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OPTIMISATION OF AN ALKALINE LYSIS PROCESS FOR A PLUG- AND PLAY PLASMID DNA PRODUCTION

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Renewed interest in plasmid DNA (pDNA) production is the result of its role in the supply chain of novel biopharmaceuticals." pDNA lies at the heart of viral and mRNA vector production because it provides the coding sequences for gene-based advanced therapy medicinal products (ATMPs). Its manufacture is therefore critical to both the supply and success of these products"[1].

One main bottleneck in processing of pDNA is the lysis step that still rely on techniques developed for laboratory-based DNA extraction. A robust process adaptable to a range of DNA sizes and scales remain to be fully realised. These include control of the alkaline environment, potential heterogeneity in a large vessel, change in the rheological properties of the process fluid and potentially shear impact if the molecule is large. Here, we focused on a batch lysis operation in a 1 L reactor. Three parameters studied were pH, mixing time and agitation speed. Using a central composite design for optimisation of these factors, we obtained the maximum plasmid yield (13 mg/gcells, R2=92%) and supercoiling content (91%, R2=95%) at 0.2M NaOH, mixing time of 15 minutes and tip speed of 2.3 m/s. These results will benchmark the design of plug- and- play platforms.

[1].T. Hitchcock 2021, Genetic Engineering & Biotechnology News 2nd August.