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Varied productivity according to the differences between targeted locations of antibody expression vectors in Chinese Hamster Ovary cells

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Chinese hamster ovary (CHO) cell lines are widely used in the pharmaceutical industry to produce therapeutic antibodies. However, trial and error cell selection methods are still used to construct high-producing cell lines. Exogenous genes are predicted to express differently depending on the expression vector integration sites. Chromosomal instability is one of the characteristics in CHO cells. We have previously constructed the CHO genomic bacterial artificial chromosome (BAC) library that is expected to cover entire the CHO-DG44 genome (Omasa *et al*., Biotechnol. Bioeng., 104, 986-994, 2009). The BAC-based physical map is a powerful tool to identify each chromosome and analyze chromosome rearrangement of CHO cell lines. According to the previous results, stability of each chromosome in a CHO cell differs. In this study, we constructed antibody producing cell lines using gene-targeting methods, and investigated the effect of targeting sites differences on the protein production.

IgG1 expression vectors and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 (CRISPR-associated proteins) vectors, which cut target sites, were co-transfected into CHO-DG44 cell line. The stably conserved chromosome and the other chromosome were selected as targeting sites. The targeting sequences were obtained from the CHO genomic BAC library: Cg0160L03 (Cao *et al*., Biotechnol. Bioeng., 109, 1357-1367, 2012) for the conserved chromosome; Cg0031N14, identified to contain exogenous gene amplified region with a large palindrome structure (Park *et al*., J. Biosci. Bioeng., 109, 504-511, 2010), for the other chromosome. The result showed that the specific antibody production rates were about 15 times higher in cell lines where the expression vectors were targeted into the conserved chromosomes than in the cell lines where expression vectors were targeted into the other chromosome. Our results indicated that the productivity varied according to the differences between targeted chromosomes.