OPTIMIZING ENZYME PRODUCTION TO SUPPORT COMMERCIAL MRNA MANUFACTURING

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In order to support pandemic-scale mRNA vaccines, the demand and quality expectations for in vitro transcription (IVT) enzymes for therapeutic mRNA production increased significantly. That included restriction enzymes for plasmid linearization, RNA polymerases, RNase inhibitors, inorganic pyrophosphatase (IPP), and DNases. Responding to the requirements of cGMP vaccine manufacturing, robust bioprocesses and enhanced quality systems for production of mRNA enzymes were developed. The biggest challenge in this work was producing enzymes at large-scale and high titer that are either toxic or unstable in culture. We leveraged our extensive experience in host cells engineering, fermentation, downstream processing and enzymology to overcome enzyme toxicity and ensure high titers and stability through the entire production process. Throughout this work, our goal was to minimize impurities while maximizing product consistency and yield. This presentation will discuss our novel approach to strain development, optimization of enzyme manufacturing, and management of enzyme instability during processing. We will share our rationale for screening both *pichia pastoris* and *e.coli* cells and how we leveraged a Quality by Design (QbD) approach with design of experiments (DOE) to select the clone and optimize the upstream process concurrently. Demonstrating that our approach enabled us to rapidly and successfully scale multiple enzymes to support COVID-19 vaccines.

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