VARIATION IN KARYOTYPE AND CHROMSOME NUMBERS IN CHO CELL LINES AND SUBCLONES

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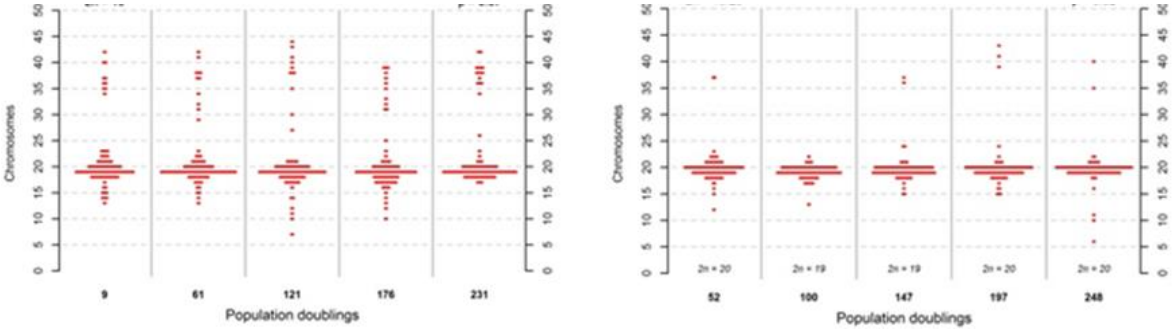
Genomic rearrangements are a common phenomenon in rapidly growing cell lines such as Chinese hamster ovary cells, a feature that, while it provides the ability to adapt to different conditions and to select the rare variants with high productivity, in the final production clone may lead to batch irreproducibility and instability. Few methods exist to assess such genome wide instability. Here we use the population distribution of chromosome numbers per cell as well as chromosome painting to identify large scale chromosomal rearrangements for quantification of karyotypic variation in several CHO cell lines, including host and recombinant cell lines, both at the pool, minipool and subclone level. Apart from investigating differences between cell lines and subclones, we followed changes in chromosome number distribution and chromosome pattern over a period of 6 months for stability assessment.

Chromosome number distribution within each population was fairly heterogeneous, typically with a subpopulation of 40-50% of single cells containing the same number of chromosomes, even though the specific complement of chromosomes present could vary, as shown by the chromosome painting results. In addition there were both cells with lower and higher numbers of chromosomes. Over the 6 months cultivation period, in some cases, a tetraploid population arose, however, this seemed to be a random event that was connected to specific cell lines, but rather occurred in some cases and not in others even for the same cell line. No differences in this distribution pattern were observed between host cell lines, engineered cell lines and subclones. Stringent sorting for a stable recombinant phenotype resulted in a slightly increased homogeneity and stability, however, subcloning the sorted cells did not lead to any further improvements. On the level of chromosome painting, similar observations were made. While the different parental cell lines, such as CHO-S and CHO-K1, had characteristic and easily distinguishable marker chromosomes, consisting of rearrangements of segments of up to 4 hamster chromosomes, the diversity within each population was comparable, both in terms of numerical abberations (consisting of different copy numbers for individual chromosomes which could or could not result in variation in the total number) and structural aberrations (containing new marker chromosomes that had formed during replication). In addition, combinations of numerical and structural aberrations were also present, in fact, after 6 months, they made up the majority of karyotypes observed in each population. This high variation in karyotype is not unique to CHO cells, as it is also observed in HEK293 cells and in primary Chinese hamster lung fibroblasts and is probably related to the high division rate of cells in culture in general.
We conclude that subcloning in itself does not contribute to population homogeneity, while the selection for a specific phenotype may inherently lead to a more homogenous population as there is selective pressure in place that may give an advantage to cells that contain a specific pattern. However, in neither case did we observe higher homogeneity in the distribution of chromosome counts within a population.



Chromosome number distribution over time in culture in CHO-K1 cells (left) and a CHO-K1 subclone (right)