# CRISPR Cell Line Knockout Report

## Project

Order CS30263
Cat. C208
Knock out gene name:JWA. Accession number: NM_006407
Cell Line name:MHCC97L(Human)， propagation media：DMEM +10%FBS + 1% P/S

## List of Deliverables

JWA CRISPR Knocked out MHCC97L(Human) Stable Cell Line P5 1xT25

## Workflow

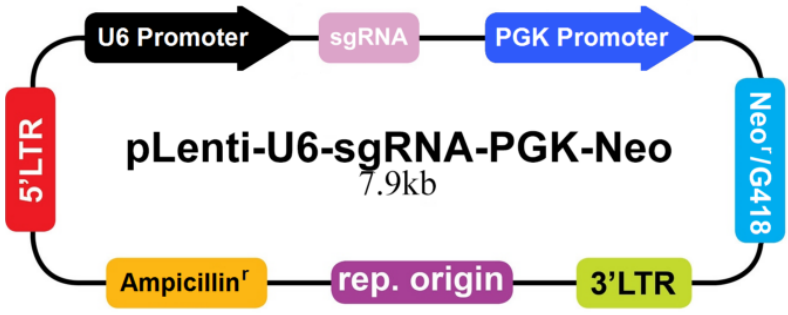
**PHASE I – sgRNA design**

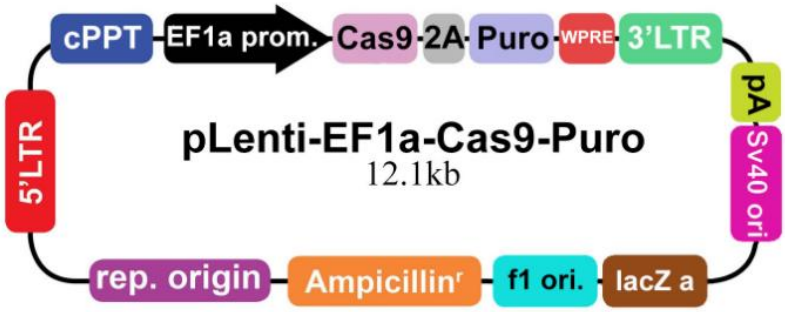
sgRNA was designed using CHOPCHOP (https://chopchop.rc.fas.harvard.edu/).

### Cas9-sgRNA Vector and sgRNA Sequences used in KO

**Transduced with Lentiviral JWA sgRNA and Cas9**

(Target sequence: AGAGCAGGTTGCTCACTACG; Homo sapiens, NM_006407)



**PHASE II – Virus Packaging Protocol**

Lentivirus packaging was performed according to abm protocol as described on the website (https://www.abmgood.com/Lentivirus-Packaging-Systems.html).

**PHASE III – Transduction and Selection of Clones**

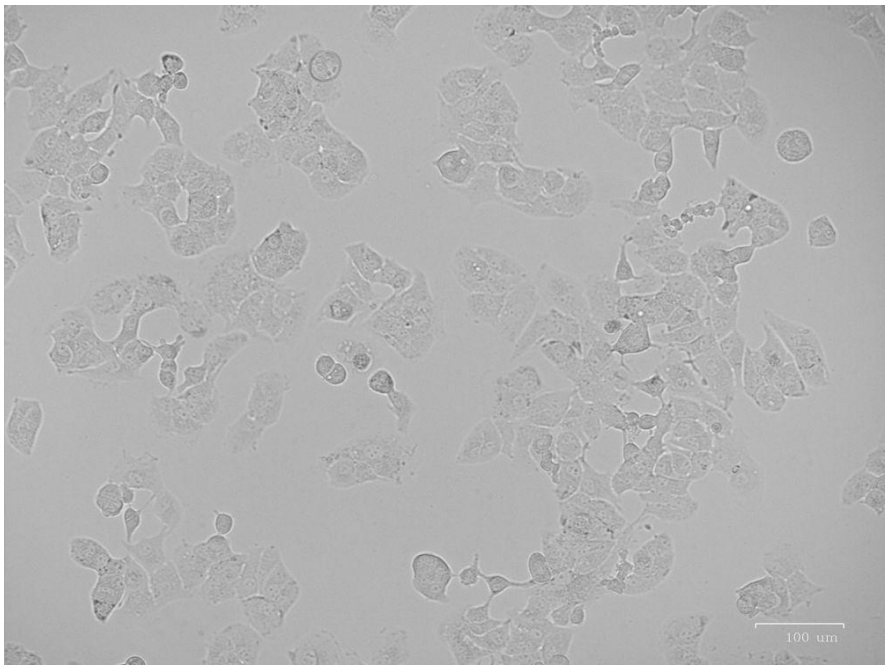
Cells were being transduced and clones were selected for further PCR, Surveyor and Sanger sequencing for verifications.

## Results – Screening

**Round I: Monoclonal Biallelic Knockout Investigation**

The selected clonal pools were used to perform serial dilutions in 96 well plates in DMEM+10% FBS+1% P/S+1.5µg/ml Puromycin+1.3µg/ml G418. G398 was for treating and decontaminate mycoplasma contamination. Wells containing single cell colonies were identified and allowed to proliferate for 28-32 days with media changes in between and expanded for further screening. Genomic DNA was extracted from 8 clones for PCR analysis. Clones with conclusive surveyor assay results for genetic editing were sent for Sanger sequenceing and compared to the reference genome.

**Clone 2C-C7** was selected for the Sanger sequencing.




**PHASE IV – Sequencing/QC Phase**

QC Method

PCR cloning after lentiviral transduction with JWA sgRNA.

PCR primers used

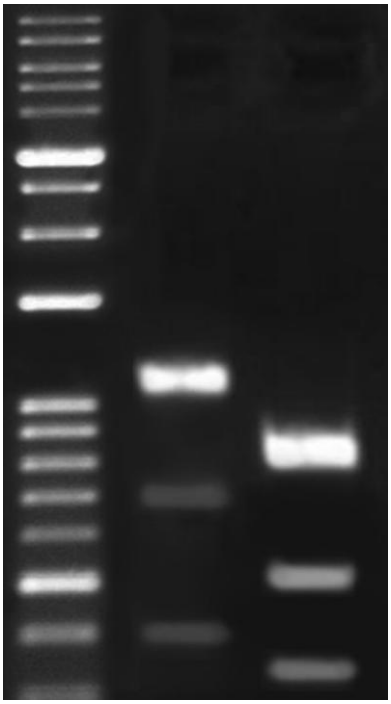
CS30263- JWA- Surveyor1-FP: AAAGGTATTTCGAAGGAGGCAGC

CS30263- JWA- Surveyor1-RP: AAGCGAAGACAGAGAAAGTGACC

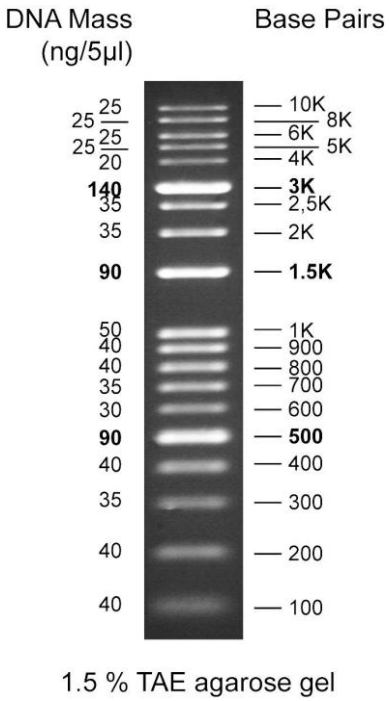
1172bp

## Results – Surveyor

Surveryor was performed as part of the verification on the nicking of the target.



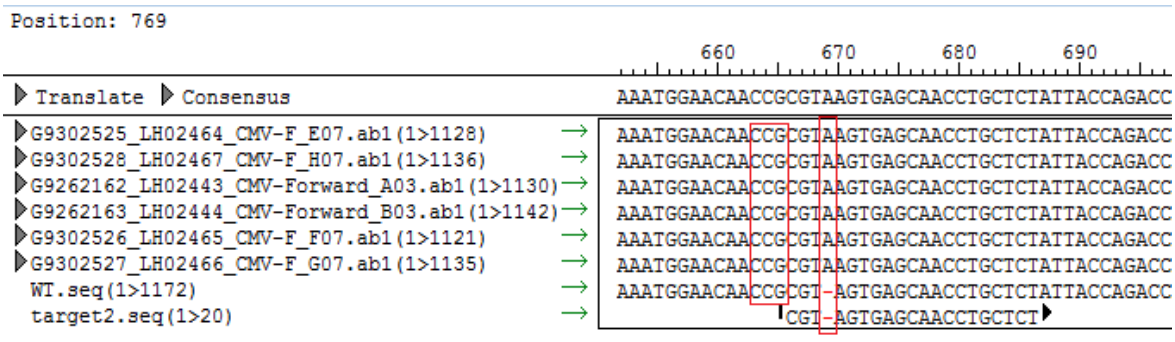
Ladder Clone 2C-C7 Control Template



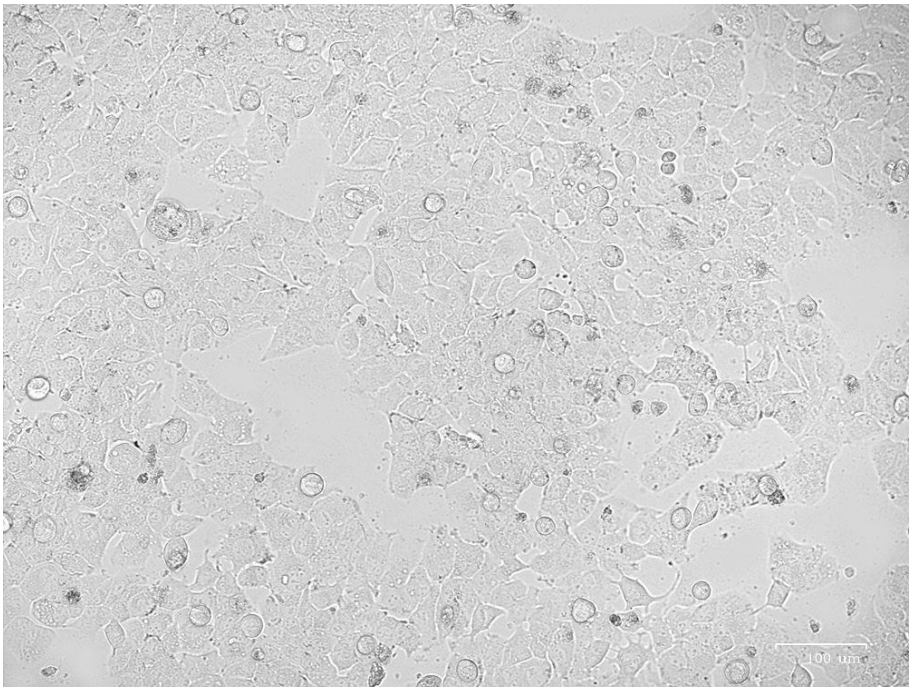

1.5 % TAE agarose gel

## Results – Sanger Sequencing

Sequencing results showing frameshift mutation resulting from successful genome editing. Cas9 Nuclease adds 1 bp upstream of the NGG PAM sequence (indicated by red box).



## Cell Morphology and microbial contamination testing




| Test for Microbial Contaminants | | |
|---|---|---|
| **Test Method** | **Results** | |
| Bacteria | Direct Culture | Not detected |
| Fungi | Direct Culture | Not detected |
| Mycoplasma | PCR | Negative |

Conclusion:
The CRIPSR knockout of gene JWA was successfully introduced via frame shift mutation in MHCC97L(Human) cell line.