Combined CRISPR - TARGATT™ Gene Editing Generates High Efficiency Knock-out, Point Mutation and Large Fragment Knock-in in Human iPS Cells

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Introduction

Gene editing in iPSCs offers great promise for personalized therapy in regenerative medicine

- CRISPR works most efficiently for gene knockout through the non-homologous end joining (NHEJ) pathway, and for point mutation/ correction through homology directed repair (HDR).
- CRISPR efficiency for large fragment DNA knock-in through nuclease-mediated HDR is very low.
- Applied StemCell's TARGATT™ integrase-based system can successfully knock-in large fragment DNA in iPSCs (up to 22 kb)
  - Site specific gene insertion with high efficiency (up to 40%)
  - Transgene integration happens at a pre-selected, transcriptionally active safe-harbor locus
- **Combined CRISPR - TARGATT™ technology broadens scope and efficiency of gene editing in human iPS cells**

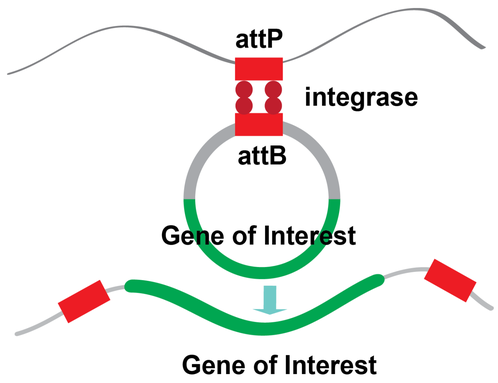
Materials and Methods

- Human iPS cells (ASC Cat# ASE-9202)
- CRISPRCLEAR™ hROSA26 Safe Harbor Knock-in Kit (ASC Cat# ASK-7041)
- Electroporation using Life Technology Neon® Transfection System

CRISPR & TARGATT™ Genome Editing Technologies

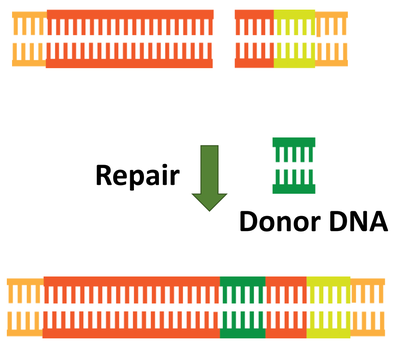
TARGATT™

Large fragment knock-in



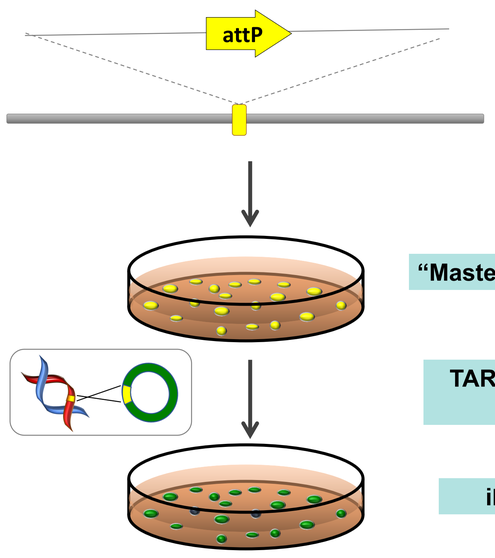
CRISPR/Cas9

Gene knock-out
Point mutation
Small DNA insertion



TARGATT™ Integrase System

Site-specific Gene Knock-in in iPSCs



Master iPSC line
TARGATT™ attP "docking" site in preselected "Safe Harbor" genomic locus using CRISPR

"Master iPSC line" with TARGATT™ attP site

TARGATT™ vector with gene of interest, eg,GFP

iPSCs expressing gene of interest

Site-Specific Knock-in Technology
TARGATT™

CRISPRCLEAR™

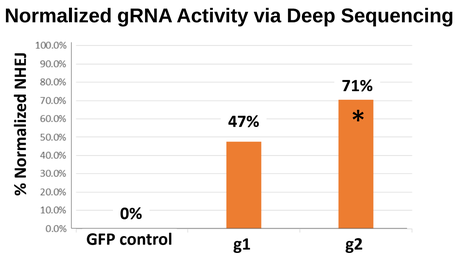
Knockout Mutation in Six Isoforms of a Gene in Human iPSCs

A

```
CTGATTCTGGGTGCCACCTGGATTTATTTGGGGTGTGATATTGACCCAATTTTCTGGAAATAACAATATATAGAA
AATTAGGGGATAGATTCTTTATCTTATGAGGGTTTGGGCAAGTCAAATACTTAGTTTCTGAGCCTTATTTTGTCT
CTAGAGCAAGAAAACTGTAAATTAGGCAGCCGGTGAGGGGCCCTCACAGCTTGGCTGTGGGTTCAGGACACCAG
ATTTCTACCACTCACTTTCTTCTTACTTCTCCTGCACAGGGATCATGGCCCAAGTTGCAATGTCCACCCTCCCCG
TTGAAGATGAGGAGTCCTCGGAGAGCAGGATGGTGGTGACATTCCTCATGTCAGCTCTCGAGTCCATGGTGAGGC
CTTCTGTTCCATCATTCCATAGTTGGGTAGGCCTGCACTGTAGATAAGGTTGATTTGTTTTTGTGGAAGATAGAA
TTTTATGGTTTTTAGTTTTAATGAGTACTTTTTCTTTACTTTTTTTTTTTTTTTTTTTTTTTTGAGACGGAGTCT
TGCTCTGTTGCCCAGGCTAGAGTGCAGTGGCGAGATCTTGGCTCACTGCAACCTTTACCTCCCGGGTTCGAGCGA
TTCTCCTGCCTTAGCCTTCCGAGTAGCTGGGATTACAGGCGCCTGCCACCACACCTGGCTAATTTTTTGTATTTT
```

Targeted Exon | Start Codon | Selected gRNA

B



C

Six Isoforms of the gene of interest shared the same ATG start codon in targeted exon. (A) gRNA target site in gene of interest. (B) Deep sequencing was used to identify the best gRNA candidate, g2 with lowest off-target and highest on-target efficiency. (C) Co-transfection of gRNA and Cas9 in hiPSCs resulted in an **8 bp deletion** in one allele and a **1 bp insertion** in second allele at the DSB site, resulting in premature stop codons in all six isoforms of the gene of interest.

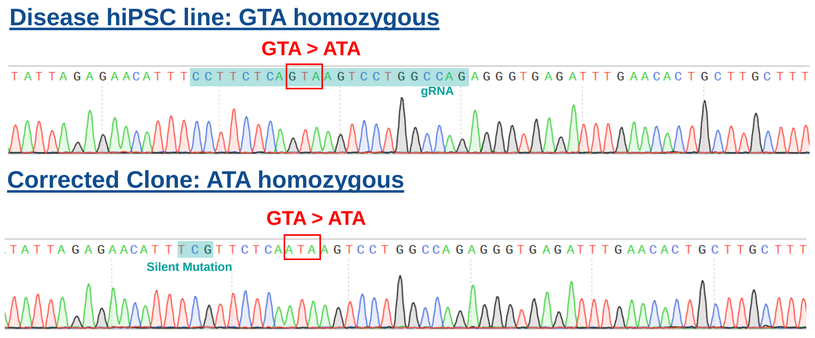
Point Mutation Correction in Human Disease iPS Cell Line

A

```
AAGTGACAAATAGCAAGTGTTGCATTTTACAAGTTATTTTTTAGGAAGCATCAAACTAATTGTGAAATTGTCTGC
CATTCTTAAAAACAAAAATGTTGTTATTTTTTATTTCAGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACA
TGCCAACAGAAGGTAAACCTACCAAGTCAACCAAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTATTG
AGAATTCACACGTGAAGAAAGATGACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAAT
ACACAGAAGGTGGAAATGCCATATTAGAGAACATTTCCTTCTCAGTAAGTCCTGGCCAGAGGGTGAGATTTGAAC
ACTGCTTGCTTTGTTAGACTGTGTTCAGTAAGTGAATCCCAGTAGCCTGAAGCAATGTGTTAGCAGAATCTATTT
```

Targeted Exon | Targeted Codon | Selected gRNA

B

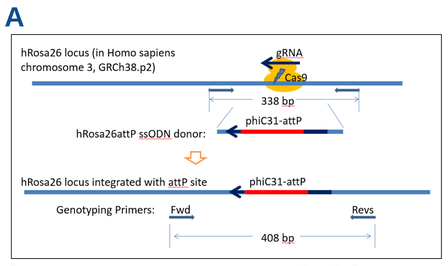


Homozygous point mutation correction in a disease human iPS Cell Line. (A) gRNA selection strategy in gene of interest. (B) Sequence chromatogram confirming **the GTA > ATA point mutation correction** in the disease cell line. The selected gRNA was transfected into patient cell line along with an ssODN containing the corrected wild type codon. Shown is a homozygous clone with the corrected mutation.

Combining CRISPR & TARGATT™ to Enable Large DNA Insertion

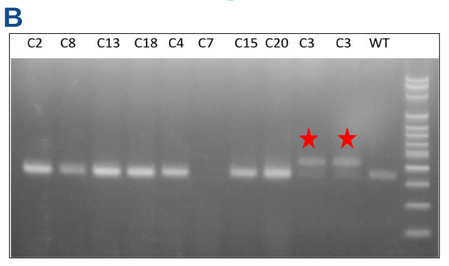
TARGATT™ Master Cell Line: Using CRISPR to Insert a "docking site"

A



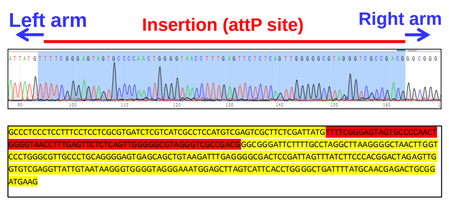
Strategy to generate attP iPS cell line

B



Clone C3 shows a heterozygous insertion

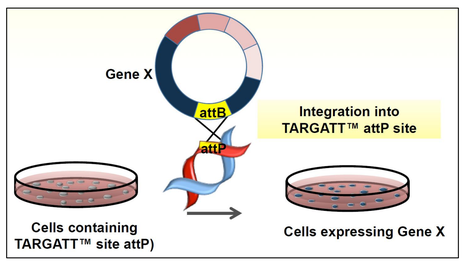
C



Sequencing of clone C3 shows insertion of 70 bp attP docking site in Rosa26 locus (red)

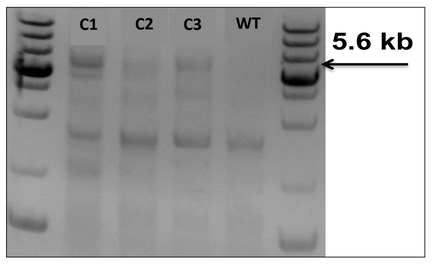
TARGATT™ Master Cell Line: Large DNA Insertion

A



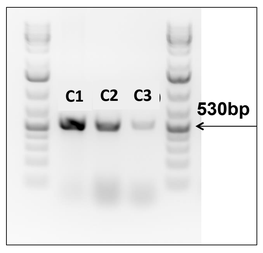
Schematic illustration of the integrase based knock-in in hiPS cells

B



PCR Gel electrophoresis to confirm insertion of 5.6 kb fragment in TARGATT™ iPS Master cell line; WT: wild type.

C



Junction PCR gel electrophoresis to confirm knock-in in Rosa26 locus

Conclusions

- Our data show that CRISPR can enable to generation of knockout and point mutation human iPS cell line models.
- Applied StemCell's TARGATT™ technology enables efficient knock-in of large DNA fragments in iPSCs at the preselected Rosa26 locus.
- Combining CRISPR & TARGATT™ technologies provides a valuable new platform for efficient genome editing in human iPSCs.

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