Chinese hamster ovary (CHO) cells, which are widely used for the production of therapeutic proteins, are prone to phenotypic instability. Cell line instability can manifest as productivity decline over time as well as unexpected changes in the amounts of process-related impurities generated. As industry moves toward implementation of longer bioprocesses (e.g., perfusion cell culture systems), it has become increasingly important to understand and address the challenges posed by cell line instability. Accordingly, site-specific integration (SSI) has recently emerged as a powerful cell line engineering technique by allowing transgenes to be targeted to pre-validated genomic loci capable of supporting high and stable expression. Despite the potential benefits of SSI, its implementation in CHO cells has been hindered by remarkably low efficiencies and relatively higher levels of off-target integration.

Here, we developed toolsets for rapidly evaluating genome editing methods that use programmable endonucleases, such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9). First, the SSIGNAL (SSI and genome alteration) reporter system was established to measure both targeted gene disruption and SSI efficiency in CHO cells. The system was used to compare existing strategies for targeted integration that employ different DNA repair pathways to identify the most efficient option to use as a basis for further method development.
Next, a second reporter system called SSIRI was developed with the added ability to quantify random integration (RI) so that SSI methods could be evaluated according to their targeting accuracies (i.e., SSI/RI ratio). The system was leveraged to develop novel methods to dramatically improve SSI efficiency and targeting accuracy as well as to probe the mechanisms that influence transgene integration in CHO cells. When the optimized methods were applied in practice, high-purity SSI cell pools were obtained and cell line development outcomes were improved. Additionally, it became possible to achieve targeted integration at two loci simultaneously.

Finally, we performed an in-depth characterization of cell line instability over time with respect to host cell protein (HCP) and recombinant protein expression for two industrially-relevant CHO cell lines. For continuous processes, which involve longer culture durations, changing concentrations of HCP impurities could be a potential challenge for downstream processing. Two complementary proteomics methods were used to quantify over 1,500 individual HCPs in bioreactor samples. To aid bioprocess improvement, we compiled a list of HCPs that exhibited age-dependent differential expression and have also been previously characterized as problematic.

Collectively, this work provides advanced tools for improved CHO cell line development and useful insights regarding cell line instability at the protein level to inform rational cell line engineering strategies and support biomanufacturing efforts.