Cloning, or the derivation of a cell line from a single cell is a critical step in the generation of a manufacturing cell line. The expectation is that the process of cloning will result in a uniform and homogeneous cell line that will ensure robust product quality over the lifetime of the product. Regulatory guidelines require the sponsors provide assurance of clonality of the production cell line and when such evidence is not available, additional studies are required to further ensure consistent long-term manufacturing of the product. One approach to characterize homogeneity of a cell line is subclone analysis where clones are generated from the original cell line and an evaluation of their similarity is performed. To study the suitability of subclone analysis to provide additional assurance that a production cell line is clonally derived, an antibody producing CHO Master Cell Bank (MCB), which was cloned by a validated FACS method and with a clear documented day 0 image was characterized. Specifically, this MCB was subcloned and imaged to assure each of the subclones were derived from a single cell. A total of 46 subclones were analyzed for growth, productivity, product quality, as well as copy number and integration site analysis. Despite demonstration of clonality for both the MCB and the subclones, significant diversity in cell growth, protein productivity, and product quality attributes was observed between the 46 subclones. The diversity in protein productivity and quality were reproduced across bioreactor scales, suggesting that albeit different, the subclones were stable populations that varied from the parental clonal cell line. Additionally, while ~2-fold shifts in copy number were seen, no significant integration site changes were observed. Our data suggest subcloning induces changes (genetic or epigenetic) outside the region of the transgene which result in the subclones exhibiting a wide diversity in cell growth protein productivity, and product quality. Transcriptomic and genomic characterization studies are underway to further characterize the differences between subclones and the MCB. Importantly, the subclones do keep their individual characteristics as they mature and stabilize, suggesting that the resulting population that grows out of a single cell is stable but with unique properties. Overall, this work adds to the growing body of work on CHO cell plasticity and suggests that subcloning is not an effective approach to demonstrate homogeneity of a cell bank.