INVESTIGATING "DIFFICULT-TO-EXPRESS" MAB FRAMEWORKS IN TRANSIENT AND SITE-SPECIFIC INTEGRATION-BASED CHO EXPRESSION SYSTEMS

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Monoclonal antibodies (mAbs) are often engineered at the sequence and structure level for improved clinical performance yet are rarely evaluated prior to candidate selection for their "developability" characteristics, namely expression, in production host cell lines, which can necessitate additional resource investments to improve the manufacturing processes for problematic mAbs. Through recent studies of "difficult-to-express" (DTE) antibodies, a strong relationship has emerged between primary amino acid sequence and mAb expression, where even single amino acid differences between mAb candidates can result in titers differing by up to an order of magnitude. Previous work has shown that these DTE phenotypes are driven by post-translational bottlenecks in antibody folding, assembly, and secretion processes which are due to the biophysical changes that arise from differences in primary sequence. However, these bottlenecks and the types of sequence variation that can create them have only been reported for a small number of cases spanning many antibody classes and cell types, making it difficult to translate these findings across cell lines and products. To better explore the relationship between mAb primary sequence and expression, we have used both transient expression systems and site-specific integration (SSI)-based tools in Chinese hamster ovary (CHO) cells to study DTE mAbs under industrially relevant conditions on a larger scale.

An extensive mAb variant expression dataset was generated by transiently expressing 178 systematically chosen variants of a model IgG1 mAb in a CHO-K1 host cell line to determine their expression level relative to the wild-type mAb in a batch process. The tested variants showed a wide range of expression compared to the wild-type mAb, with 91 mutations identified that decreased expression. Combining the expression data with the physical and chemical changes resulting from changes in sidechain chemistry revealed distinct combinations of primary sequence mutations and molecular contexts that impact mAb expression. In particular, mutations at inaccessible residues that led to decreases in residue hydrophobicity were not favorable for high expression.

To translate the results of the transient expression screen into a stable expression platform, we used a hybrid SSI approach to generate clonally derived cell lines containing a landing pad (LP) that is capable of recombinase-mediated cassette exchange (RMCE) for targeted integration of mAb gene cassettes at known genomic loci. The LP/RMCE cell lines demonstrated high reproducibility of RMCE performance, which translated to minimal differences in gene expression levels between biological replicate pools. Using this system allowed for rapid and reliable generation of stably integrated mAb-expressing pools from a consistent clonal background. Ten mAb variants identified as low-expressing in the transient screen were expressed using this SSI-based system at two different genomic loci. While absolute productivity differed between sites, the rank order of variant productivities was conserved, and 8 of the 10 variants showed significantly lower expression compared to wild-type at both sites. Further evaluation of six variants in shake flasks showed that three variants maintain this low-expressing phenotype even under fed-batch conditions.

Our work has shown that using transient and stable expression systems in tandem can increase the throughput and translatability of DTE mAb studies. The workflows presented here can be used to better understand sequence determinants of mAb expression which could improve candidate selection procedures, provide early warnings of molecules with low "developability," or construct mAb sequence frameworks optimized for high expression, all of which could contribute to accelerated process development timelines and reduced resource requirements for bringing novel antibody therapies into the clinic.