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Targeted sequencing for comprehensive genetic characterization of a recombinant CHO cell line

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Next generation sequencing has revolutionized genomics, catalyzing an era of personally-tailored therapeutics with enhanced efficacy and safety profiles. This technology also holds great promise for bioprocess and cell line development. The combination of Targeted Locus Amplification (TLA) and next generation sequencing is an emerging approach for the characterization of transgene integration and genetic stability for recombinant cell lines. TLA [de Vree *et al.*, Nature Biotechnology 32, 1019-1025 (2014)] is based on the crosslinking of physically proximal sequences and enables the targeted complete amplification and sequencing of transgenes and their integration sites with greatly increased sequence coverage and depth. Information about integration regions, Single Nucleotide Variants (SNVs), and structural changes in the transgene sequences can be tracked across different clones, over the course of multiple cell line generations and processes.

TLA sequencing was successfully applied for the comprehensive genetic characterization of a recombinant monoclonal antibody-expressing CHO cell line. A single transgene integration region with three genome-transgene breakpoints was identified within the host genome. Evidence of genetic rearrangements including vector amplification, duplication, and inversion was found by mapping genome-transgene breakpoints and specific patterns of transgene vector-vector concatamers. A copy number >20 transgene insertions was calculated, and PCR on both genomic DNA and cDNA verified a subset of vector fusions. Transgene sequencing to a median coverage of 2,388 reads per base pair also determined a limited number of homozygous and heterozygous SNVs.

To further investigate the cell line's genetic "fingerprint," ~30 research cell bank sub-clones were examined after shake flask and bioreactor expansion. A highly specific and conserved transgene vector signature was identified, signifying stability. The targeted sequencing approach applied here can efficiently provide extensive genetic sequence and linkage insight for complex transgene integration regions of recombinant cell lines, enabling improved strategies for constructing and ensuring high producing and stable cell line platforms.