# A MATHEMATICAL MODELING FRAMEWORK FOR DETERMINING THE PROBABILITY OF OBTAINING A CLONALLY-DERIVED MAMMALIAN CELL LINE

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Ensuring a high probability of clonally-derived mammalian cell lines for biopharmaceutical production is an expectation from regulatory agencies. To date, multiple approaches have been reported to estimate the probability of clonality (PoC) associated with various cloning workflows. These approaches use varying assumptions, and even different definitions of the PoC. Thus, it is challenging to make comparisons across studies, and it can also limit the ability to have rigorous discussions with regulatory agencies in the context of a regulatory filing. There is clearly a need for a universal mathematical modeling framework that enables an aligned definition of PoC and a systematic analysis to enable comparing PoC across multiple approaches that can guide clone selection and regulatory filings.

$$P_{\mu}(k) = \frac{\mu^k}{k!}e^{-\mu}$$

$$G_k = 1-(1-a)^k, where\ k = 0, 1, 2, 3 \ldots$$

$$W = \sum_k \left(N \cdot P_{\mu}(k) \cdot G_k\right) = \sum_k \left(N \cdot \frac{\mu^k}{k!}e^{-\mu} \cdot (1-(1-a)^k\right)$$

$$PoC = \frac{P_{\mu}(1)\cdot a}{\sum_k (P_{\mu}(k)\cdot G_k)} = \frac{a \cdot ue^{-\mu}}{\sum_k (\frac{\mu^k}{k!}e^{-\mu}\cdot(1-(1-a)^k)}$$

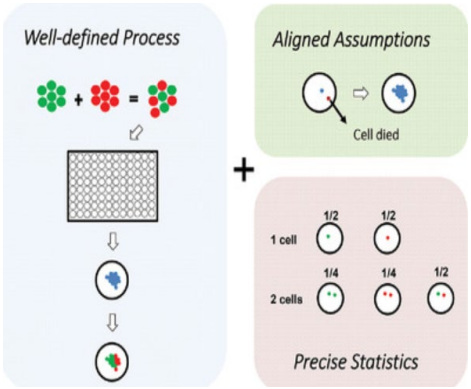


*Figure 1 – A snapshot of the modeling framework for estimating the probability of clonality*

We have proposed a mathematical modeling framework that attempts to define and align the PoC estimation under multiple cloning conditions reflective of real-world laboratory conditions (with or without direct imaging evidence, with or without considering cell survival statistics). This framework provided novel insights into the PoC associated previously reported cloning workflows. Specifically, we observed that the PoC associated with Limiting Dilution Cloning (LDC) were 47.5% - 63.3% and 72.4% - 86.5%, after 1- and 2-rounds, respectively, significantly lower than previously reported values. However, when the fact that not all individual cells after single cell cloning will recover was accounted for, the resulting PoCs were increased to 75.7% - 86.5%, and 94.1% - 99.98%, respectively. We also observed that with an automated imaging and single cell determination workflow, one round of LDC resulted in a 92.5% PoC, which increases to 97.3% after inclusion of cell survival statistics in the model. These observations support the use of direct imaging evidence coupled with 1-round LDC or FACS to obtain clonally-derived cell lines for biopharmaceutical production. As single cell deposition and high-resolution imaging technologies continue to advance, the cloning workflow will evolve, and the mathematical modeling framework developed in this study can enable aligned determination of the PoC associated with these multiple cloning approaches. This will enable comparison of cloning workflows across the biopharmaceutical industry and can simplify and streamline regulatory filings.