Identification of H11 locus in CHO-S genomic DNA

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. (A) CHO parental cell line with 5-arm primers; (B) CHO master cell line (4-6) with 5-arm primers; (C) CHO parental cell line with 3-arm primers; (D) CHO master cell line (4-6) with 3-arm primers.

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

A Schematic representation of recombinant cell lines generation and consistent protein production as a potential valuable approach for therapeutic biomanufacturing.

B 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). Genotyping of knock-in cell lines with 5- arm insertion site primer and site-specific recombination at the attP and attB sites with RT-PCR analysis shows the expected DNA product sizes on agarose gel.

Analysis of TARGATT™ Knock-in Cell Line by FACS

A 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 signal was detected by fluorescent microscopy 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). Genotyping of knock-in cell lines with 5- arm insertion site primer and site-specific recombination at the attP and attB sites with RT-PCR analysis shows the expected DNA product sizes on agarose gel.

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.