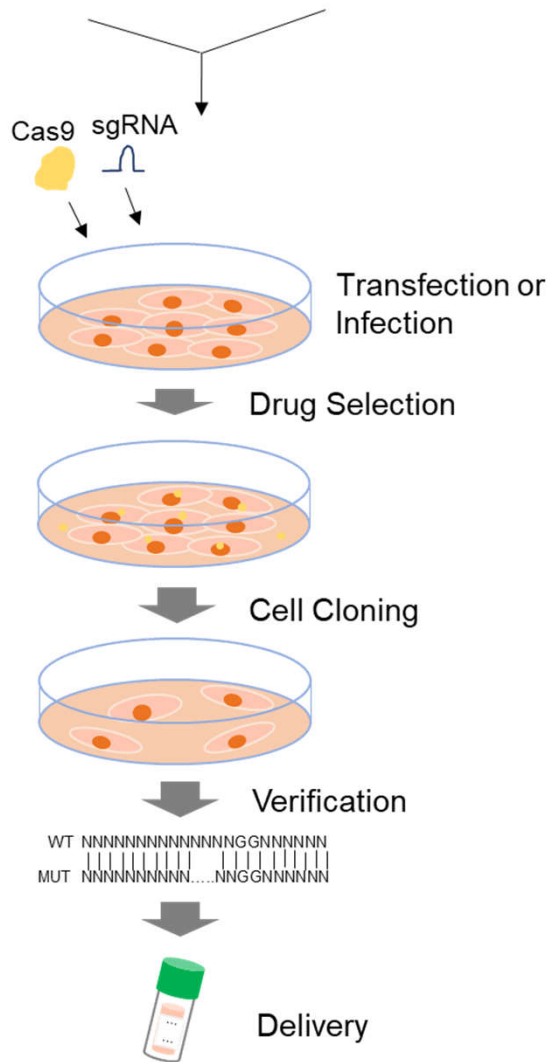


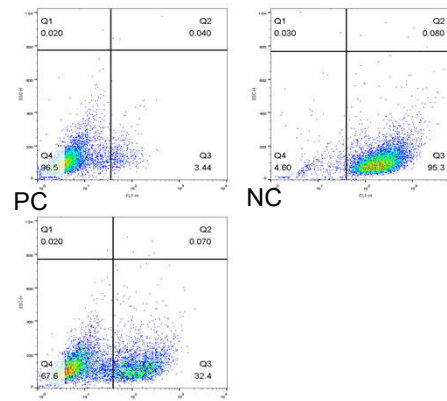
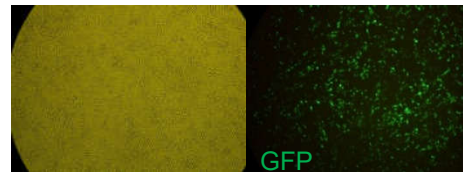
# Custom Knockout Cell Line Generation by CRISPR/Cas9 Platform

## Cell Line Characterization SgRNA Design and Construction

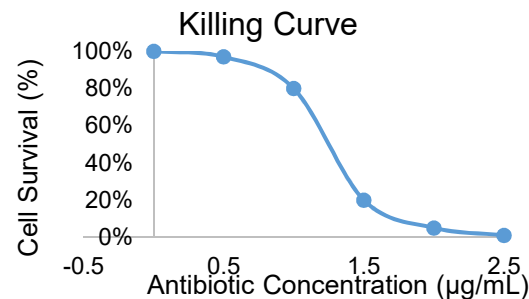


## Cell Line Evaluation

1. Transfection Efficiency
  - ✓ Liposome-mediated Transfection
  - ✓ Electroporation
  - ✓ Lentiviral Infection



## 2. Antibiotic Killing Curve



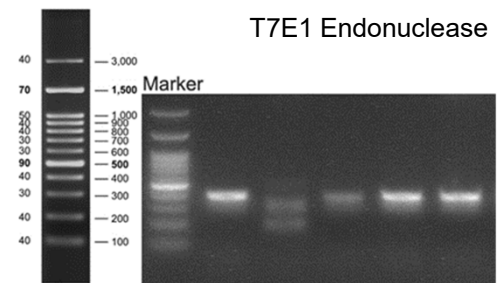
## 3. Clonality

## SgRNA Design and Vector Construction

1. SgRNA Design
  - At least 3 sgRNA are selected for a gene.
2. Vector Construction
  - ✓ SgRNA Synthesis
  - ✓ Construction
3. Validation of sgRNA efficiency

## Cell Line Generation

1. Transfection cells with sgRNA/Cas9 mix
2. Drug Selection
3. Cell Cloning and expansion
4. Genetic screening by PCR and sequencing



## Sequencing

WT: CTCGCTCCGTGGCCTTAG--CTGTGCTCGCGCTA  
 MUT: CTCGCTCCGTGGCCTTAGGGCTGTGCTCGCGCTA

## 5. Positive clone expansion and cell cryopreservation

## 6. Delivery

A vial of homozygous knockout cells, which contains more than  $1 \times 10^6$  cells, will be delivered with the project report.