OMICS-BASED CHARACTERIZATION OF RANDOM TRANSGENE INTEGRATION SITES FROM CHO PRODUCTION CELL LINES

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The transgene integration site in CHO biologics-producing cell lines can influence productivity and stability. To identify features of desirable integration sites, a high-throughput, in-house integration site analysis pipeline was applied to clonally-derived production cell lines. Integration sites were identified for top clones from 7 different antibody-expression programs. From the 40 clones characterized, over 100 unique integration sites were identified, with individual clones having from 1 to 19 different genomic integration sites (Figure 1). To characterize these integration sites, multiple omics technologies were utilized including Whole Genome Sequencing (WGS), RNA sequencing (RNAseq), and Assay for Transposase Accessible Sequencing (ATACseq). These omics data were linked to phenotypic characteristics of the clones such as productivity and stability to identify features of desirable integration sites. These features were used to screen clones and select candidate integration sites for targeted integration.

Top candidate genomic sites were targeted for site-specific integration using a genomic homology arm plasmid expressing both heavy and light chain antibody proteins. Optimal conditions for integration of the plasmid were identified, and pools were generated for each integration site as well as for combinations of multiple integration sites. The pools were then cloned and characterized for on vs. off-target integration, and the clones were analyzed for antibody expression levels and consistency relative to clones derived from random integration. Data from these experiments was used to evaluate the suitability of implementing targeted integration into our cell line development platform.

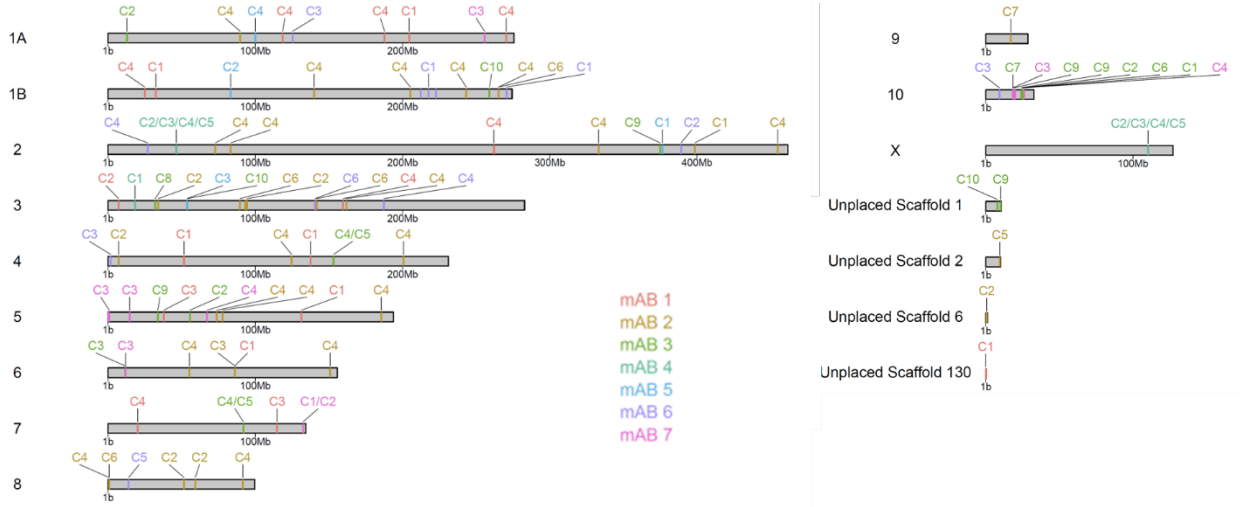

Figure 1 – Locations of identified integration sites from top clones from 7 different programs across the genome. Chromosomes are plotted to scale based on their assembled length in the CriGri-PICRH-1.0 genome. Chromosome 1 is shown in two fragments, labeled 1A and 1B. Unplaced scaffolds containing integration sites are also shown. Integration locations are labeled with the clone number and colored based on the monoclonal antibody (mAB) the clone is producing.