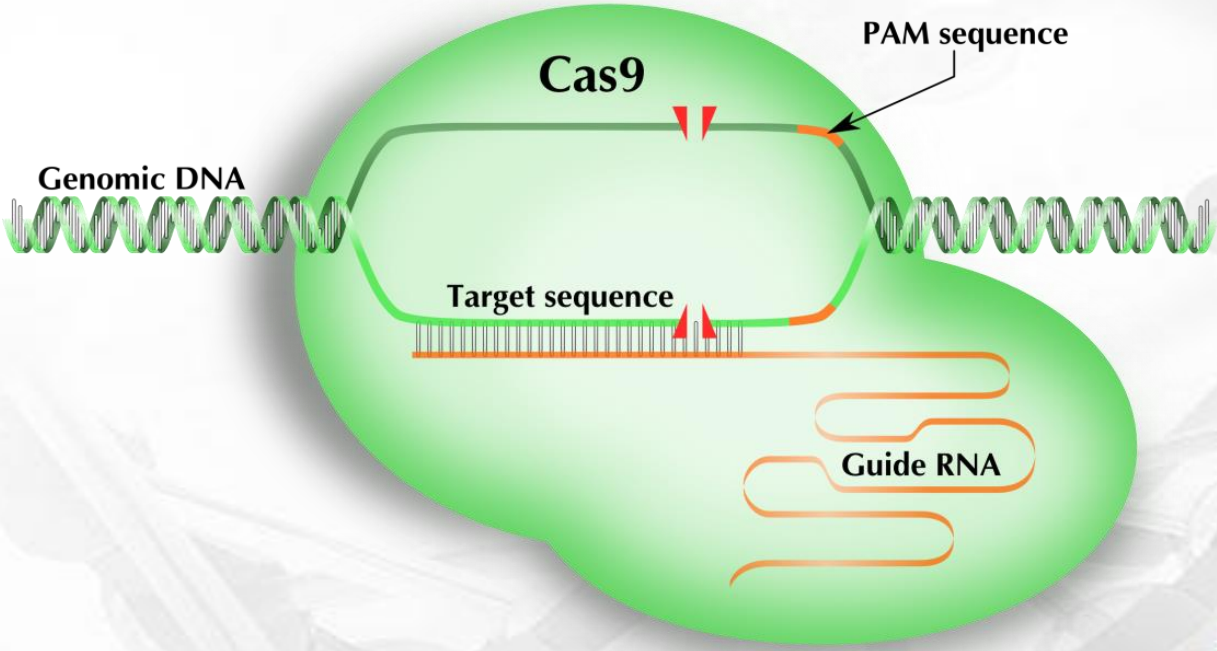
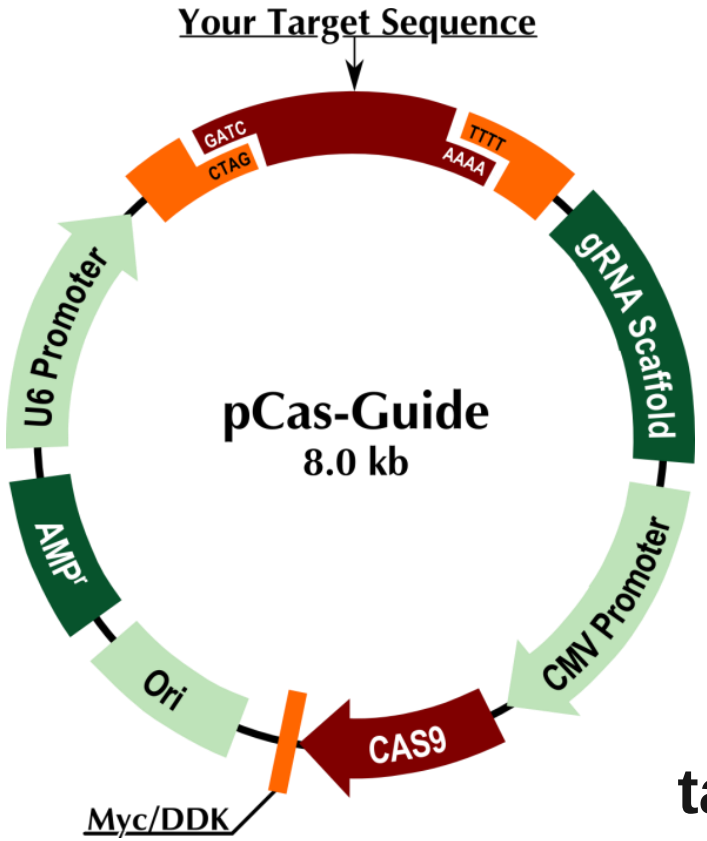
KN2.0 CRISPR Knockout Kit Validation



All-in-one CRISPR/Cas9 vector



pCas-Guide

- Target sequence cloning
- Cas9 expression

Cas9 + sequence specific gRNA

↓

targeted double-stranded break

KN2.0 CRISPR Knockout Kit

- ✓ 2nd generation CRISPR knockout kit
- ✓ Higher knockout efficiency
- ✓ EF1a-GFP-P2A-Puro
- ✓ A specific kit for every gene locus

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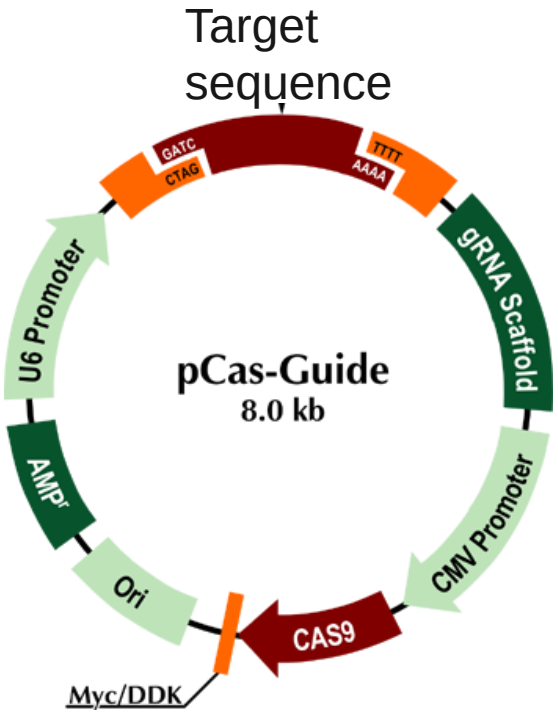
KN2.0 CRISPR Knockout Kit

Components:

- 2 gRNA vectors in pCas-Guide
- Linear donor DNA containing EF1a-GFP-P2A-Puro



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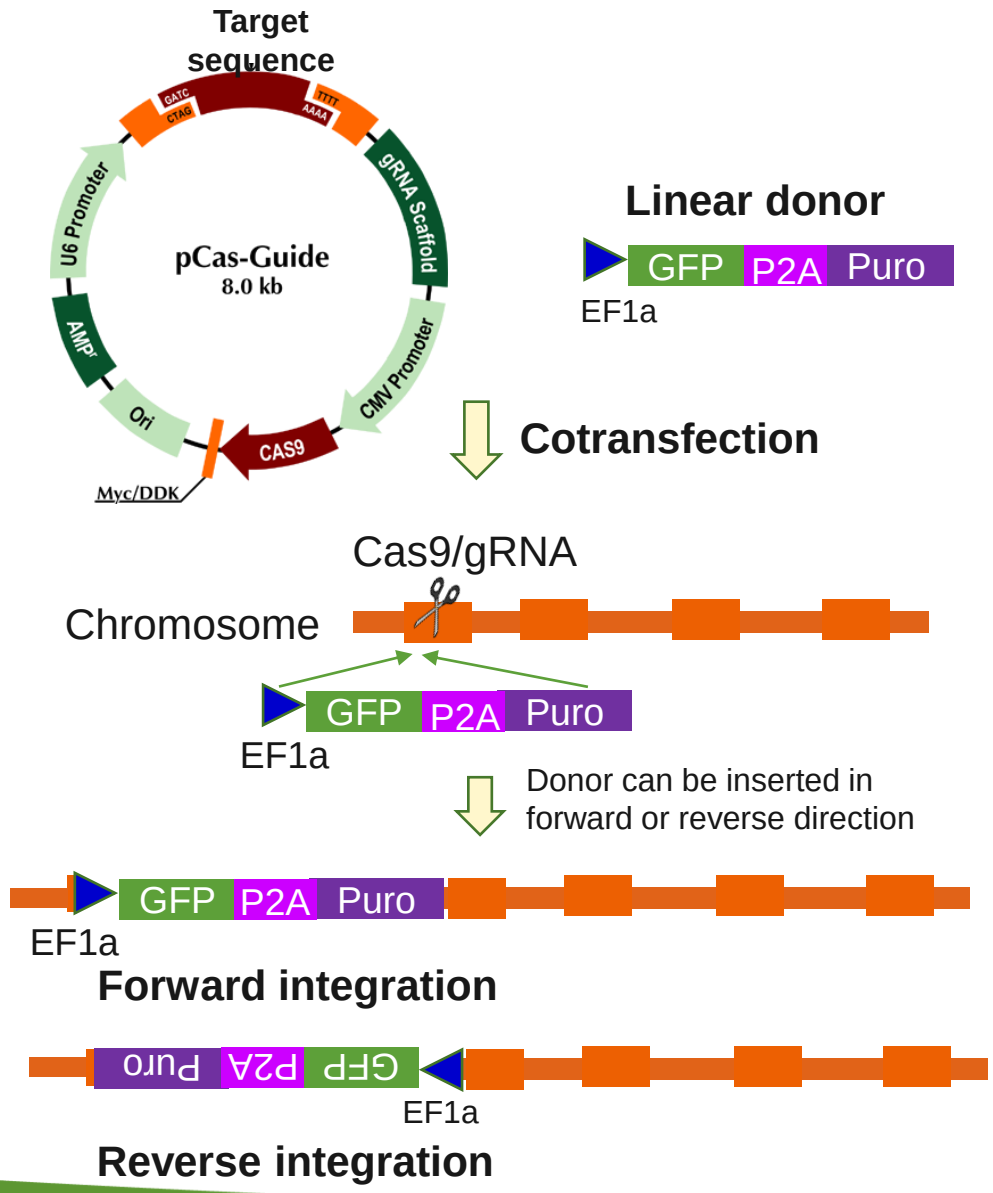
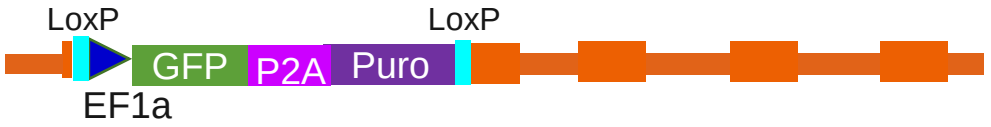
Scheme of KN2.0 CRISPR Knockout Kit



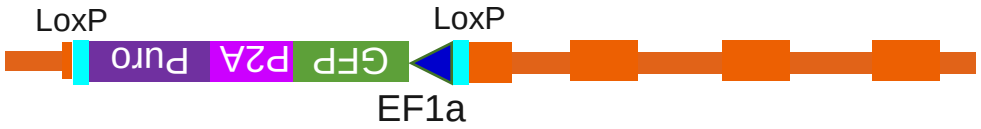
KN2.0 Edited Chromosome

- ✓ **Donor inserted at the gRNA cutting site**
- ✓ **Endogenous gene knocked out**
- ✓ **GFP under EF1a**
- ✓ **Puro with P2A**

Forward integration

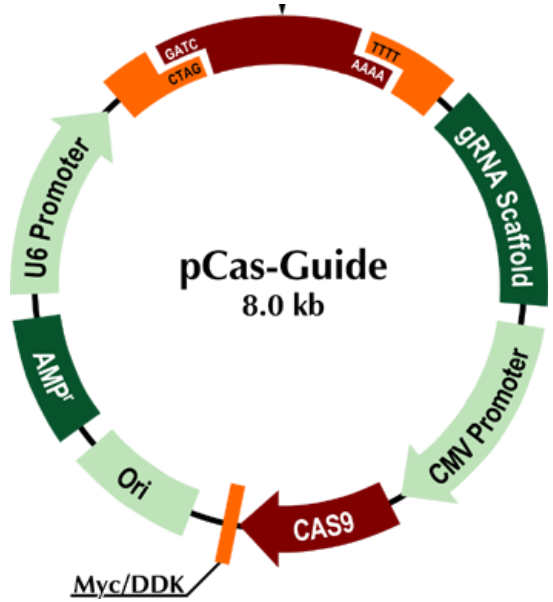


Reverse integration



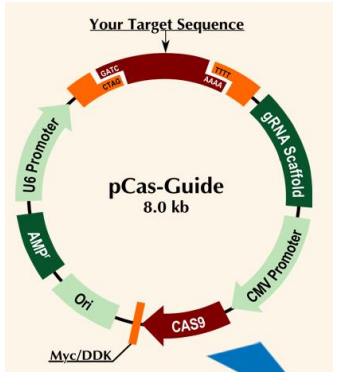
Human ATG5 Knockout using KN2.0 in HEK293

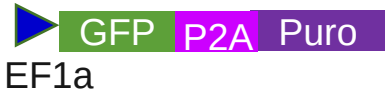
- **gRNA sequence: AACTTGTTTCACGCTATATC**
- **Linear donor:**



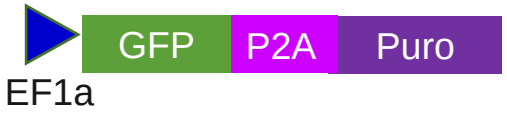
Protocols for Targeted Gene Knockout

1. Cotransfection: gRNA plasmid + donor



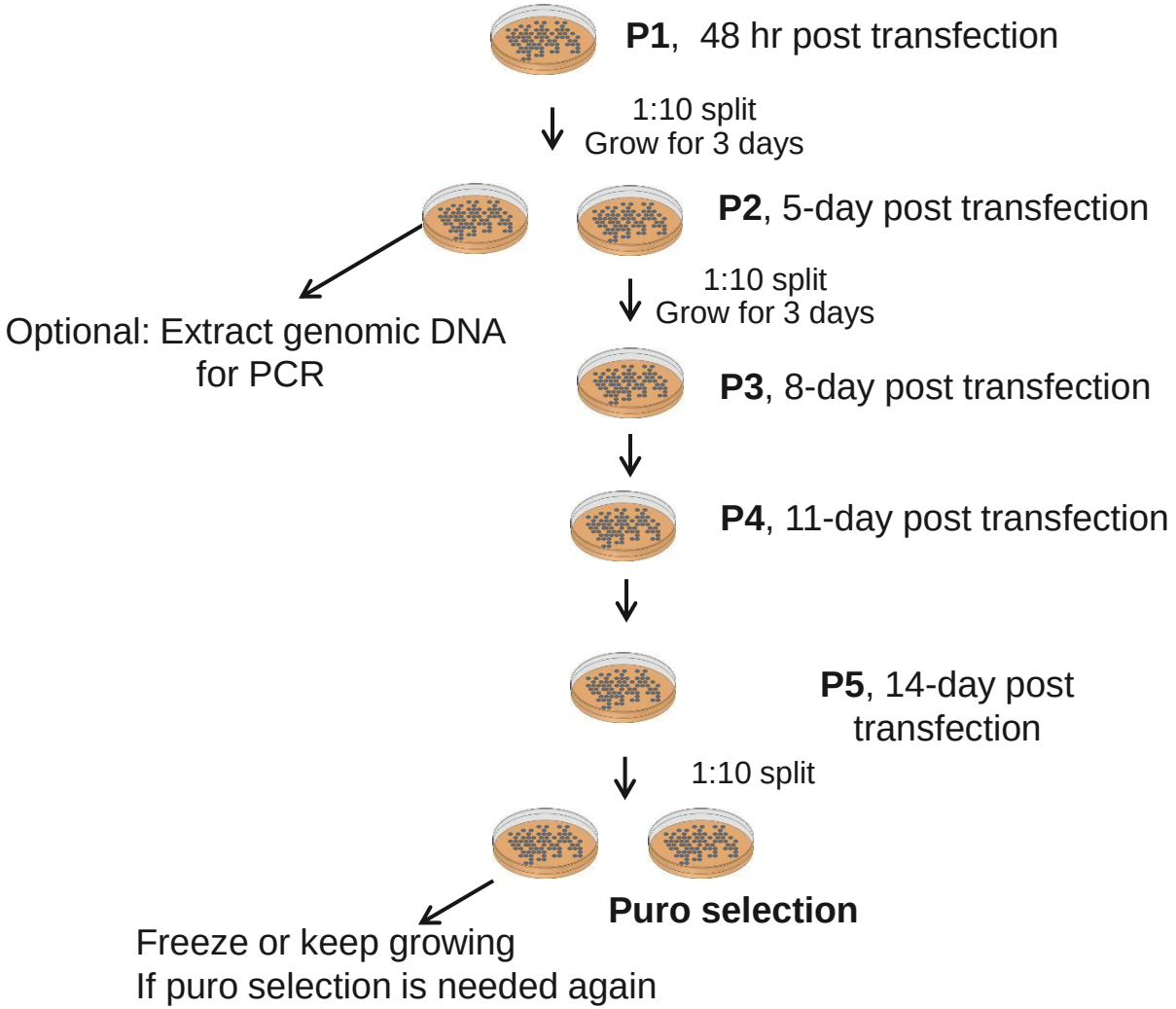


2. Dilute cells after transfection ~ 20 days before puro selection



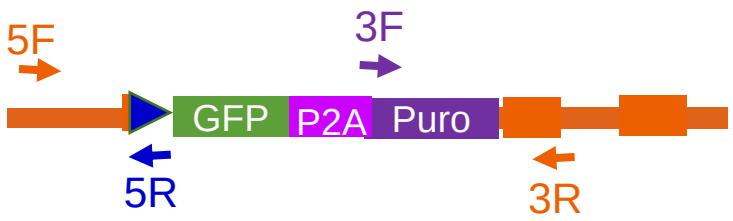
Note: Since puro selection marker is under P2A and EF1a promoter,
Episomal and randomly integrated donor vector will also give puro resistance.

Diagram of Cell Passaging After Transfection



Verification of Donor Integration by Genomic PCR

Forward integration



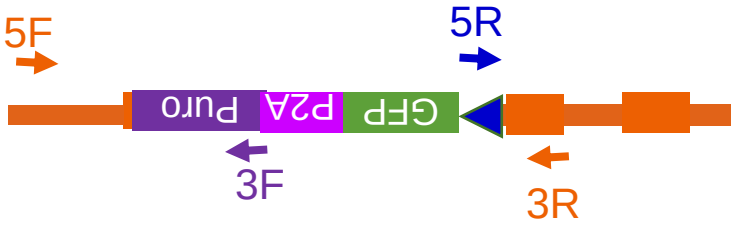
5’ Junction (0.8 kb)

5F: AGTTGGACTGTCAGGATTCACA
5R: CAGGTGGAAGTAATTCAAGGCAC

3’ Junction (0.9 kb)

3F: CCTATGACCGAGTACAAGCCC
3R: CCAGAACGCATCATGACAACA

Reverse integration



5’ Junction (1.2 kb)

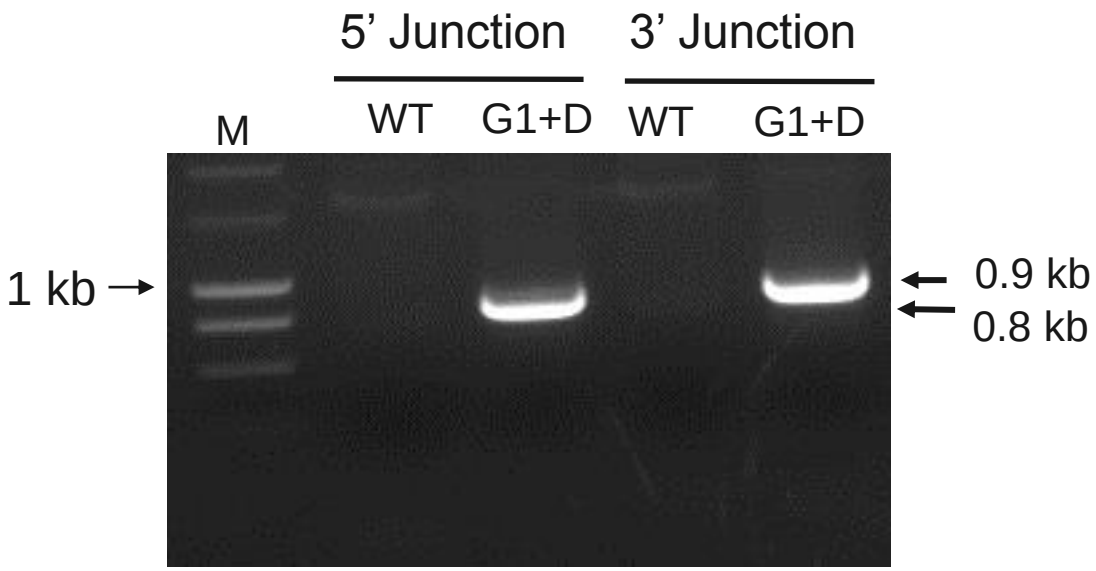
5F : AGTTGGACTGTCAGGATTCACA
3F: CCTATGACCGAGTACAAGCCC

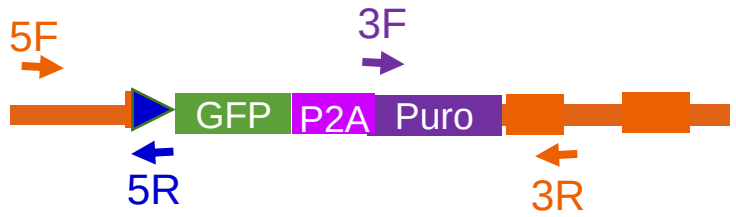
3’ Junction (0.7 kb)

5R: CAGGTGGAAGTAATTCAAGGCAC
3R: CCAGAACGCATCATGACAACA

- Please note 5F and 3R are gene specific, and they should be designed based on the target genome sequence.

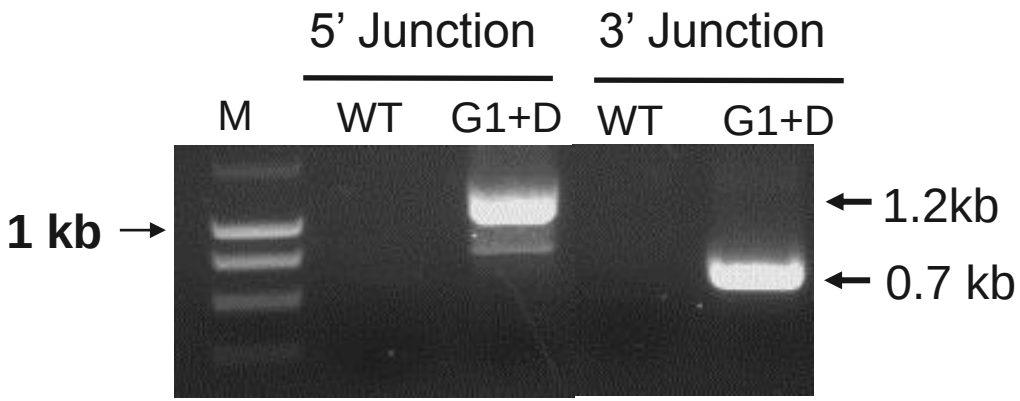
Verification of Donor Insertion in Forward Direction

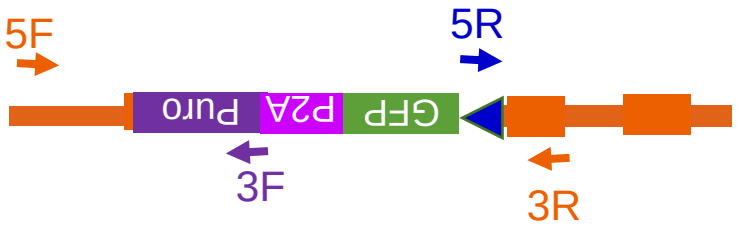




Three days after transfection, un-transfected (WT) and transfected (G1 and donor) HEK293 cells were harvested and genomic DNA was extracted. For forward donor integration, PCR was performed using primer pairs *5F and 5R (5’ junction); 3F and 3R (3’ junction).*

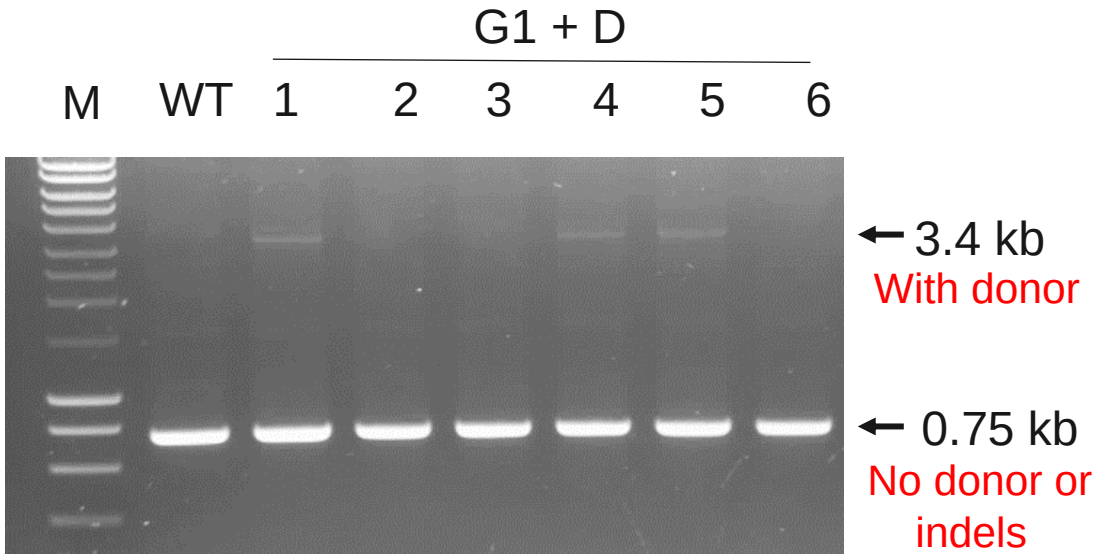
Verification of Donor Insertion in Reverse Direction



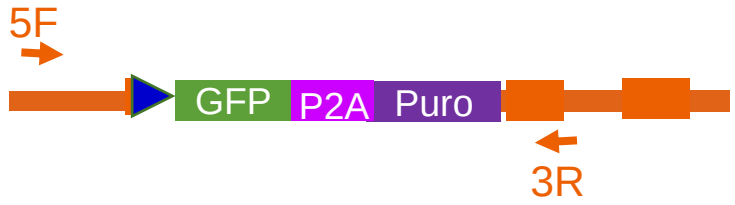


Three days after transfection, un-transfected (WT) and transfected (G1 and donor) HEK293 cells were harvested and genomic DNA was extracted. For donor reverse integration, PCR was performed using primer pairs *5F and 3F (5’ junction*); *5R and 3R (3’ junction).*

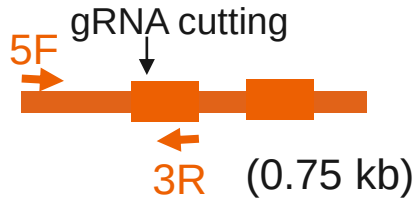
50% of Cell Clones Contain Donor Insertion



With Donor
3.4 kb (5F and 3R)

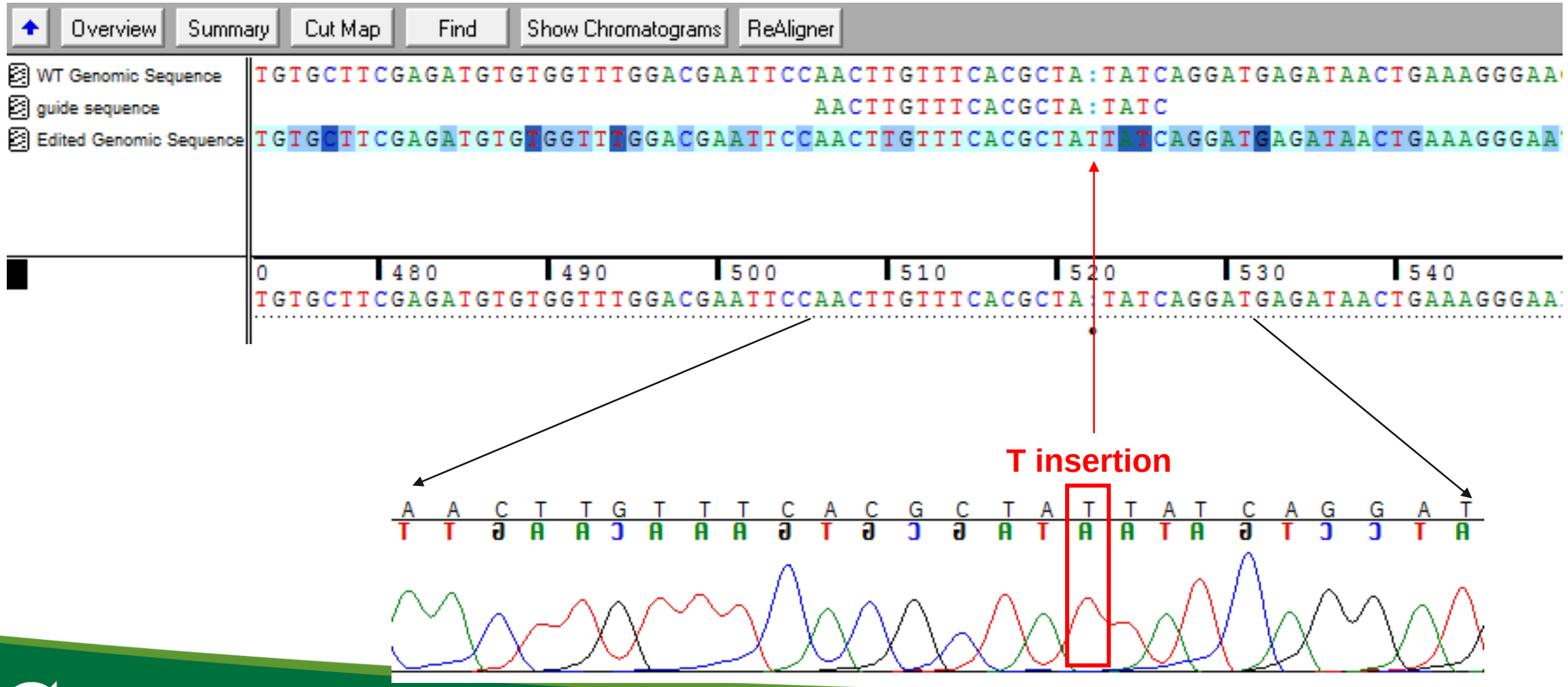


No donor
or indel
0.75 kb



Single HEK293T cell clones were isolated after puromycin selection. Genomic DNA was extracted and PCR was performed using primer pair 5F and 3R. WT: untransfected cells.
1, 2, 3, 4, 5, 6: single colonies of G1 and donor transfected.

0.75 kb PCR Fragments have Indels



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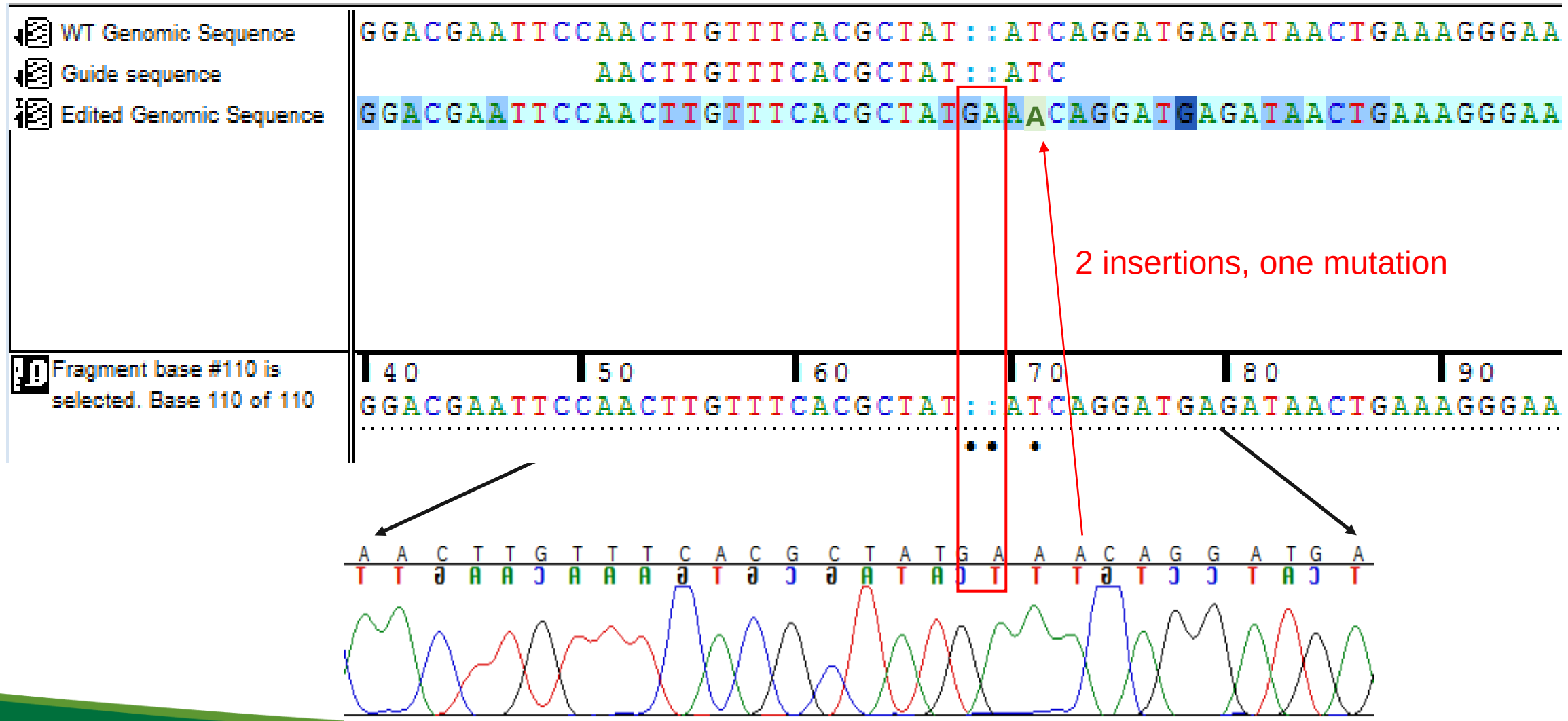
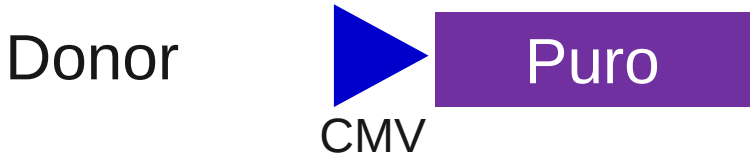
0.75 kb PCR Fragments have Indels



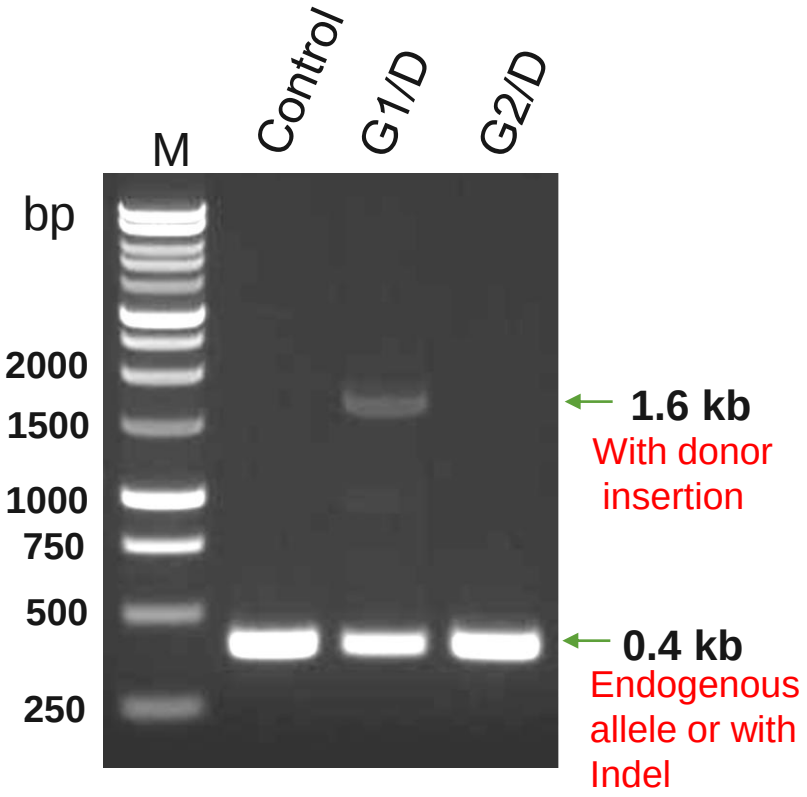
Human SHMT1 Knockout using KN2.0 in MIA PaCa-2

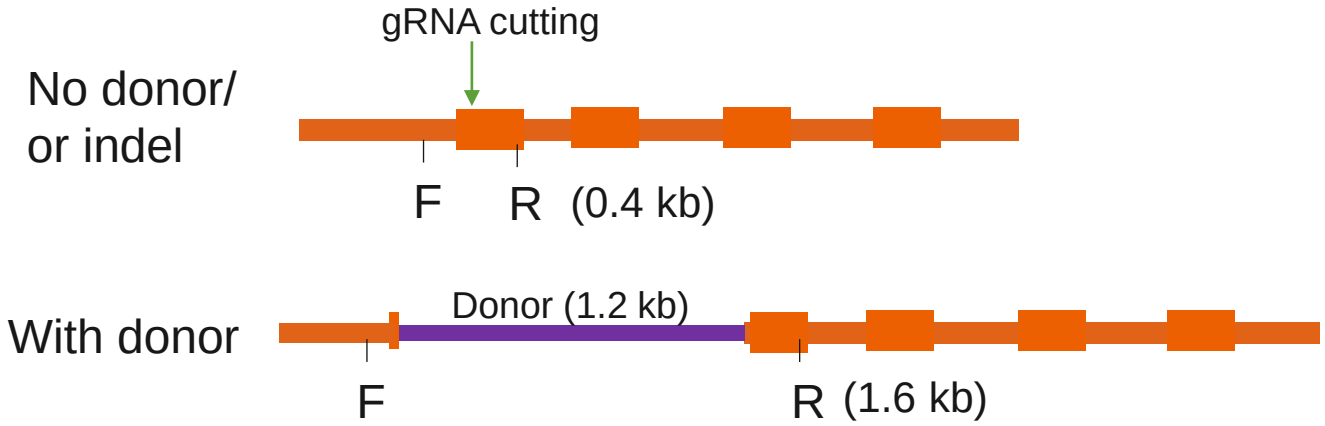
gRNA 1: TCAACATCACTGTCTTTGAG
gRNA 2: ACCACAGGTCAGCATCCTTG



This KN2.0 validation data are provided by a customer

Genomic PCR from Stable Pools

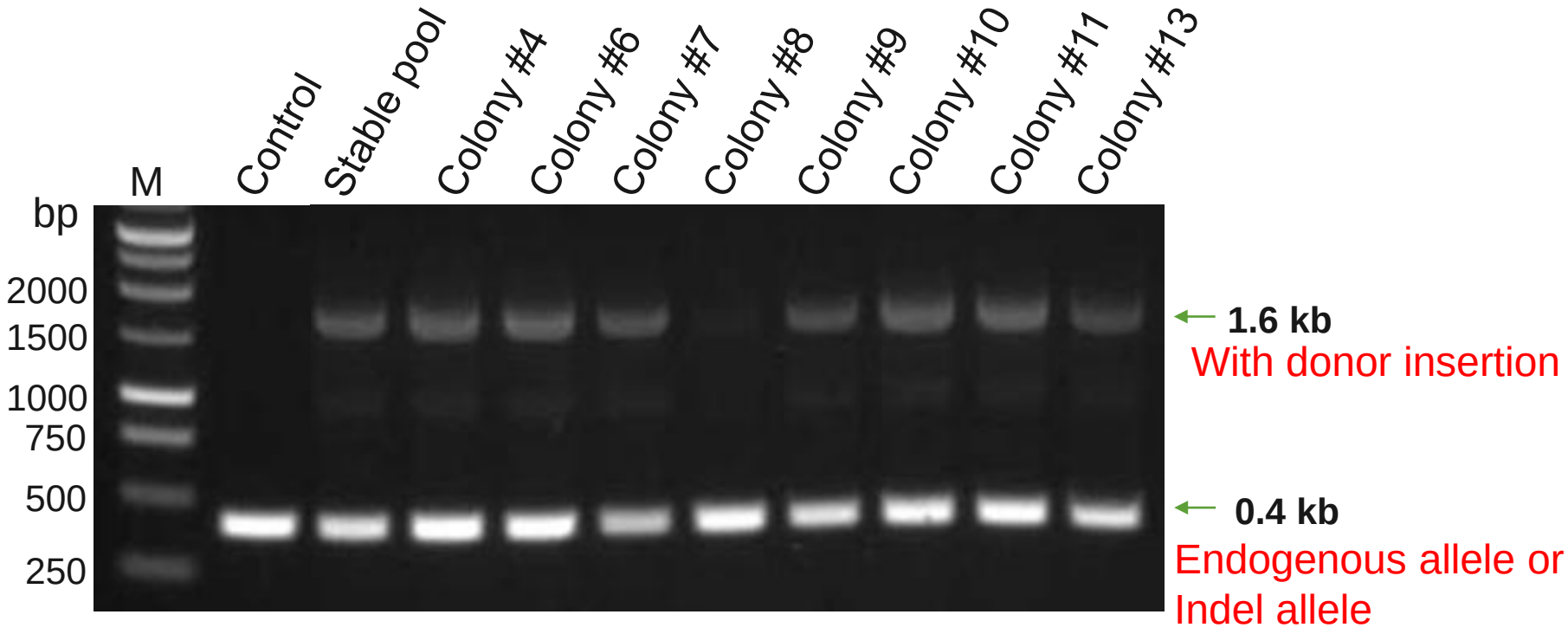




F: Forward primer
R: Reverse primer

MIA PaCa-2 cells were transfected with gRNA and donor DNA using lipofectamine 3000, selected with puromycin after passaging for 8 times. Genomic DNA was extracted from the stable pools and genomic PCR was performed using primers flanking the integration site (PCR fragment from endogenous locus will be 413 bp, with donor insertion will be 1.6 kb). Control DNA is from non-transfected cells.

7 out of 8 Single Cell Clones (87%) Have Donor Insertion



Single cell clones were isolated from gRNA 1 and donor transfected MIA PaCa-2 stable pools.
Genomic PCR was performed using primers flanking the integration site.
Control DNA is genomic DNA from non-transfected cells.

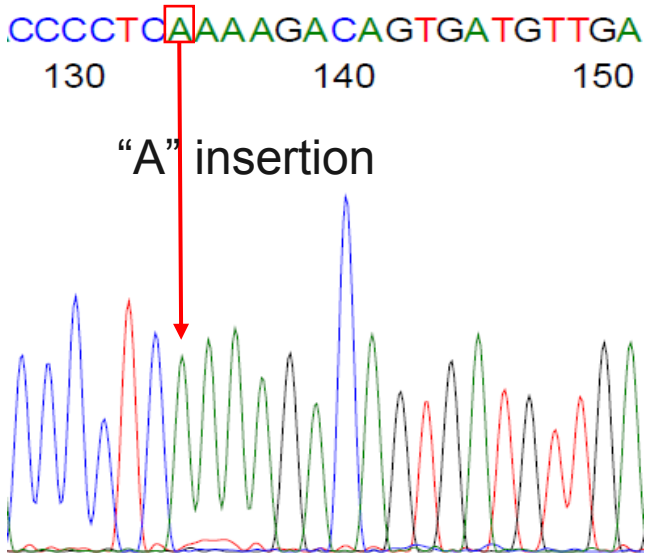
The 0.4 kb Fragment Has Insertion of “A”

TGGAAGCTACACATGTTTTTCCCATTTTTTTAGGCAGCTTCGAACCAGTGCAATGACGATGCCAGTCAACGGGGCCC
ACAAGGATGCTGACCTGTGGTCCTCACATGACAAGATGCTGGCACAACCCCTCAAAGACAGTGATGTTGAGGTGAGA
TTTTTGGGGTCTTCACAGATTTTTTTATGTTGGAGGCCTTCATTTAATCTTTAGTTCTAATTACAAATTAATTAGGG
ACAGCCTTGAAATGAGTATTATCCTGCTGGATTTAGAGGTGGTGGCAGACAAAATGGCTACAAATCCTTTGAGGGTA
AATTTAAAGATTGCTGGGTTTCTTCAAAGTGTGGAGCACTGTGCAGGGTGCTCTGGAGAAAAGCGAGAAGGGGAGAG
ATCACTGACAGTTGACCCTTTCCTGACA

PAM Indel site

F-primer TGGAAGCTACACATGTTTTTCCC
R-primer ACAGTTGACCCTTTCCTGACA

gRNA1 CTCAAAGACAGTGATGTTGA
gRNA2 CAAGGATGCTGACCTGTGGT



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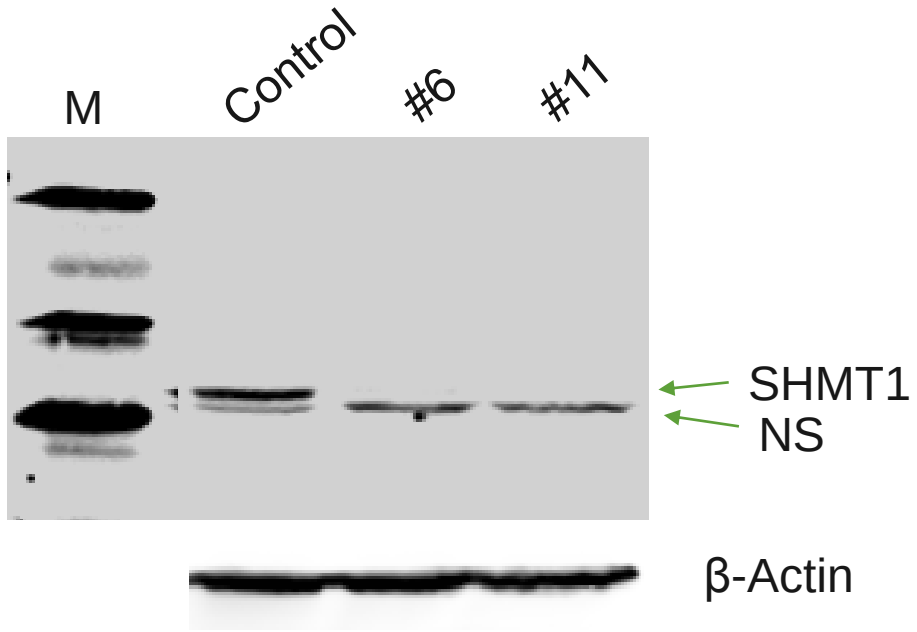
SHMT1 Biallelic Knockout was Confirmed on WB



Single clones, #6 and $11, and non-transfected MIA PaCa-2 cells (control) were subjected to WB with SHMT1 polyclonal antibody. NS- non-specific band.
The same blot was blotted with β -actin antibody as sample loading control.

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