TRANSPOSASE-MEDIATED TRANSGENE INTEGRATION FOR RAPID GENERATION OF HIGH-PRODUCING STABLE CELL LINES

Lauren M. Kraft, Ph.D., Janssen R&D
Elizabeth Sorichillo, Janssen R&D
Thomas Kelly, Janssen R&D

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There is significant pressure in the biopharmaceutical industry to reduce timelines from DNA to IND, and a large portion of the timeline on the critical path is the cell line development (CLD) process. Traditionally for CLD, the method of transgene introduction into the genome of the CHO host cell has been random integration. This method has obvious downsides as there is not control over where the transgene integrates, so many transfectants integrate the transgene in regions of the genome that are not conducive to high, sustainable expression, usually resulting in low productivity and poor expression stability. This necessitates the inclusion of multiple time and resource-intensive steps in the CLD process to screen for clones that have high expression and long-term stability. A number of industry members have overcome the issues of random integration by developing targeted-integration strategies where the host cell line has a landing pad engineered into the genome that is then retargeted for transgene integration through recombinase mediate cassette exchange. This method also has its limitations as significant effort is required to generate this host cell line with a single copy landing pad in a region of the genome that can function as a suitable hot spot. High transgene expression typically correlates with high copy number and it has proven very difficult to identify regions of the genome where one copy of the transgene results in sufficiently high titer to support commercial manufacturing. The development of methods for multiple copy integrations at a single-landing pad or simultaneous utilization of multiple landing pads have similarly proven to be very difficult and time consuming. Finally, the growing prevalence of multi-chain biotherapeutics adds additional complexity to targeted integration strategies that must be overcome if a precise chain expression ratio is not known in advance.

The Janssen R&D Cell Line Development group has partnered with ATUM to evaluate the utility of a transposase-mediated transgene integration process for cell line development. In contrast to classical recombinases, transposase recognition sequences can be much shorter in length and are found thousands of times in any host cell. In addition, transposase tends to target open, active regions of the genome due to chromatin accessibility. For transposase-mediated transgene integration, the plasmid is cloned so that the transgene is flanked with the transposase recognition sequences, then a co-transfection of plasmid DNA and transposase RNA is performed, and this finally results in cutting-and-pasting of the transgene into the naturally occurring transposase recognition hot spots throughout the host cell genome. Integration copy number and chain ratio are easily tunable based on transfection and selection conditions. We have tested transposase-mediated integration with mAb and non-mAb projects and found that it can generate cell lines with very high productivity, good growth and viability, and with good expression stability. Furthermore, we have optimized and tested other vector components such as promoters and insulators to further improve copy number, titer, and stability. Not only do clones generated using transposase meet our strict criteria for manufacturing, but transfection pools also have high titers, high viability, and stability, allowing for large-scale protein generation earlier in the process to support other functions at Janssen. High-producing pools allow us to screen fewer clones in less time, so we have the capacity to generate cell lines for more molecules. We have determined that by incorporating transposase into our cell line development process, we can significantly shorten the overall cell line development timeline and reduce screening and resource requirements without compromising productivity or product quality in our production cell lines.