

ENHANCING THE YIELD AND QUALITY OF SUPERCOILED PLASMID THROUGH PLASMID ENGINEERING

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There has been a rise in the interest of plasmid DNA as therapeutics. This is evident in the number of ongoing clinical trials involving the use of plasmid DNA. To be effective as therapeutics, high yield and high level of supercoiling are required from the bioprocessing point of view. We have approached meeting these requirements through plasmid engineering. 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 pSV β -Gal. Four *E. coli* strains were transformed with both the modified pSV β -Gal398 plasmid and pSV β -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 pSV β -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 pSV β -Gal398 maintaining a supercoil density of -0.022 and pSV β -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 pSV β -Gal398 was more compact with a D_h of 40-59nm compared to pSV β -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:

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