ENHANCING THE PRODUCTIVITY OF SUPERCOILED PLASMID UPSTREAM BIOPROCESSING THROUGH PLASMID ENGINEERING.

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This study was set out to develop an approach for producing highly supercoiled plasmid DNA. Potentially, the level of supercoiling can have an impact on ease of downstream processing. A 7.2kb plasmid was developed by cloning of Bacteriophage-Mu Strong gyrase-binding sequence (Mu-SGS) into 6.8kb pSVβ-Gal. Four *E. coli* strains were transformed with both the modified pSVβ-Gal398 plasmid and pSVβ-Gal. Small scale fermentations and analysis were carried out in triplicate cultures to screen for best performing strains. Two of the four strains selected amplified the plasmids efficiently. There was over 20% increase in the total plasmid yield with pSVβ-Gal398 in both strains. The supercoiled topoisomer content was increased by 5% in both strains leading to a 27% increase in the overall yield. The two strains were investigated further in shake flasks. Increases in supercoiling and plasmid yield were also observed. The extent of supercoiling was examined by superhelical density quantification, with pSVβ-Gal398 maintaining a supercoil density of -0.022 and pSVβ-Gal -0.019 in both strains. The compactness of the plasmid DNA was also quantified by hydrodynamic diameter measurement using the Nanoparticle Tracking Analysis (NTA) and it was observed that pSVβ-Gal398 was more compact with a D_h of 40-59nm compared to pSVβ-Gal with D_h of 70-90nm for both strains examined. The report of this study has shown that plasmid engineered to contain the Mu-phage SGS sequence has a beneficial effect on improving not only the yield of total plasmid but also the supercoiled topoisomer content of therapeutic plasmid DNA during bioprocessing.

References:

Hassan, S., Keshavarz-Moore, E., & Ward, J. (2016). A cell engineering strategy to enhance supercoiled plasmid DNA production for gene therapy. *Biotechnology and bioengineering*, *113*(9), 2064-2071.

Yau, S. Y., Keshavarz-Moore, E., & Ward, J. (2008). Host strain influences on supercoiled plasmid DNA production in Escherichia coli: Implications for efficient design of large-scale processes. *Biotechnology and bioengineering*, *101*(3), 529-544.