TARGATT™: Rapid and Efficient Transgene Integration Technology to Develop Large Mammalian Cell-Based Screening Libraries

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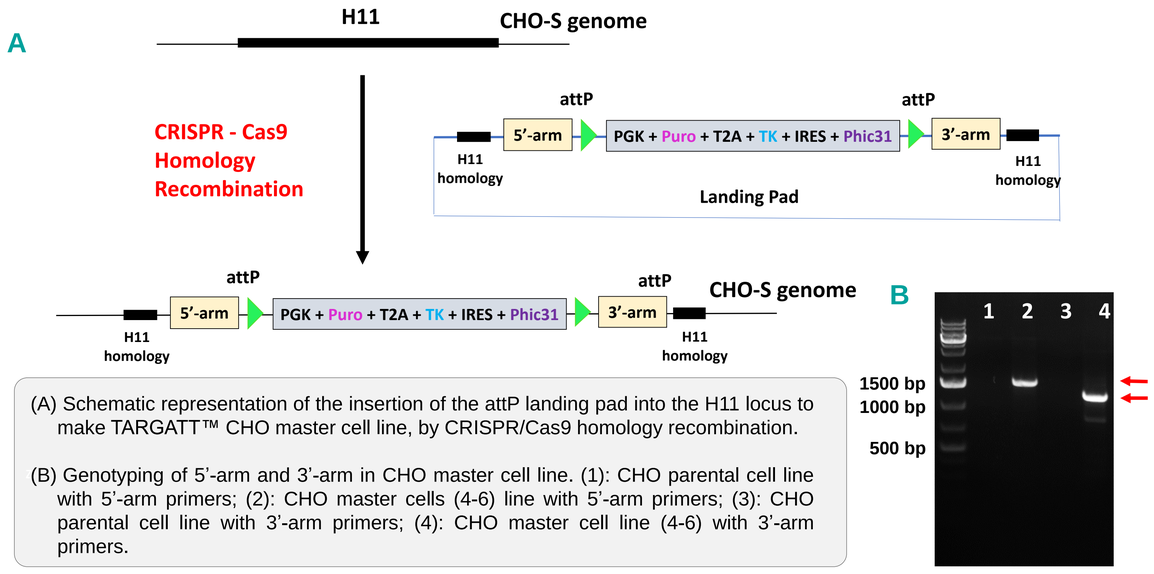
ABSTRACT

- Mammalian cell-based libraries offer an affordable and physiologically relevant context for high throughput screening.
- Current technologies to develop mammalian cell display libraries: (1) result in cells with multiple copies of genes; (2) are inefficient to prepare large library screens; (3) may carry vector backbone.
- The TARGATT™ site-specific, integrase-mediated gene integration technology provides an efficient strategy for integrating a gene of interest into a preselected, transcriptionally active locus:
  - 1:1 variant-to-cell ratio: a single cell containing a single copy of transgene inserted at a single docking site.
  - Very efficient, uniform transgene expression
  - Reproducibility for constructing large isogenic cell libraries for rapid, high throughput screening.
- We engineered a TARGATT™-HEK293 and TARGATT™-CHO Master Cell line containing an integrase recognition landing pad in the ROSA26 and Hipp11 safe harbor locus in HEK293 cells and CHO cells, respectively; and in combination with an HSV-TK/GCV negative selection or promoter-less reporter gene to enrich for cells that have library plasmid integrated.
- These master cell lines showed a > 12% knock-in efficiency without selection; with selection, virtually 100% efficiency can be achieved.
- The TARGATT™ Master Cell Lines thus enables stable cell line generation, and 4-8x larger library sizes than currently available technologies. The TARGATT™ technology is an efficient system for creating large cell-based library screens for applications in directed evolution (such as vaccine development, drug screening), genome wide screening, and biotherapeutic drug discovery and biomanufacturing.

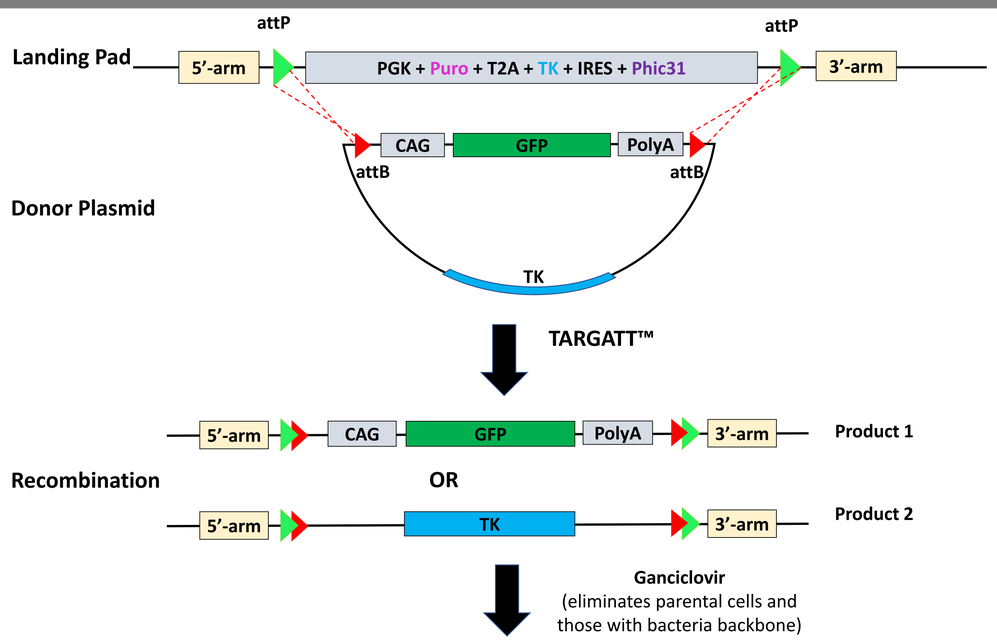
CHO-S Mater Cell Line Generation: Insertion of Landing Pad into H11 Locus and Genotyping



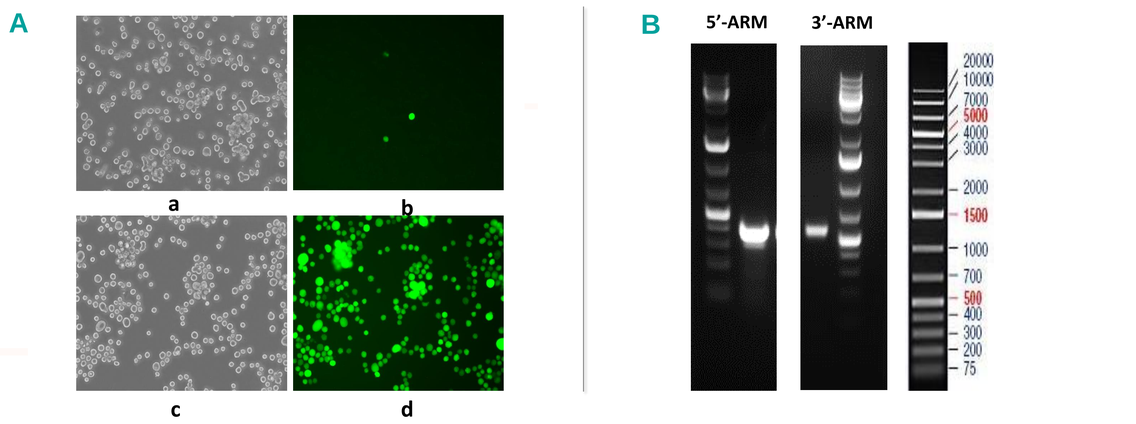
(A) Schematic representation of the insertion of the attP landing pad into the H11 locus to make TARGATT™ CHO master cell line, by CRISPR/Cas9 homology recombination.

(B) Genotyping of 5'-arm and 3'-arm in CHO master cell line. (1): CHO parental cell line with 5'-arm primers; (2): CHO master cells (4-6) line with 5'-arm primers; (3): CHO parental cell line with 3'-arm primers; (4): CHO master cell line (4-6) with 3'-arm primers.

TARGATT™ System: Site-Specific Integration

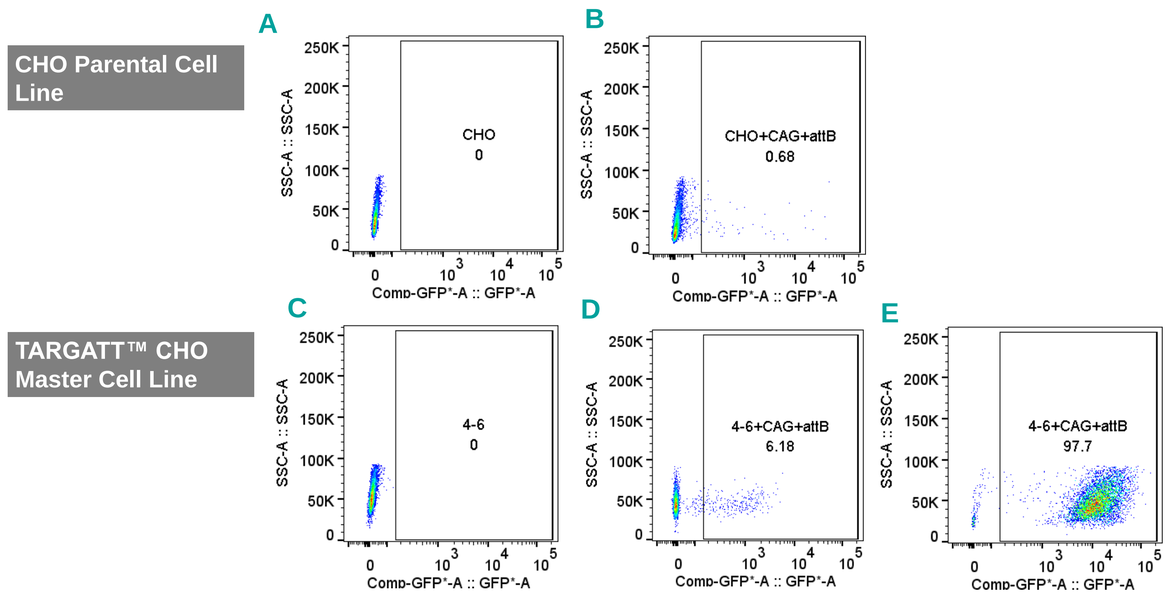


TARGATT™ CHO Master Cell Line Evaluation: Uniform Integration of Gene of Interest in H11 locus



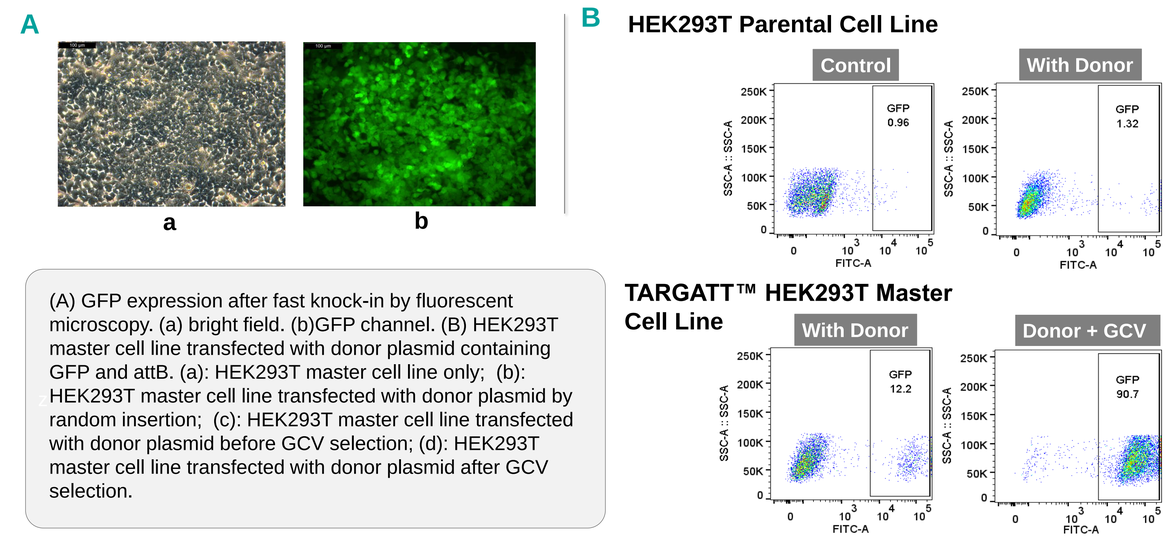
(A) GFP signal was detected by fluorescence imaging after transfection with donor construct by random insertion in bright field (a) and GFP channel (b), and TARGATT™ integration plus GCV selection in bright field (c) and GFP channel (d). (B) Genotyping of knock-in cell line with 5'-arm and 3'-arm primer sets to confirm the correct recombination of attP and attB sites. PCR analysis shows the expected DNA product sizes on agarose gel.

TARGATT™ CHO Master Cell Line Evaluation: High Integration Rate of the Gene of Interest



FACS analysis of GFP expression level in CHO cells. (A) CHO parental cell line only; (B) CHO parental cell line randomly transfected with donor plasmid; (C) CHO master cell line only (4-6); (D) CHO master cell line (4-6) transfected with donor plasmid before GCV selection; (E) CHO master cell line (4-6) transfected with donor plasmid after GCV selection.

High Integration Rate of Gene of Interest in TARGATT™ HEK293T Master Cell Line



(A) GFP expression after fast knock-in by fluorescent microscopy. (a) bright field. (b)GFP channel. (B) HEK293T master cell line transfected with donor plasmid containing GFP and attB. (a): HEK293T master cell line only; (b): HEK293T master cell line transfected with donor plasmid by random insertion; (c): HEK293T master cell line transfected with donor plasmid before GCV selection; (d): HEK293T master cell line transfected with donor plasmid after GCV selection.

Comparison of Lentivirus and TARGATT™ Mammalian Cell Libraries

	Lentivirus	TARGATT™
Site-specific gene insertion	No	Yes
Protein level	Varies	Consistent
Size of inserted gene	Limited	Less Limited
Selectable marker	Depends	No
Cost	High	Low
Time	Long	Short
Copy no. of inserted gene	Varies	Single Copy

Potential Applications in Antibody Discovery

scFv library screening

Bioprocessing

Antibody engineering

Off-target screening with membrane protein library

Other Library Screening:
- CAR efficiency, specificity and safety screening
- Discover novel immune targets, checkpoints
- Ion channels
- GPCR

Protein Evolution
- Enzyme activity and specificity
- AAV capsid specificity and efficiency

CONCLUSION

- An efficient site-specific integration (TARGATT™) coupled with short-term HSV-TK/GCV negative selection system was developed for precise and stable gene insertion in HEK293T & CHO-S cell lines.
- H11 locus was newly identified as a safe harbor site for target gene knock-in in CHO-S genomic DNA.
- The TARGATT™ plus HSV-TK/GCV negative selection system was successfully validated in the TARGATT™ Master Cell Lines.
- The system provides a robust and efficient integration platform for generating a uniform cell population with stable transgene expression. This platform paves the way for homogeneous expression of GOI and subsequent biotherapeutic protein screening and production.

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2018-ADC