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Cre-*loxP*–controlled cell-cycle checkpoint engineering in Chinese hamster ovary cells

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The gene amplification system is widely used in Chinese hamster ovary (CHO) cells for the productive cell line construction of therapeutic proteins. To enhance the efficiency of conventional gene amplification systems, we previously presented a novel method using cell-cycle checkpoint engineering[1)]. Here, we constructed high-producing and stable cells by the conditional expression of mutant cell division cycle 25 homolog B (CDC25B) using the Cre-*loxP* system[2)]. A bispecific antibody-producing CHO DG44-derived cell line was transfected with floxed mutant CDC25B. After inducing gene amplification in the presence of 250 nM methotrexate, mutant CDC25B sequence was removed by Cre recombinase protein expression. Overexpression of the floxed mutant CDC25B significantly enhanced the efficiency of transgene amplification and productivity. Moreover, the specific production rate of the isolated clone CHO Cre-1 and Cre-2 were approximately 11-fold and 15-fold higher than that of mock-transfected clone CHO Mock-S. Chromosomal aneuploidy was increased by mutant CDC25B overexpression, but Cre-1 and Cre-2 did not show any changes in chromosome number during long-term cultivation, as is the case with CHO Mock-S. Our results suggest that high-producing and stable cells can be constructed by conditionally controlling a cell-cycle checkpoint integrated in conventional gene amplification systems.

1) Lee,KH et al., Appl. Microbiol. Biotechnol., **97:** 5731-5741(2013).
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