# TARGATT™: A Robust System for Site-Specific Integration of Transgenes in Mammalian Cells

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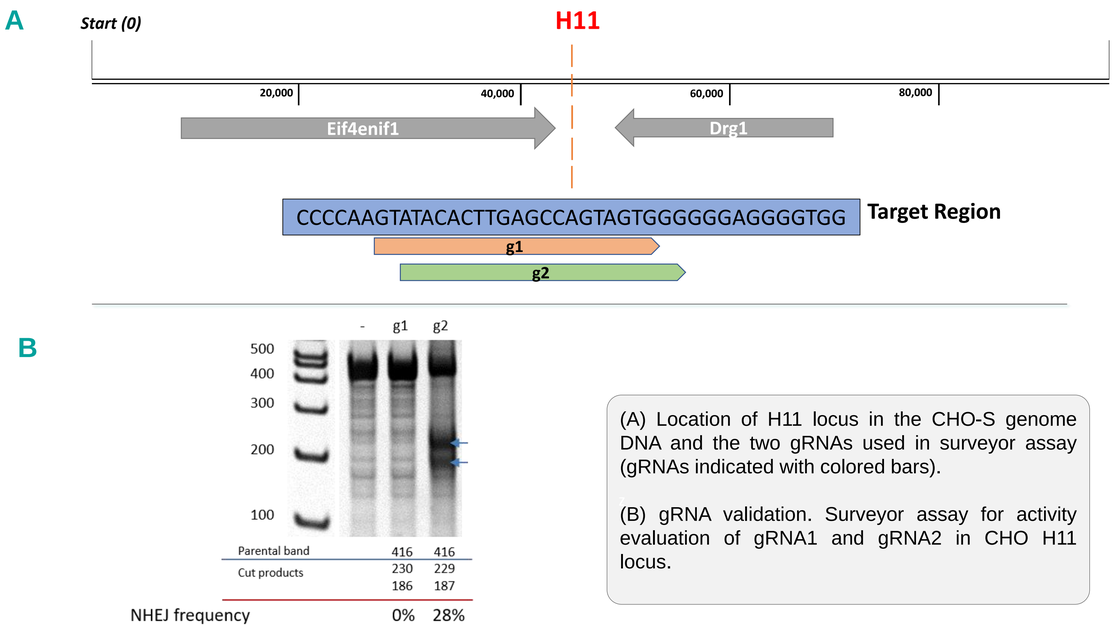
## ABSTRACT

Mammalian expression system had been the major sophisticate host for biotherapeutic production. However, development of recombinant mammalian cell lines has been restrained due to unstable and variable transgene expression caused by random integration. Here, we introduced an efficient strategy of integrating a gene of interest (GOI) by site-specific integration, TARGATT™, followed by HSV-TK/GCV negative selection. This method has enabled rapid and precise insertion of a gene expression cassette at a defined loci in CHO-S cells, resulting in homogeneous transgene expression. The integrate efficiency can reach up to 97.7% in CHO-S cells based on the FACS analysis of the GFP gene as a reporter. The efficacy of this system was further validated in HEK293T cells. Taken together, the results displayed here enables the application of an accurate and cost-effective protocol for recombinant cell lines generation and consistent protein production, as a potential valuable approach for therapeutic biomanufacturing.

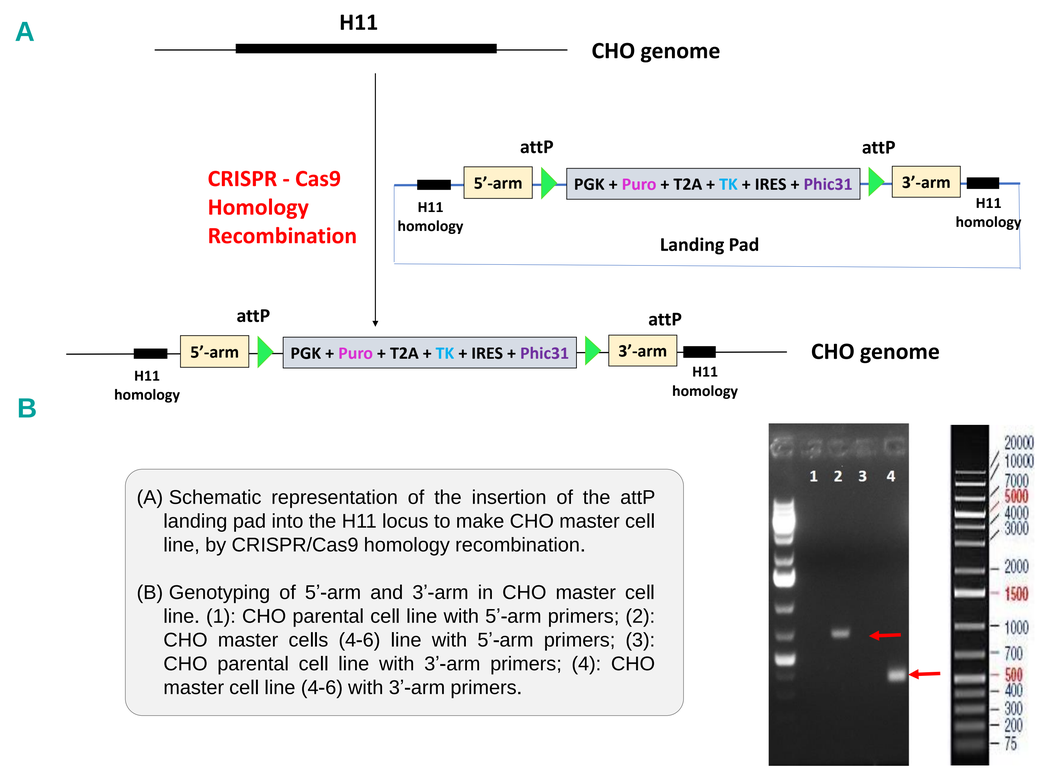
## Identification of H11 locus in CHO-S genomic DNA




(A) Location of H11 locus in the CHO-S genome DNA and the two gRNAs used in surveyor assay (gRNAs indicated with colored bars).

(B) gRNA validation. Surveyor assay for activity evaluation of gRNA1 and gRNA2 in CHO H11 locus.

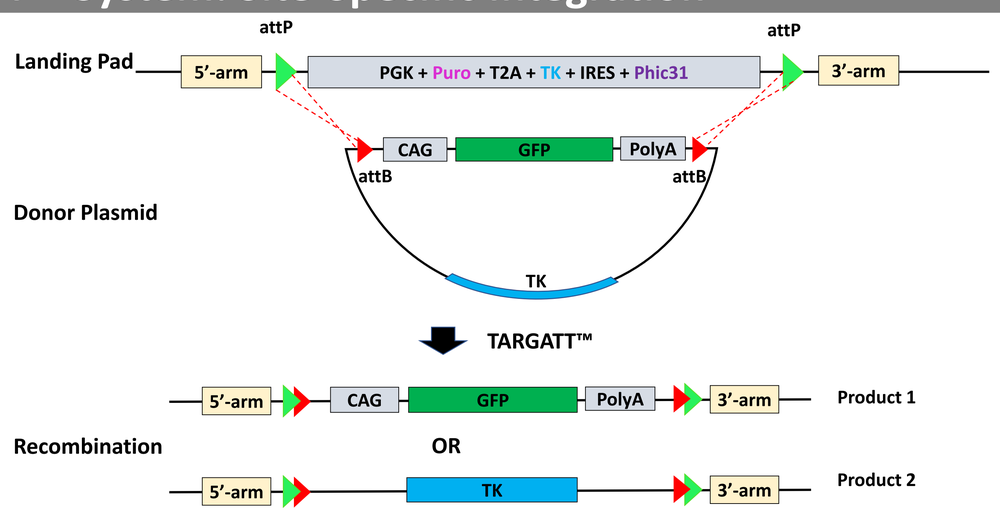
## CHO-S Mater Cell Line Generation: Insertion of Landing Pad into H11 Locus and Genotyping



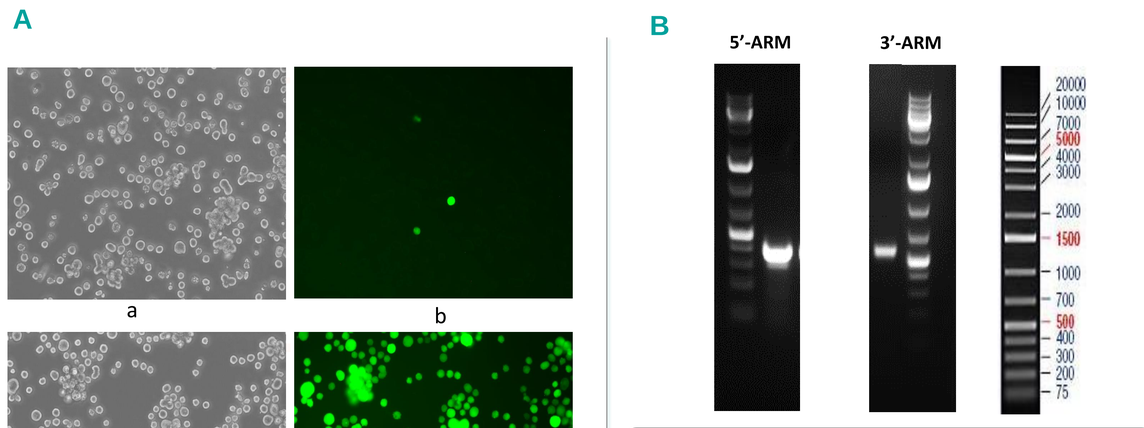

(A) Schematic representation of the insertion of the attP landing pad into the H11 locus to make CHO master cell line, by CRISPR/Cas9 homology recombination.

(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

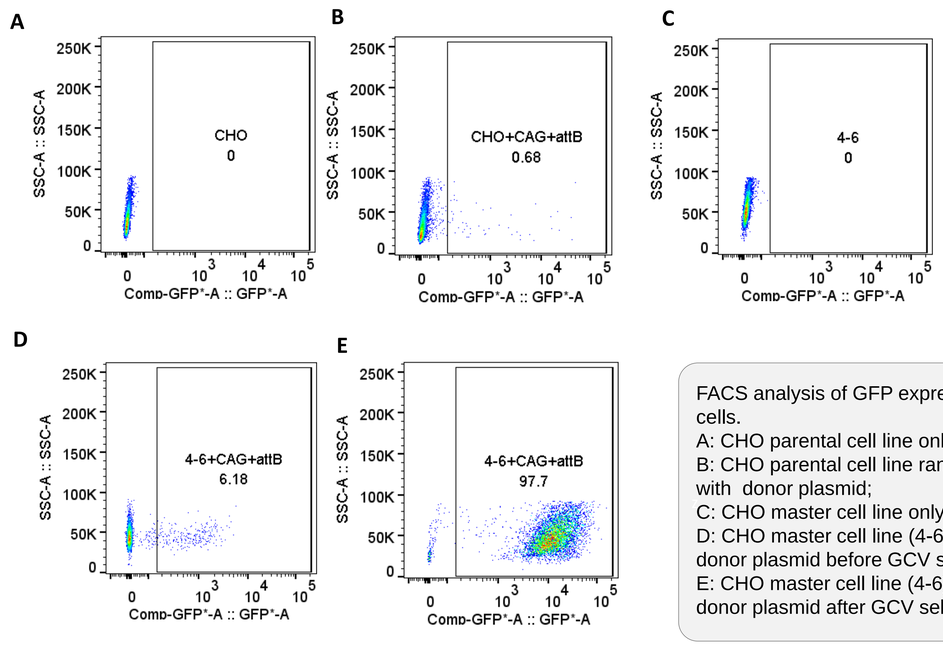



## Analysis of TARGATT™ Knock-in Cell Line by Fluorescent Microscopy and Genotyping



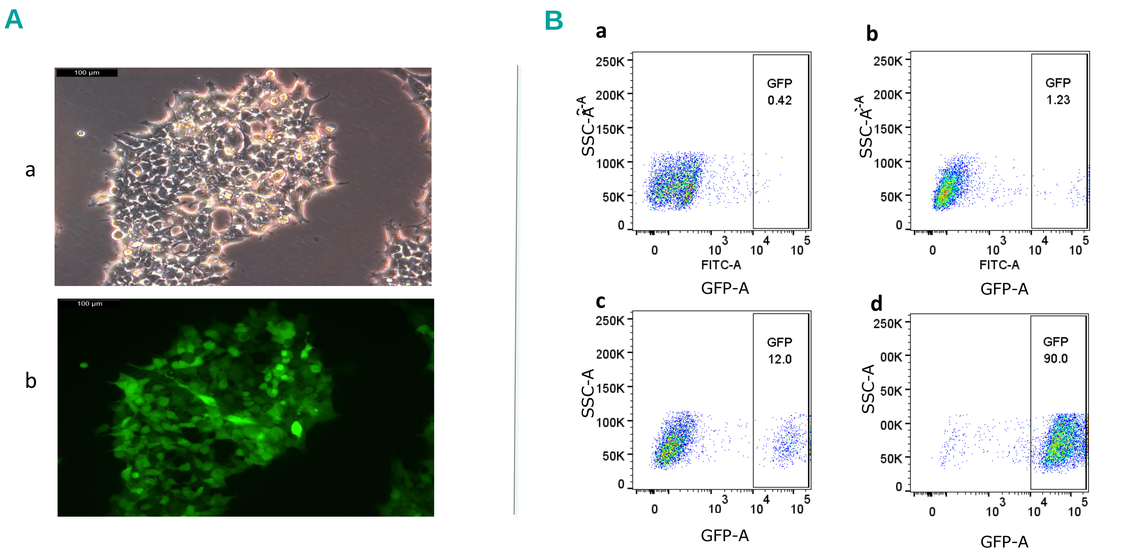

(A) GFP signal was detected by fluorescence imaging after transfection with donor construct by random insertion in bright field (a) and GFP channel (b), and TARGATT™ integration plus GCV selection in bright field (c) and GFP channel (d). (B) Genotyping of knock-in cell line with 5'-arm and 3'-arm primer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

## Analysis of TARGATT™ Knock-in Cell Line by FACS




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.

## TARGATT™ Knock-in into 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.

## 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 CHO-S cell line.
- 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 HEK293T cell.
- The system provides a robust, fast 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 production.