DEVELOPMENT OF A PLATFORM EXPRESSION SYSTEM USING TARGETED INTEGRATION IN CHINESE HAMSTER OVARY CELLS

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Key Words: Targeted Integration, Landing Pad, CHO

In recent years the biomanufacturing industry has seen significant improvements in recombinant protein production titers due to advancements in protein expression technologies as well as media, feed and manufacturing process development. However, the standard methods of recombinant cell line development have remained relatively unchanged. The majority of the biopharma industry introduces transgenes into Chinese Hamster Ovary (CHO) cells using mechanical or chemical transfection processes followed by metabolic or antibiotic selection of stable recombinant pools. Through this process, the transgene(s) are randomly integrated into the genome, often times resulting in significant heterogeneity within the stable pools. Individual recombinant CHO cells within the pools can vary greatly in their growth and productivity profiles, product quality attributes, and genetic stability. To isolate and identify the best performers, the time and resource consuming process of single cell clone generation and characterization is used, commonly requiring hundreds to thousands of clones to be characterized to find those suitable for manufacturing processes. In contrast, the use of targeted gene integration in cell line development programs will shorten timelines and reduce the burden of clone screening and characterization. The ability to integrate transgenes at a well-characterized and stable site will decrease heterogeneity in stable pools and lead to more consistent clone performance and product quality.

Targeted Integration can also enable researchers to perform specific modifications of glycosylation and metabolic pathways but this abstract will focus more on improving cell line development and manufacturing applications.

In this abstract we describe our strategy for developing a CHO expression platform that enables targeted and site specific integration of transgenes. We have generated clonal cell lines in which a landing pad has been randomly integrated into the CHOZN® genome at a low copy number. The landing pad contains a recombinant IgG expression cassette enabling us to screen for clones that support high and stable recombinant protein expression. Following this approach, we have identified and characterized several high expressing landing pad clones with performance characteristics suitable for commercial manufacturing processes. To bring these clones to the CHO industry we must first remove our IgG cassette from the landing pad and exchange it with a regulatory friendly and easy to screen GFP reporter. The landing pad was designed with Lox sites flanking the IgG cassette so that it could be excised using Cre Recombinase Mediated Cassette Exchange (RMCE). The landing pad itself remains integrated in the genome and acts as a placeholder for future site specific integration. Following another round of single cell cloning and characterization we can ensure that the original IgG cassette was cleared from the CHO genome and only the GFP reporter is expressed at the landing pad site.

Our team is currently testing all of the top clones that have been derived from this process to select a single clone that will be taken forward for commercialization. We are hoping that upstream biopharma teams will soon be able to integrate their clinical molecules into a landing pad site available in our CHO cell line. The ability to integrate a transgene at a single genomic locus should decrease off target effects and increase the homogeneity of the resulting pools, thus reducing the burden and time required for clone screening and stability studies. Biopharma teams using such a platform expression system will be able to generate more high producing clones with fewer resources and get pipeline molecules to clinic more quickly.