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ASSESSMENT OF GENOMIC INSTABILITY IN CHINESE HAMSTER OVARY (CHO) CELLS

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CHO cells are the number one production system for therapeutic proteins due to their ease of handling, their fast growth in suspension culture and their capability to perform complex protein folding and human-like post-translational modifications. This flexibility is in part due to, but at the same time set off by the frequent occurrence of chromosomal rearrangements and other genomic variants, which influences individual cell line performance and the stability of industrial producer cell lines, resulting in prolonged screening phases in order to isolate cells with sufficiently stable properties. Furthermore producer cell properties are also frequently lost again over time and properties within clones derived of the same cell population may vary significantly.
The present work focuses on methods for quantification of the rate of chromosomal rearrangements in a given cell line over time in culture. The methods tested include Amplified Fragment Length Polymorphism (AFLP), Chromosome Painting and Chromosome Counting.
The principle of AFLP is a restriction enzyme digest of genomic DNA, followed by ligation of the fragments to adapters with a predefined sequence. DNA amplification of restriction fragments is performed using selective AFLP primers complementary to the annealed adapter sequence, but containing extra nucleotides. An initial pattern of bands of digested genomic DNA is defined which allows quantification of chromosomal changes over time using sophisticated statistical techniques.
The second technique used is Chromosome Counting of metaphase spreads from a statistically significant number of cells (50-100) in a CHO cell population, with a focus on the spread of counts and ploidy and on how that changes over time. Finally, using chromosome painting, translocations within and across chromosomes and the variation in individual cells within a population can be observed in fine detail.
A variety of CHO host cell lines, both pools and subclones were analyzed over a period of six-months in culture. With AFLP we could identify genomic rearrangements for each cell line over time revealing different rates of genomic changes in the analyzed cell lines as well as degrees of relationship between the cell lines and clones at the starting point. Chromosome Counting indicated that the chromosome number and its variation in a CHO cell population differs not only within a population over time, but also between different CHO cell lines. Furthermore the chromosome number of a CHO cell culture changes over time. The older a culture, the more variation and diversity within the population is observed, frequently with a clear tetraploid sub-population appearing after several months in culture. Chromosome painting reveals appearance of new chromosome variants over time, but typically not within the entire population.
Overall we can conclude that CHO cells are highly rearranged and that the genomic stability over a production process cannot be guaranteed.