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Abstract:

Sequence variants (SV) are protein products that contain unintended substitutions in the amino acid sequence. SV are one source of heterogeneity inherent to biological systems. They can arise from multiple sources within CHO cells including mutations in genomic DNA, transcription and/or translation errors, splice variants, and post translational modifications. This poster focuses on SV resulting from single base changes in a copy of the integrated exogenous gene in genomic DNA of single cell cloned CHO cell lines.

The increased sensitivity and selectivity of analytical instruments and methods has revealed across industry that a number of cloned CHO cell lines, including clinical cell lines, contain sequence variants. SV that result in a single amino acid change typically originate in a single base change in the coding sequence of the DNA. At Pfizer, analytical and genetic sequencing methods are now in place to screen out clones containing detectable SV. Extensive clonal sequencing (ECS) of exogenous gene transcripts in over 200 cloned CHO cell lines reveals that the variants are randomly located and are not associated with any base change bias or codon position.

Digital Droplet PCR (ddPCR) analysis has been utilized to further characterize SV in transcripts and trans-gene DNA sequences from four different cloned cell lines to study the impact of cell age as well as cell culture process conditions on the level of SV in both mRNA and genomic DNA. SV levels in in the protein product were also characterized using LC/MS to link SV levels in DNA, mRNA and protein. Data so far suggest SV-containing cloned cell lines fall into two distinct categories; variants that are present in only a sub-population of cells and variants present in the whole population of cells. Results of this study with hypotheses to support population dynamic models will be presented.