Development of recombinase-based targeted integration systems for production of exogenous proteins using transposon-mediated landing pads

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Background and novelty

Current methods for stably expressing recombinant protein therapeutics in CHO cells often rely on random genomic integration events which result in a widely heterogeneous cell population. Consequently, a significant portion of cell line development efforts involves extensive pool and clone screening to identify clones with high expression, growth, and product quality. Additionally, these integration methods preclude experiments such as variant library screening that involve stably overexpressing pools or libraries of molecules in a single cell culture. In these cases, it is difficult to determine whether differences in library member behavior (yield, degree of library enrichment, etc.) are due to true differences inherent to each variant or merely to due to variable genomic integration site(s).

Experimental approach

We developed two targeted integration systems that express high levels of recombinant protein in CHO cells. We first generated two clonal cell lines stably expressing enhanced green fluorescent protein (eGFP) reporter landing pads in genomic hot spots. Subsequently, three therapeutic protein molecules were used to test targeted integration, which were assayed for yield and productivity as well as characterized for landing pad copy number and integration fidelity by targeted locus amplification (TLA) and PCR. We additionally test for enrichment of cell subpopulations with fully saturated landing pads with ganciclovir (GCV) counterselection. Finally, we developed a combinatorial antibody library of 100 variants through random pairing of 10 unique light chains and 10 unique heavy chains. We transfected this library into a cell line containing a single copy landing pad wherein each cell line would express a single variant. We performed puromycin selection for cells that had successfully taken up one of the variants and cell-sorted for variants that successfully paired and expressed. We determined the identity of successful chain pairs with next generation sequencing.

Figure 1. Design of targeted integration systems. PhiC31-mediated integration and Cre-dependent recombinase-mediated cassette exchange (RMCE) were both evaluated in this work. Note that PhiC31-mediated integration in this case is a two-step process as compared to RMCE.

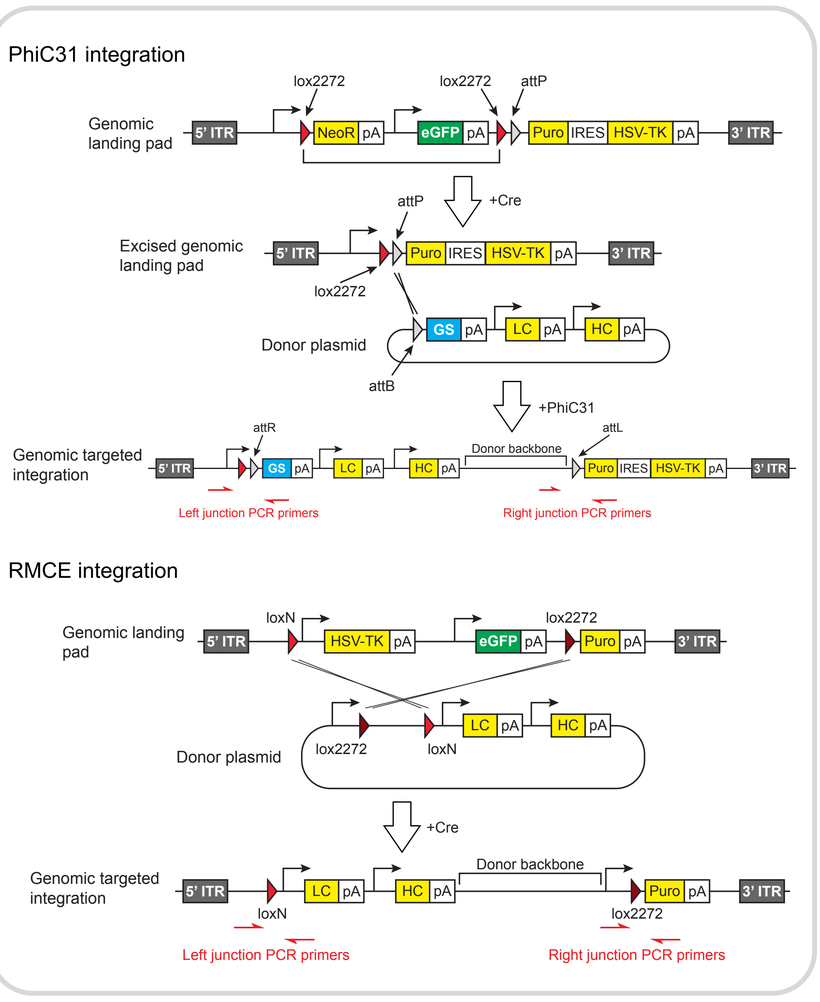


Figure 2. Serial sort enrichment of highly expressing cells and productivity of several therapeutic antibodies or Fc-fusion molecules without counterselection. GFP-expressing reporter landing pad cells were serially sorted to enrich for highly expressing cells. One RMCE clone and two PhiC31 clones (158 and 215) were isolated. Mean titer and productivity (qp) are shown for all three cell lines with various protein therapeutic molecules.

Figure 3. PCR and Sanger sequencing characterization of targeted integration landing pads. Junction PCR analysis was performed after PhiC31 landing pad reporter excision, PhiC31 integration, and Cre RMCE integration. Sanger sequencing confirmed the identities junction PCR products and the accuracy of donor integration.

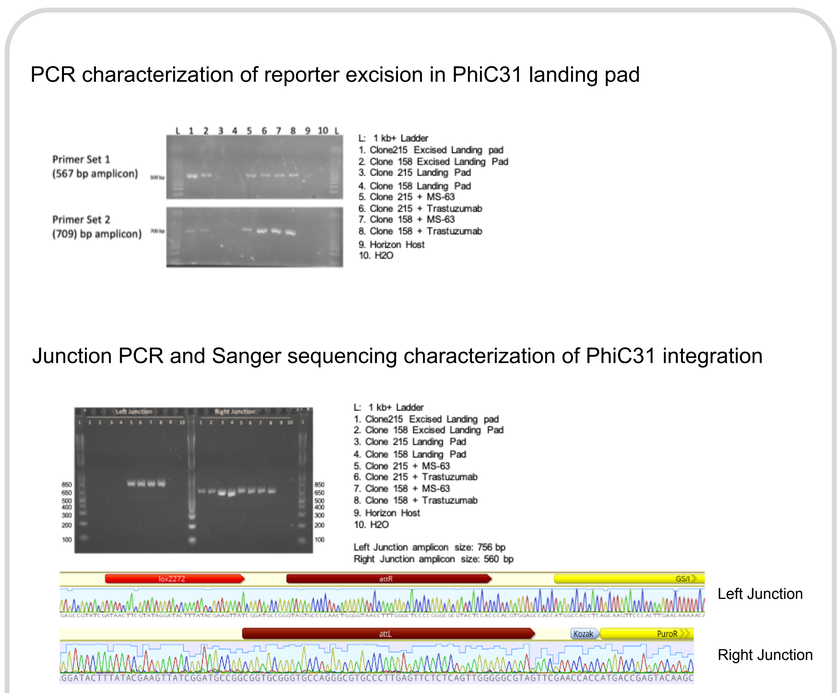


Figure 4. RMCE post-integration cells were counterselected with GCV to enrich for cells with all genomic landing pads integrated. Pre- and post-GCV selected cells show marked reduction of GFP⁺ cells as expected. Yield and productivity correspondingly increased after GCV counterselection.

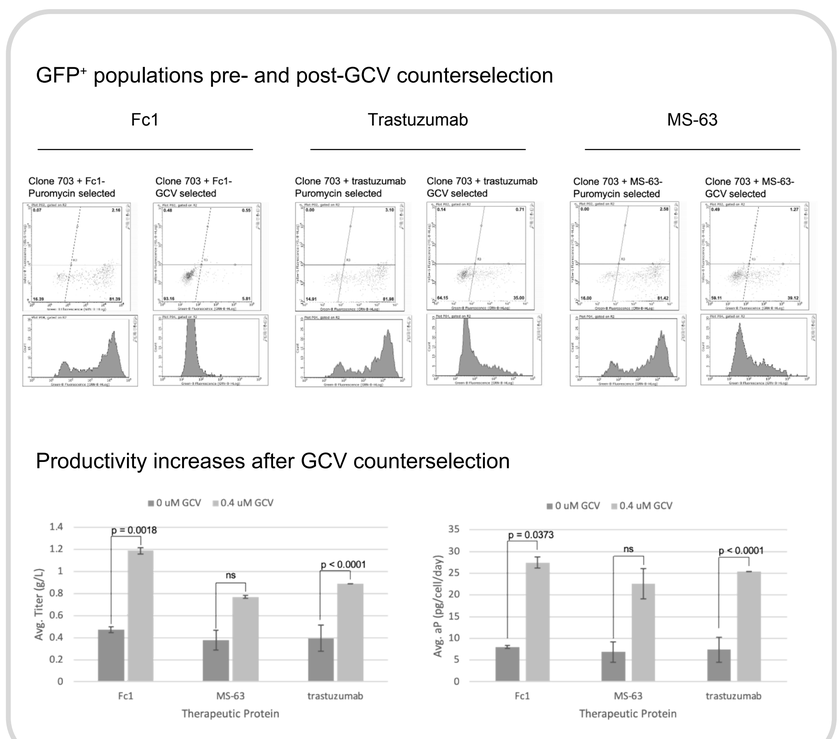


Figure 5. Proof of principle CHO display using single-copy RMCE cell line and fed-batch evaluation of individual library members using traditional non-targeted integration expression system.

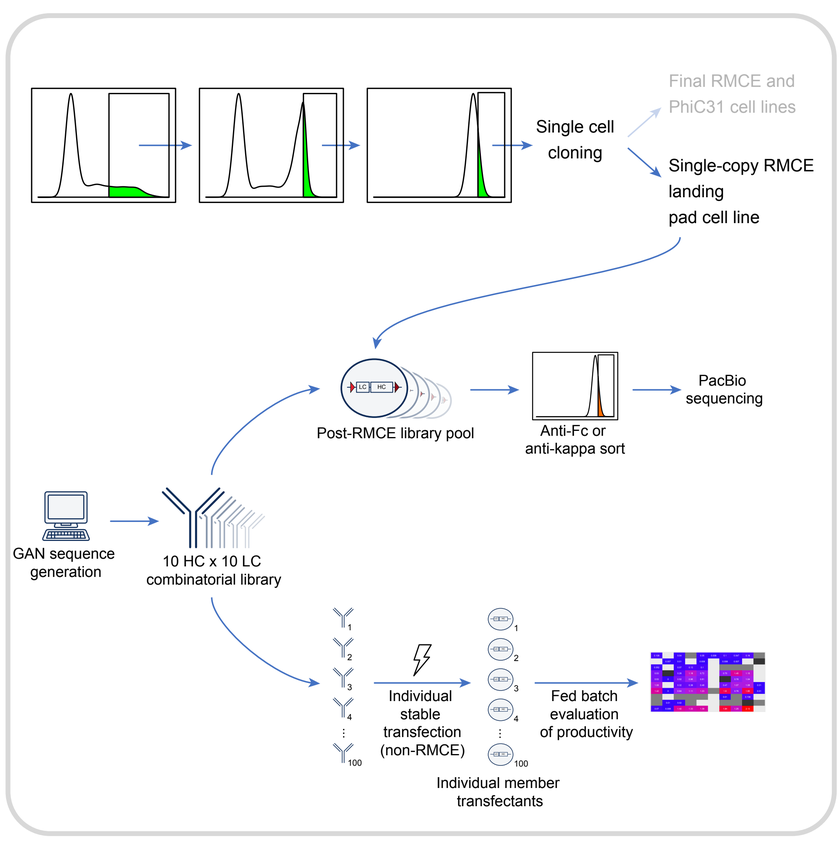


Figure 6. Fed-batch plate-scale evaluation of productivities of GAN96 library using conventional, non-targeted integration methods. Titers are noted in text and in heatmap color.

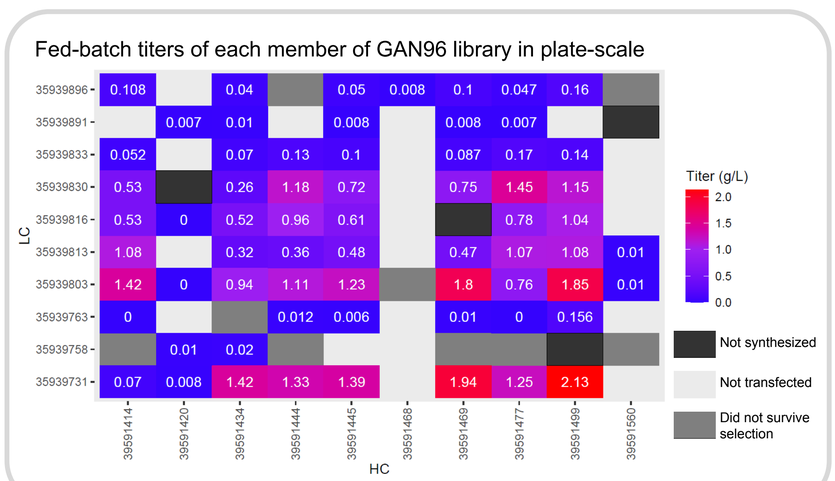
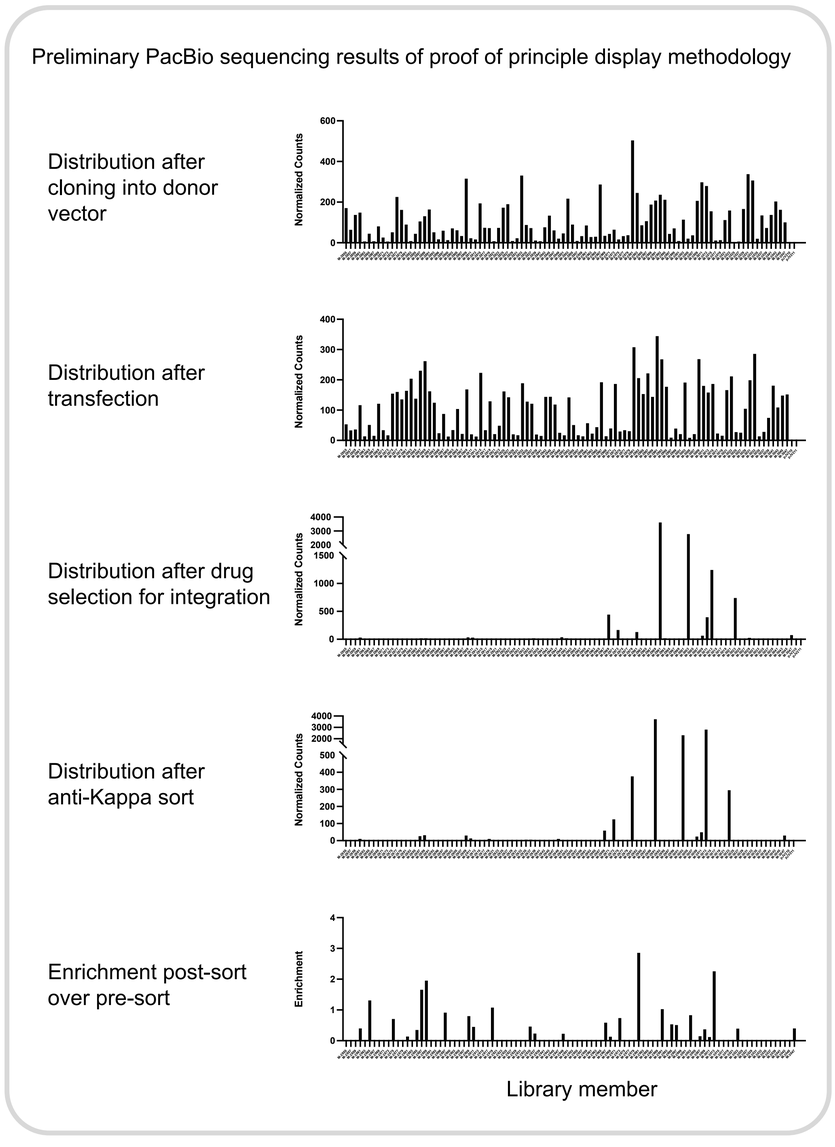


Figure 7. GAN96 PacBio sequencing data. Samples were collected a various points throughout the display/enrichment experiment and sequenced. Distributions of detected antibody sequences (read counts normalized to total counts) are shown for the first four graphs, and enrichment due to display sorting is shown in the bottom graph.



Summary

In this study, we demonstrated the development of two targeted integration systems that support high expression of recombinant protein therapeutics in CHO-K1 cell lines. We first isolated clonal landing pad cell lines inferred to have high expression based on eGFP fluorescence. We then demonstrated the integration of different donor molecules in both systems. Despite incomplete excision or incomplete exchange of the eGFP cassette in the landing pad seen after transfection and selection, fed--batch production of integrated clones showed moderate productivity of donor molecules and similar expression levels across varying recombinant proteins. Subsequently, we observed that enrichment of cells through GCV counterselection improved population purity and increased cell productivity of RMCE-integrated molecules. Additionally, we isolated a single-copy landing pad clone and showed proof of principle of its use in library screening as a system that eliminates confounding influences from variable integration copies and genomic integration sites. We evaluated the productivity of each member in this library as well as demonstrated feasibility of a display-based method for enriching and isolating antibodies that are predisposed to high expression as confirmed by next-gen sequencing.

References

Reese and Ku, *Curr Res Biotechnol,* 3 p269 (2021)

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