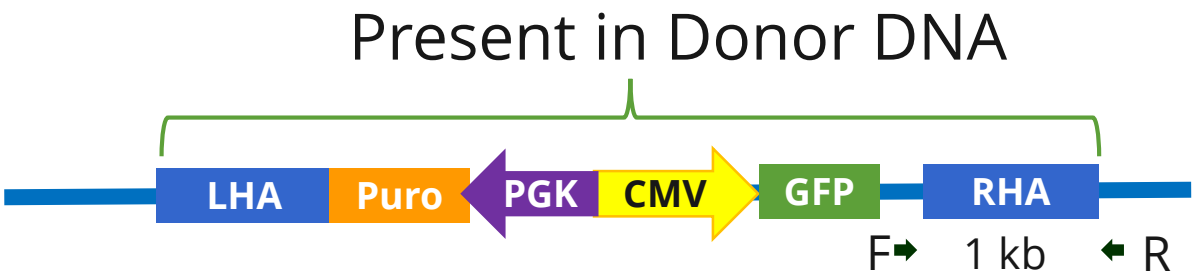


Green fluorescence



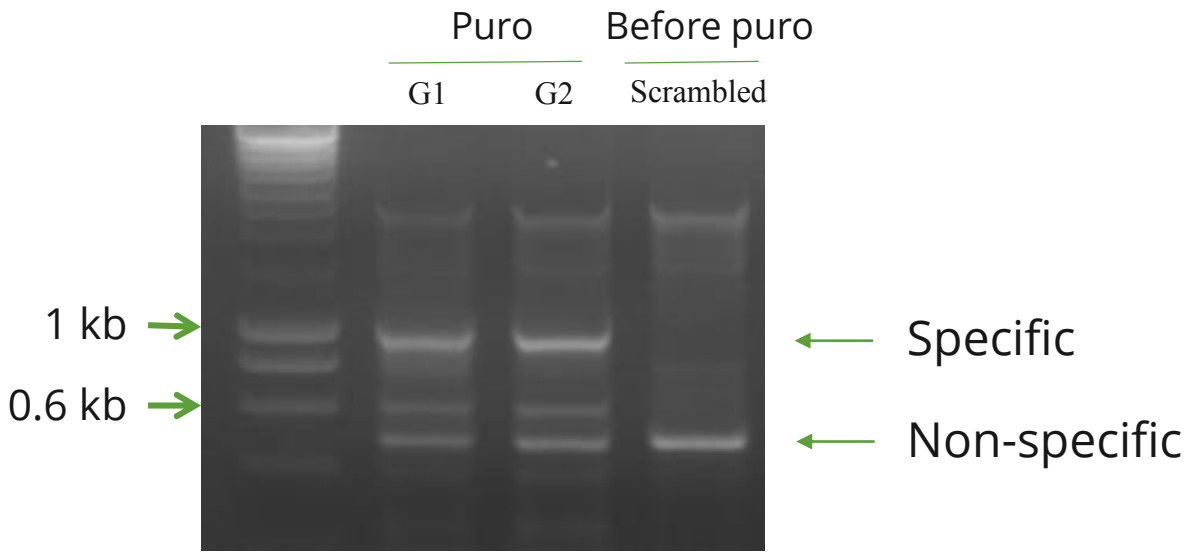
Neuro-2a cells were transfected with ROSA26 gRNA 1 and GFP ROSA26 donor. 2 weeks post transfection, cells were selected with puromycin at 1ug/uL. Images were taken 1 week after selection.

Genomic PCR Primers to Detect the Right Integration Junction



F: forward primer: 5′ CCATGCCCAACCGGTGCCAGAGAG 3′
R: reverse primer: 5′ GCCACCTCTCCAGCCCCTGGATAG 3′

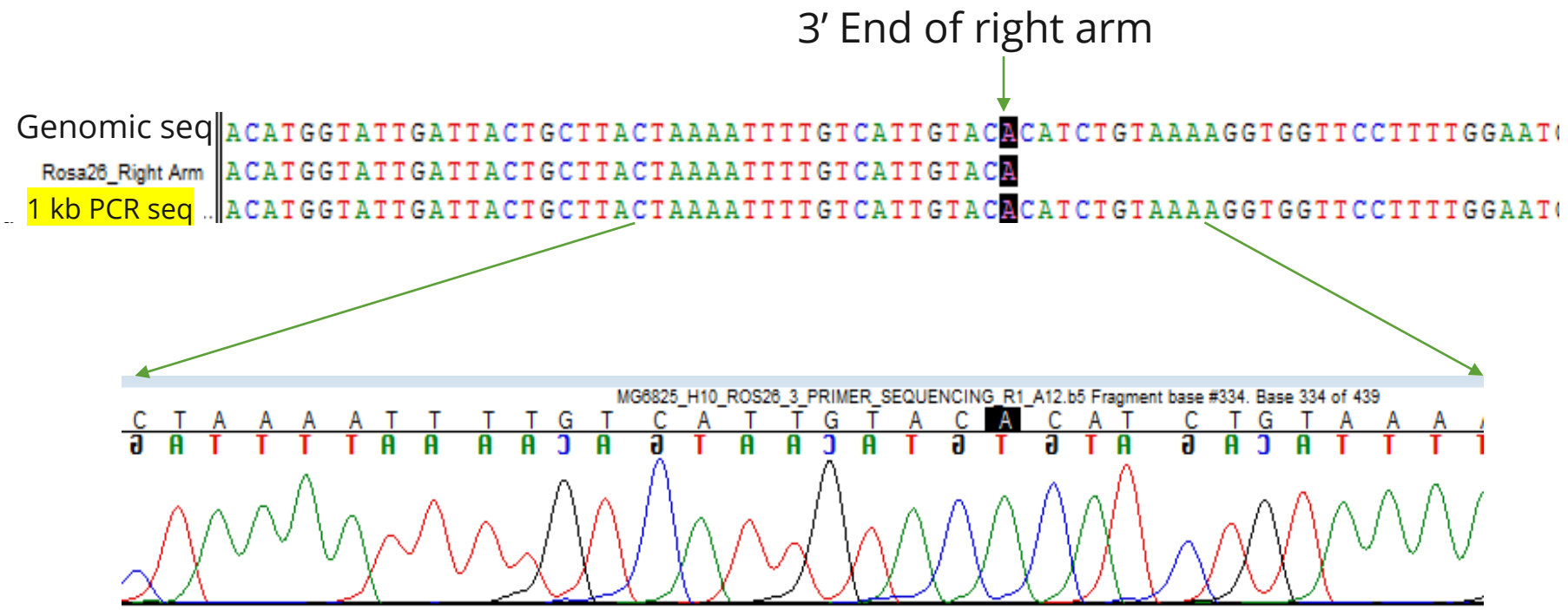
Correct size of PCR Fragment was Amplified



Neuro-2a cells were transfected with gRNA scramble or two ROSA26 gRNAs (G1 or G2) with donor plasmid. 2 weeks after transfection, cells were selected with 1 ug/uL puromycin. Genomic DNA was extracted from the stable pool and genomic PCR was performed. The 1kb PCR fragment was gel purified and sent for sequencing.

Note: after puro selection, most cell died in scramble gRNA transfected cells. Scrambled sample is genomic DNA before puro selection.

GFP Donor Cassette was Correctly Integrated at ROSA26 Locus



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