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Cell Line Development at Aragen: Research cell banks in 5 months

Biological therapeutics have become more essential in the battle against many diseases, including cancer, autoimmune disorders, and other ailments that small-molecule medications have failed to address effectively. The primary goal of biologics development projects is to establish monoclonal cell lines that are stable and consistently express specific biomolecules of excellent quality and in high quantities while employing a cost-effective and efficient manufacturing process.

The process of creating a cell line and finding a high-producing clone is time-consuming and labour-intensive. But despite the many challenges and obstacles, different strategies can be implemented to accelerate the associated timelines. Technological advancements in expression cell line engineering, vector design, and clone screening have all contributed to the fast expansion of biological therapies.

Here, we describe the cell line development process (Illustration in Figure 1) and strategies leveraged at Aragen that allow us to produce novel cell lines in affordable timelines.

Sequence optimization and vector construction

The cell line development (CLD) process starts with vector construction where our skilled molecular biologists perform in silico studies to do codon optimization and synthesis based on the sequence given followed by transfection in Aragen's proprietary vectors.

Depending on the project, we do several transfections. The transfected cultures are incubated for 72 hours post which they are pooled together, called "bulk transfection."

Selection of the transfected cells

To identify the best transfected cells or hot spots the bulk transfection is divided into several tiny populations called minipools and the leftover is referred as the bulkpool. Minipools help identify the high producing cells. The minipools are plated in 96 well plates and later they are screened in 24 well stage, after which the top 20 minipools are identified and are evaluated in shaker flask. It takes around six weeks to complete the selection process.

Significance of bulkpool

The bulk pools are seeded on T75 cell culture flasks and are purified after two weeks. Bulkpool is used for a fed-batch production run to generate materials that is purified and analysed as an analytical standard or benchmark against the expected product quality.

Single Cell imaging and printing

After shaker flask evaluation, the top performing clones are taken for single cell printing and imaging. Based on the titer data, top few mini-pools are plated for single cell printing,

using a microfluidic-based platform, CYTENA, which ensures that each well has only one cell. Cells are left to divide for 3–4 days, and the cell population is confirmed using the Solentim imager.

Expansion screening and analytics

Post the shaker flask evaluation, the protein is purified, and the analytics and characterization are performed to calculate parameters like cell viability, metabolic profile, titer, and quality of protein and three clones are selected for the research cell banking (RCB).

The top 20 cell clones which are fully adopted in the shaker flask are banked in three vials each and they are referred as safety cell bank.

Bioreactor evaluation and safety studies

For manufacturing the proteins in large quantities, we use bioreactor evaluation where all the parameters like temperature, pH, dissolved oxygen, agitation can be controlled. We start with 1L bioreactor and is scalable to 2L, 5L, 10L, 50L to 2000L. The selected RCB undergoes stability studies for 60 to 100 generations. The total time taken to achieve the RCBs is around 5 months.

In cases where the timelines are challenging, the process development in bioreactors can be conducted with parent minipools. This way, the process development, and MCB generation move forward in parallel, cutting the timelines by around two months. Cell line stability studies can also be run in parallel with master cell bank generation using interim clones, further reducing the timelines.

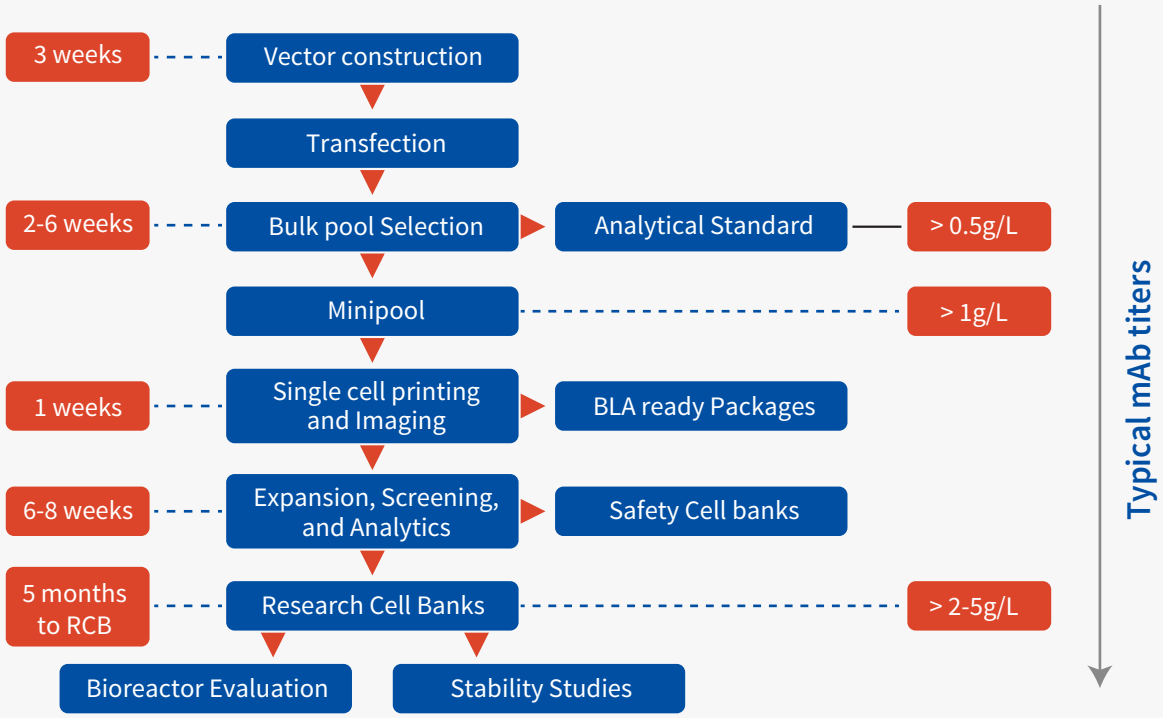

Figure 1: Illustration of Cell Line development process at Aragen.

Aragen has over two decades experience of successfully delivering integrated and standalone solutions in research, optimization, and development of biological solutions. Our depth of expertise enables us to produce novel cell lines with maximum yield, while reducing processing time. We believe that every minute of our clients' and, more significantly, every minute of our patients' unmet medical requirements is crucial. Our decades of experience on more than 120 biomolecules and engineering numerous cell lines makes us one of the most sought partners in life sciences.