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ACCELERATED HOMOLOGY-DIRECTED TARGETED INTEGRATION OF TRANSGENES IN CHO CELLS VIA CRISPR/CAS9 AND FLUORESCENT ENRICHMENT

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Development of recombinant CHO cell lines has been hampered by unstable and variable transgene expression caused by random integration. With draft genome of several CHO cell lines and targeted genome editing technologies, rCHO cell line development based on site-specific integration has the potential to overcome the limitations of clonal heterogeneity. In a previous study, we demonstrated efficient and precise targeted gene integration in CHO cells using CRISPR/Cas9 technology and homology-directed repair (HDR) pathway¹.

However, it requires a selection process, which limits targeted integration of multiple transgenes at multiple sites due to a limited number of selection markers and a lengthy selection process.

Here, we improved the targeted integration platform by applying fluorescent enrichment of transfected cells. The improved system is based on a fluorescent protein A linked Cas9 together with sgRNA towards the integration site and donor DNA harboring a fluorescent gene B outside homology regions. Involvement of fluorescent markers in constructs confers FACS enrichment of cells transfected with both Cas9 and donor DNA.

Simultaneous introduction of GFP 2A peptide-linked Cas9 and sgRNA expression vectors together with donor plasmid has enabled precise targeted integration of large transgenes encoding model proteins including antibody, following transient expression and FACS enrichment. Subsequent selection of non-fluorescent clonal cells allowed for excluding cells with randomly integrated donor DNA. In this way, we not only shortened the clone screening time, but also generated clonal CHO cell lines with site-specific, marker-free (no antibiotic selection needed), and clean (no unwanted DNA present) targeted integration of GOI. Further improvement in targeted integration efficiency was additionally assessed by chemical treatment toward cell cycle arrest or nonhomologous end joining inhibition combined with fluorescent enrichment.

Taken together, the present platform has the huge potential to accelerate targeted generation of stable production CHO cell lines in a rational way.

Reference

1) Lee, J. S., Kallehauge, T. B., Pedersen, L. E., Kildegaard, H. F., Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway. *Sci. Rep.* 2015, 5, 8572.