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Mammalian cell lines used for manufacturing recombinant therapeutic proteins need to have high productivity, long term stable production and good product quality. The process of cell line development currently used in industry is based on random integration of the plasmid vectors into genome. Due to clonal variation, hundreds to thousands of clones are screened through multiple stages of evaluation. The whole process takes at least a few months and is extremely tedious. Targeted integration-based cell line development is attractive as the plasmid vectors integrate into predetermined active sites in genomes. All targeted cell lines have same genetic background and thus similar behavior. As such, the process of clone screening could be avoided. Targeted integration-based cell line development usually prefers integration of one recombinant gene copy into a single site as targeting one site is more efficient than targeting multiple sites. Single gene copy also avoids repeat-induced gene silencing and has lower risk of sequence variants than multiple copies. However, obtaining high productivity from a single gene copy is extremely difficult if not impossible. We have developed a FRT/FLP based cassette exchange strategy for efficient targeting of monoclonal antibody (mAb) genes into predetermined multiple sites in the CHO genome. Clones were first generated by random integration of an optimized tagging vector containing FRT sequences, a GFP reporter gene, and a stringent selection marker. Subsequent FACS screening was next carried out to identify master clones with high and homogeneous GFP expression. Homogeneous expression of a clone is an indicator for long term stable production. These identified master clones were further screened for their capacity to provide high expression in targeted cells using a targeting vector carrying an easily detectable DsRed gene. The top ranked master clones with inheritable high expression were finally tested for long term production stability and validated for generation of mAb producing cell lines. The targeting vector for mAb expression was designed to express the light chain and heavy chain at balanced ratios and optimized for enhancing the selection stringency of complete cassette exchange. All master clones that are able to provide high expression in targeted cells have multiple integration sites. The percentage of targeted cells with complete cassette exchange reached from 30% to 80% in targeted pools after drug selection depending on the master clones. The process from transfection to obtain high titer clones in shake flask takes about 6 weeks. The isolated targeted clones had mAb titer over 2 g/L in 14-day shake flask fed-batch cultures. Moreover, the targeted cell lines have low mAb aggregation (<1%), similar productivity, similar glycosylation, and stable production over 90 generations.