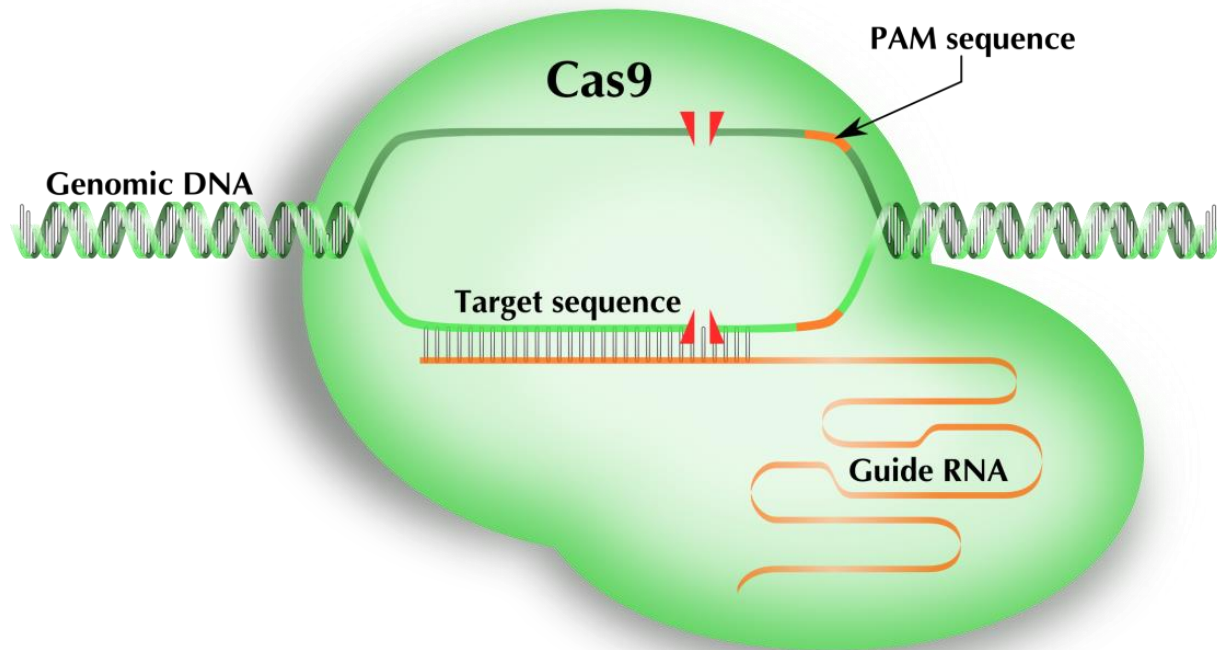


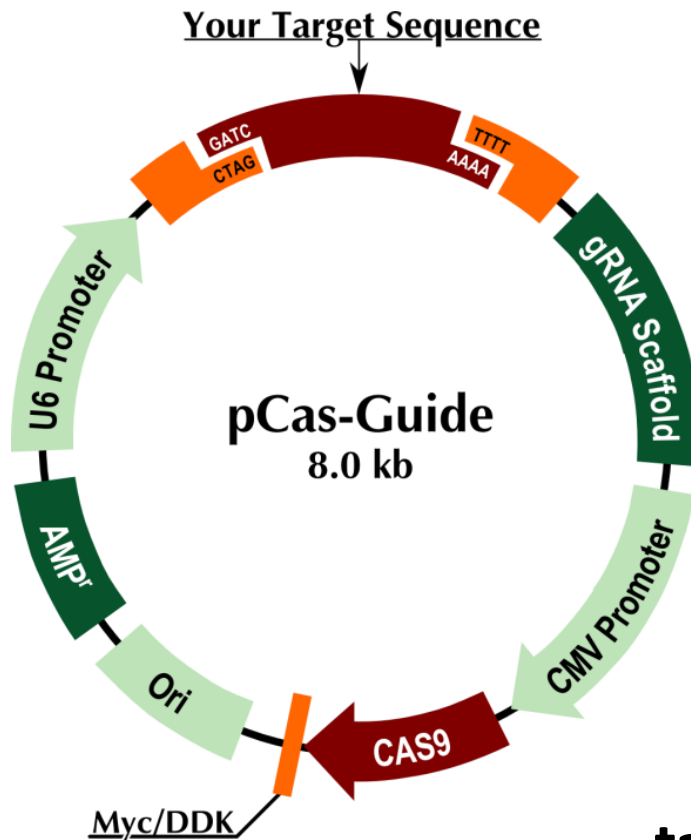
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# CRISPR Knockout / Knockin kit Validation





# All-in-one CRISPR/Cas9 vector



## pCas-Guide

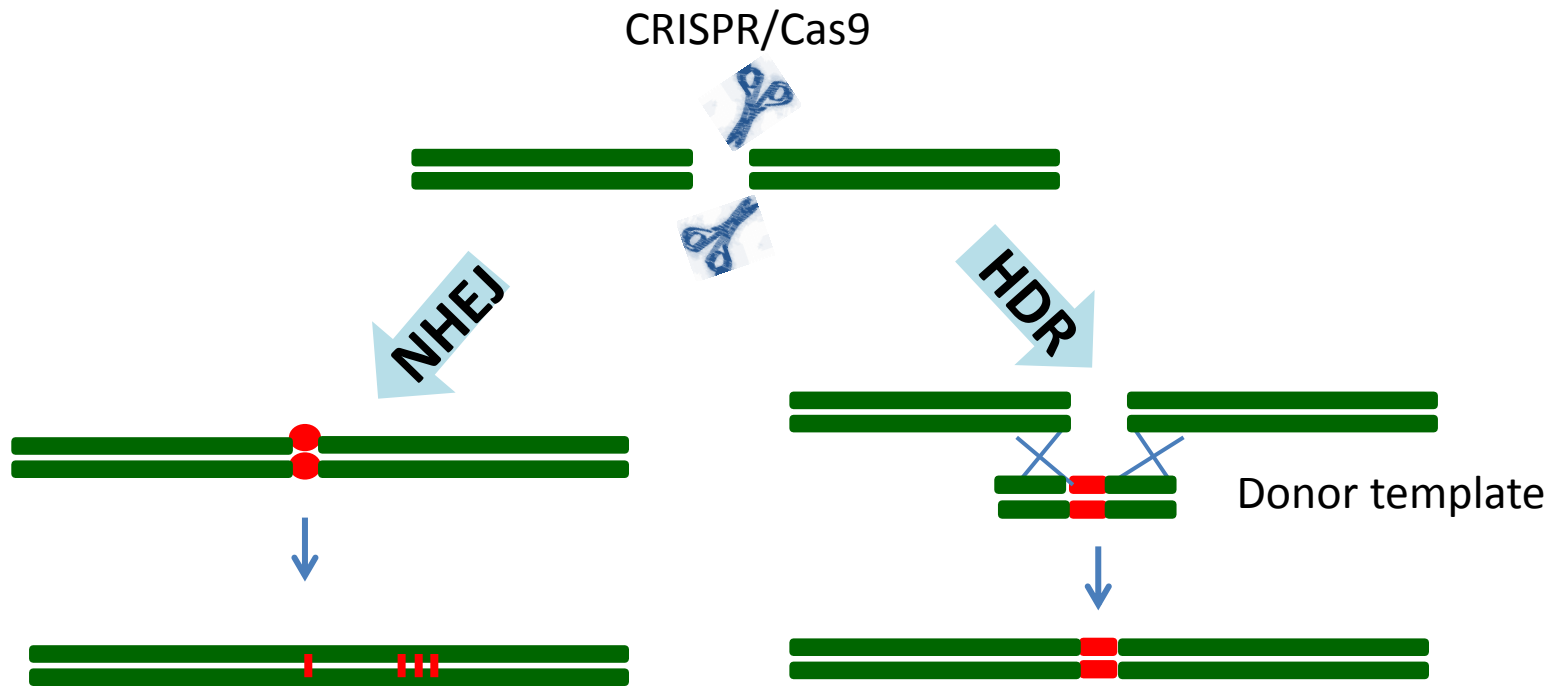
- Target sequence cloning
- Expresses Cas9

**Cas9 + sequence specific gRNA**



**targeted double-stranded break**

# Genome Editing Is Achieved via Repair



**Unpredicted indels**  
mutations  
Insertions/ deletions  
Gene knockout

**Desired**  
Gene knock-out  
Specific mutations/SNP  
Deletion/insertion/tagging genes  
Knock-in (reporter gene)  
Promoter study



# CRISPR/Cas9 Tools

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- **CRISPR/Cas vectors**
- **Pre-designed donor vectors**
- **Genome-editing Knockout kit via CRISPR, genome-wide**
  - ✓ **2 guide RNA vectors**
  - ✓ **1 GFP-puro donor vector**  
(gene specific homologous arms cloned)
  - ✓ **1 scramble control**

# KN210563 Was Used For Validation

## ATG5 - human gene knockout kit via CRISPR

Specifications		Related Products	Validation Data	FAQ
SKU	Description	Price	Availability	Manual
KN210563	ATG5 - human gene knockout kit via CRISPR	\$1200	4 Weeks	 

### Also for ATG5 (Locus ID 9474)

[cDNA Clone](#)[shRNA/siRNA](#)[Primer Pair](#)[Protein Request](#)[Antibody](#)

### Kit Components

**KN210563G1**, ATG5 gRNA vector 1 in [pCas-Guide vector](#), Target Sequence: AACTTGTTTCACGCTATATC

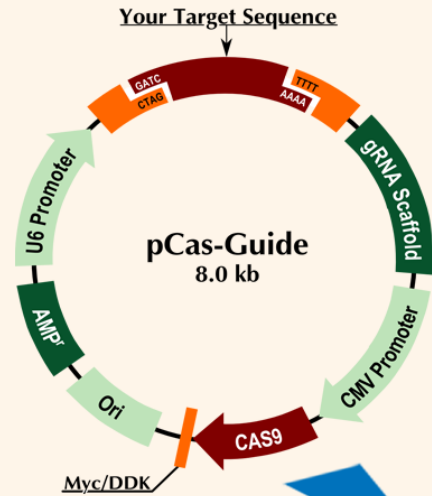
**KN210563G2**, ATG5 gRNA vector 2 in [pCas-Guide vector](#), Target Sequence: AAGATGTGCTTCGAGATGTG

**KN210563D**, donor vector containing Left and right homologous arms and GFP-Puro functional cassette.  
[Homologous arm and GFP-puro sequences](#)

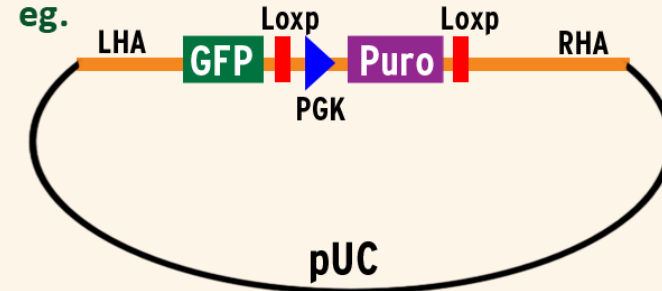
**GE100003**, scramble sequence in pCas-Guide vector

# Diagram of CRISPR Knockout Kit

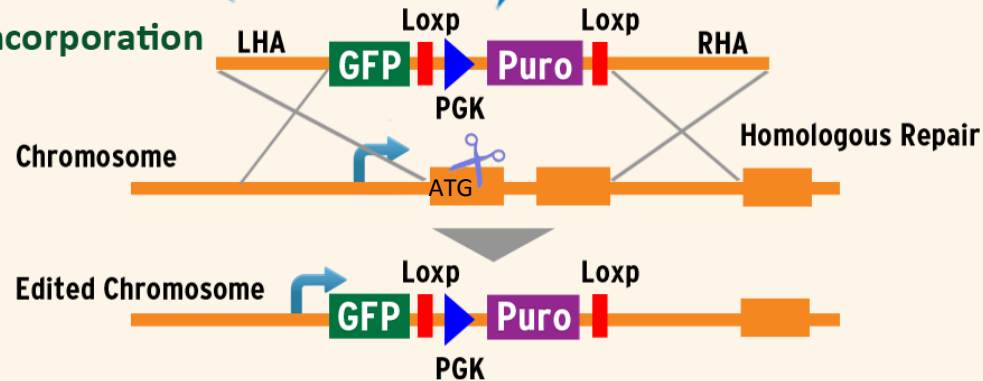
## 1 Target Sequence Cloned In pCas Guide Vector



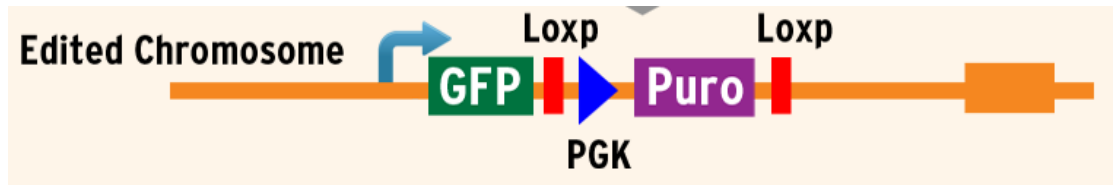
## 2 Donor Template DNA Containing Homologous Arms & Functional Cassette



## 3 Genome Incorporation



# Edited Chromosome – gene knockout / GFP-Puro knockin



- ✓ Target gene is knocked out
- ✓ GFP under endogenous gene promoter
- ✓ Puromycin selection marker under PGK promoter

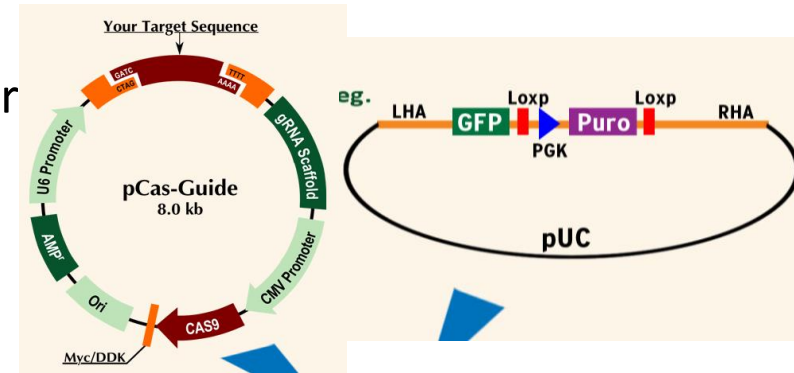


# Protocols for targeted gene knockout using CRISPR Knockout / Knockin Kit

1. Cotransfection: one of the gRNA vector + donor

*Controls: 1). Scramble control + donor vector*

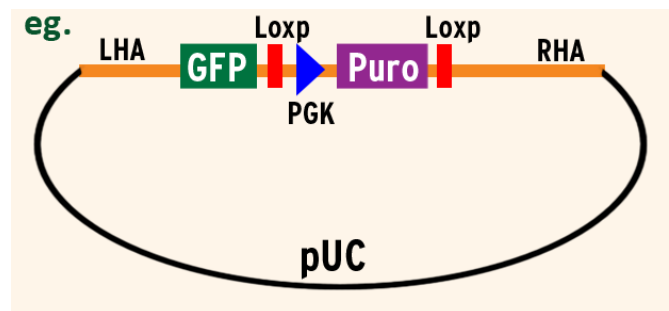
*2). Donor only*



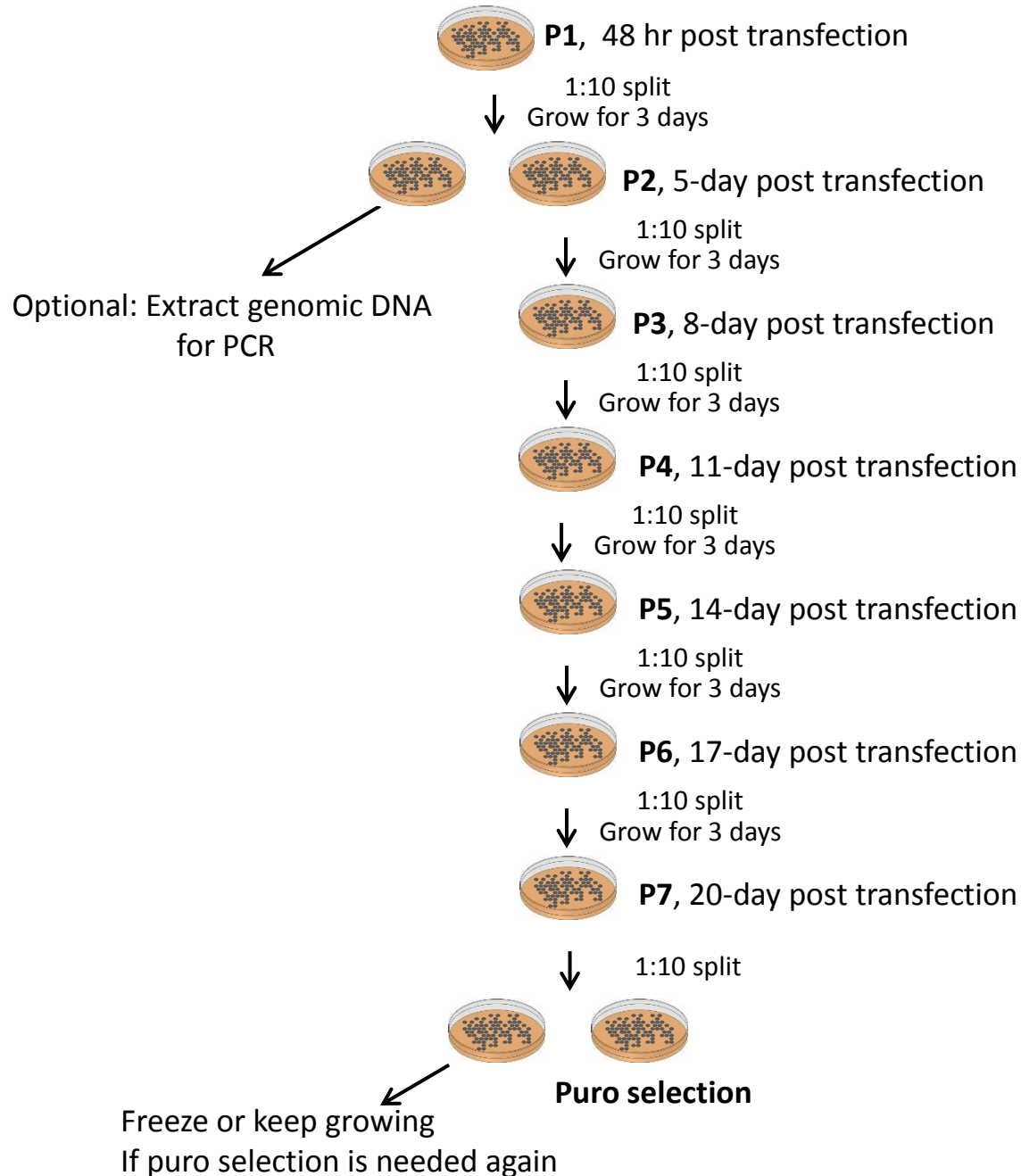
2. Dilute cells containing donor vector ~ 20 days before puro selection

*Note: Since puro selection marker is under PGK promotion,*

*Episomal and randomly integrated donor vector will also give puro resistance.*



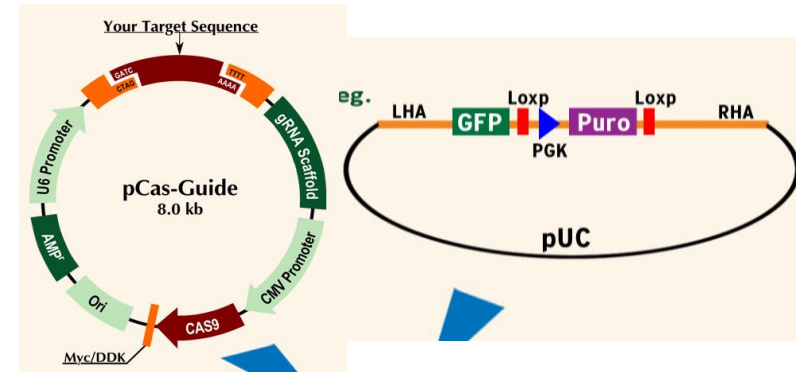
# Diagram of diluting cells before puro selection



# Protocols for targeted gene knockout using CRISPR Knockout / Knockin Kit

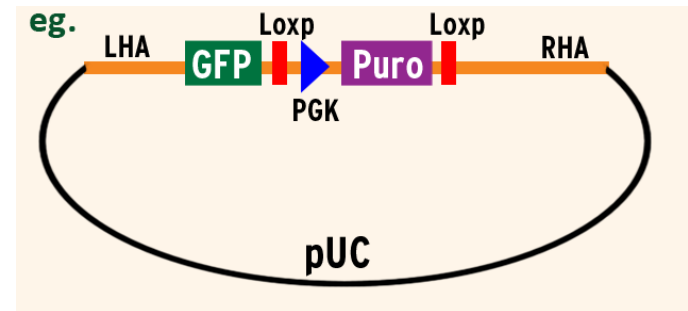
## 1. Cotransfection: gRNA vector + donor vector.

Controls: 1). Scramble control + donor vector  
2). Donor only



## 2. Dilute cells containing episomal donor vector ~ 20 days post transfection

Note: Since puro selection marker under PGK promotion, Episomal and randomly integrated donor vector will also give puro resistance.



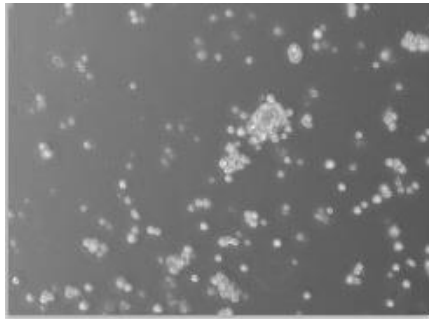
## 3. Apply Puro selection. Isolate individual cell colonies

- Note. Doses need to be determined by kill curve for each cell line
- Donor vector alone can randomly integrate into the genome, but the efficiency should be much lower

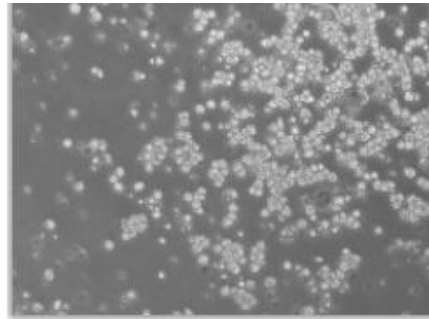
# Puromycin selection

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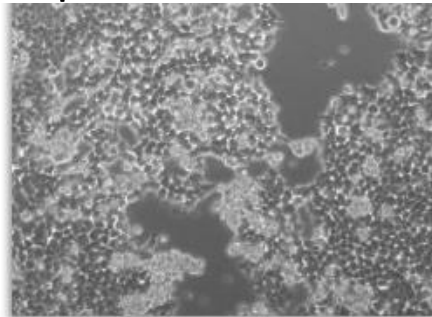
Donor only



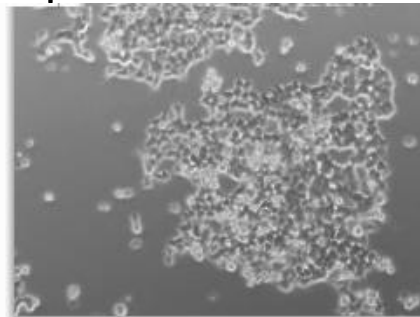
pCas-Scrambled +Donor



pCas-T1 +Donor



pCas-T2 +Donor

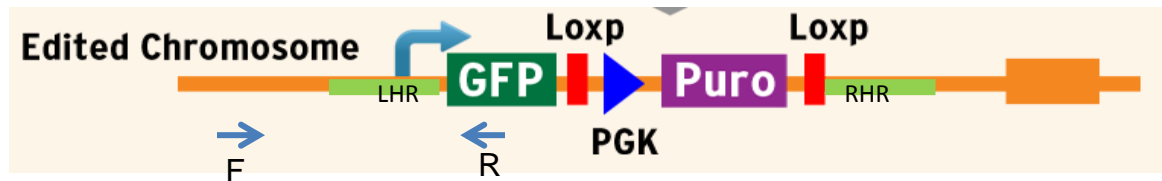


After 5 splits, HEK293 cells were selected under 1  $\mu\text{g}/\text{mL}$  puromycin for 5 days

# Protocols -- continue

## 4. Analyze puro positive cells.

- A. WB to detect the knockout effect (better with single colonies)
- B. Genomic PCR to verify GFP-puro integration, sequence the PCR products to confirm the integration.

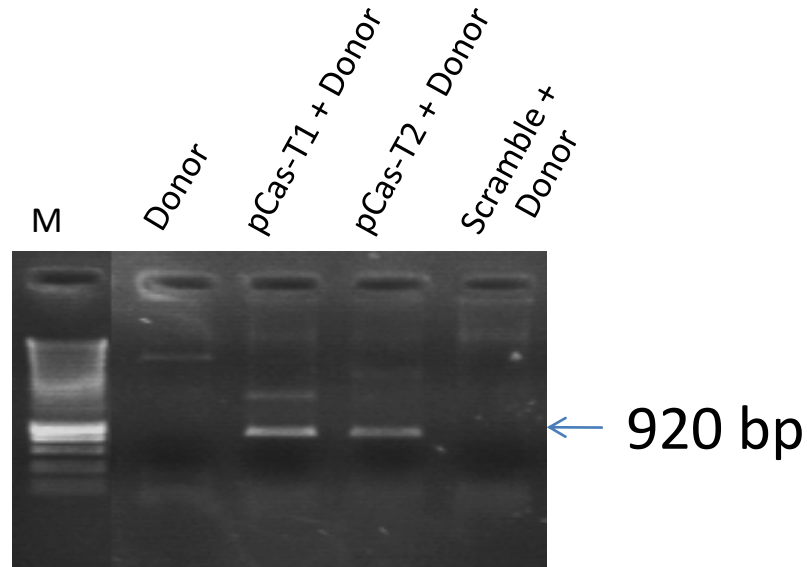


### Avoid Donor DNA contamination:

- F primer: upstream of the 5' end of left arm
- Reverse primer: GFP region

# Genomic PCR of GFP-puro Integration

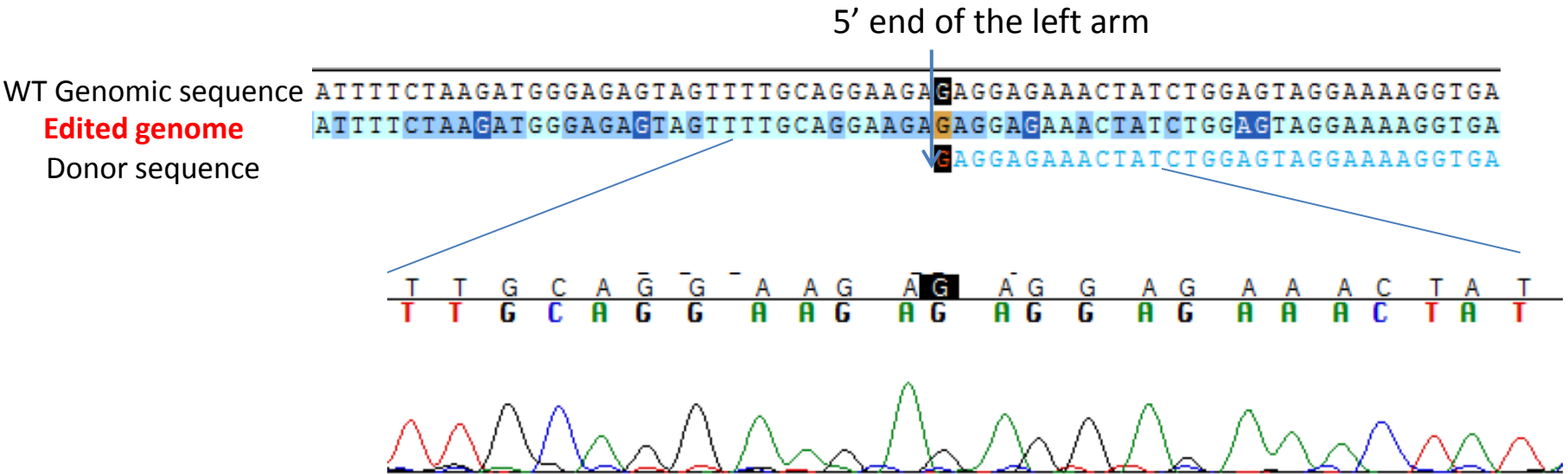
KN210563 genomic\_F GGATACAGAGAAAGGTGTTTCAGG  
tGFP-integration\_3R TAGGTGCCGAAGTGGTAGAAGC



Genomic DNA was extracted from cells 5 days post transfection before puro selection

# Sequencing Using The Forward Primer

Correct integration at 5' end of left arm

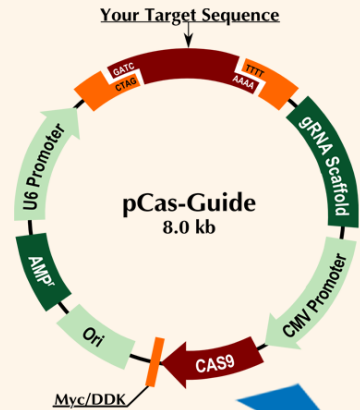




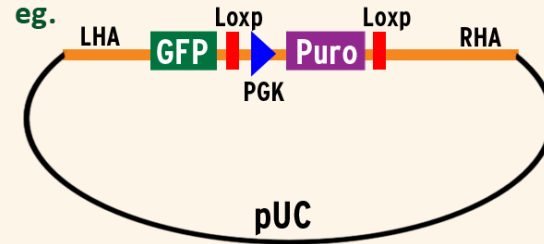


# Other Donor Vectors with different FP or Luciferase

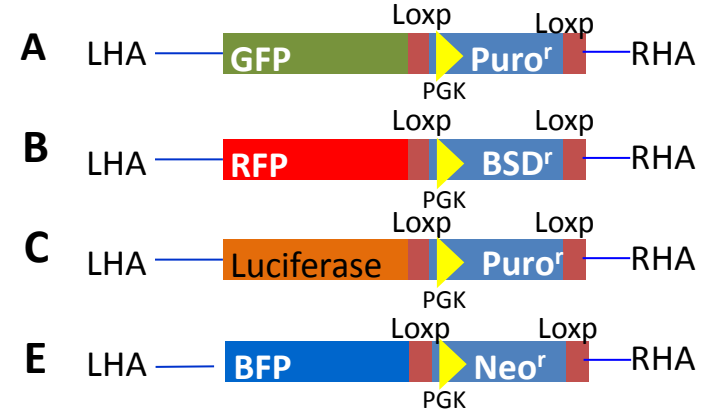
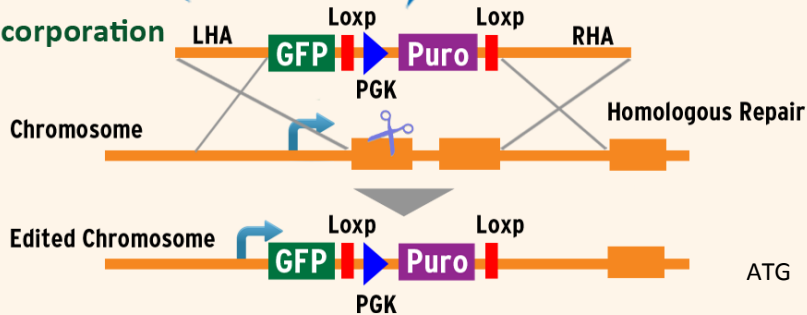
## 1 Target Sequence Cloned In pCas Guide Vector



## 2 Donor Template DNA Containing Homologous Arms & Functional Cassette



## 3 Genome Incorporation





Please visit us

[www.origene.com](http://www.origene.com)

[techsupport@origene.com](mailto:techsupport@origene.com)