

DEVELOPMENT OF HIGH-PRODUCING CHO CELL LINES THROUGH TARGET-DESIGNED STRATEGY

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Productivity and stability are critical for the protein drug producing cell lines for manufacturing. Given that the integration sites of gene of interest (GOI) could contribute remarkable effect on the productivity and stability of GOI expression, we intended to develop a targeting-designed approach to generate the high-producing cell lines in a time-saving and less labor-intensive method through targeting the active and stable regions. To identify the active and stable regions located in CHO genome, two approaches were applied in our experiments. Firstly, the integration sites of GOI in cell clones developed by random integration were identified by whole genome sequencing. Secondly, we developed transposon-mediated low copy integration to discover novel active region located in CHO genome. It is interesting that the productivity per integrated GOI in cell clones developed by transposon system was more than two times to that in cell clones developed by random integration (random integration: 20-40 mg/L/copy; transposon-mediated integration: 40-140mg/L/copy). In addition, about 80% of cell clones developed by transposon system maintained the stability of antibody titer after culturing for 60 generations. These results implied that the potential active and stable integration region in the cell clones developed by transposon system. The identified integration regions could be applied for target integration. In order to verify the expression activity and stability of the integration sites, we employed CRISPR/Cas9 to specifically integrate the antibody gene into CHO genome for expression. Our data showed the cell pool generated by knock-in of expression vector into the IS1 integration site present higher expression titer than cell pools generated by integration into other sites or random integration. We further cultured the single cell clones derived from this cell pool by Clonepix and limiting dilution. These single cell clones have high expression titer ranging from 254 to 804 mg/L in batch culture of after 6 Days. A single cell clone(376 mg/L in batch culture) can reached 2 g/L in fed-batch culture. The stability analysis showed this clone maintain stable expression of GOI after 60 generation. Here, we demonstrated the generation of stable cell line with high protein expression by CRISPR/Cas9 mediated target integration. This approach will cost less time and labor than traditional method.