Benchmarking and optimization of a Next Generation Sequencing based method for transgene Sequence Variant Analysis in Biotherapeutic Cell Line Development

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Abstract

In recent years Next-Generation Sequencing (NGS) based methods to detect mutations in biotherapeutic transgene products have become a key quality step deployed during the development of manufacturing cell line clones. Previously we reported on a higher throughput, rapid mutation detection method based on amplicon sequencing (targeting transgene RNA) and detailed its implementation to facilitate cell line clone selection. By gaining experience with our assay in a diverse set of cell line development programs, we improved the computational analysis as well as experimental protocols. Here we report on these improvements as well as on a comprehensive benchmarking of our assay. We evaluated assay performance by mixing amplicon samples of a verified mutated antibody clone with a non-mutated antibody clone to generate spike-in mutations from ˜60% down to ˜0.3% frequencies. We subsequently tested the effect of 16 different sample and NGS library preparation protocols on the assay's ability to quantify mutations and on the occurrence of false-positive background error mutations (artifacts). Our evaluation confirmed assay robustness, established a high confidence limit of detection of ˜0.6%, and identified protocols that reduce error levels thereby significantly reducing a source of false positives that bottlenecked the identification of low-level true mutations.

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