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A regulatory requirement for the production of therapeutic proteins from mammalian cells is that the production cell line is clonal, that is, derived from a single progenitor cell. It is therefore standard procedure to include at least one cloning step during the development of a recombinant cell line for therapeutic protein production. Numerous techniques can be employed for cloning cell lines, but regardless of the cloning method used there should be appropriate evidence to support that the method is fit for purpose. A point highlighted by the increasing interest from regulatory bodies regarding the cloning method used and the probability of monoclonality (P(monoclonality)) achieved during cell line development (CLD).

FUJIFILM Diosynth Biotechnologies have thoroughly considered the cloning approach used during CLD: A two-step cloning strategy employed which combines the ClonePix™ as a cloning and screening tool followed by a second cloning step using the industrially accepted method of limiting dilution cloning will be discussed. A collaboration with statisticians led to the development of a method to estimate the resulting P(monoclonality) of cell lines generated using the ClonePix™ and experimental data to support this statistical method was generated, thereby ensuring that the ClonePix™ cloning step is robust. We will highlight the challenges of using the ClonePix™ for a single round of cloning and the advantages of combining it with a second cloning step. We will demonstrate how we achieve a minimum probability of monoclonality of ≥99.78% and typically achieve a P(monoclonality) of 99.9% using a two-step cloning strategy.