In-process controls for plasmid isoform characterization

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During plasmid production, the purity of the plasmid DNA increases with progressing downstream processing stage. Apart from the major contaminants such as chromosomal DNA and RNA, plasmid isoforms are also present and need to be removed. The covalently closed circular conformation of plasmid is highly efficient in traversing the cell and nuclear membrane resulting in better transfection rates. Even where the plasmid DNA acts as a starting material, for example for the *in vitro* production of mRNA, the ccc conformation ensures that no single strand break is present in critical points of the plasmid that could otherwise lead to premature length mRNA. Thus it is very important to analyze plasmid quality during production through in-process controls so that the percentage of supercoiled plasmid can be continuously monitored and to ensure that impurities are contained within the limits defined for the corresponding downstream stage. Capillary Gel Electrophoresis is the established gold standard method for this analysis which gives a clear result in percentage of ccc and open circular (oc) form of plasmid. As an auxiliary method, High Performance Liquid Chromatography has been tested in this work to explore its suitability as a method for routine analysis. The results show that with the current conditions, it is possible to demonstrate a good resolution between the ccc and oc forms of the final plasmid product. Initial quantitative measurements of the respective isoforms showed correlation with the CGE data. Adapting this method as an in-process control will involve testing samples of varying purity levels.

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