MSS2024
HYBRID

Purification of linearized template plasmid DNA decreases double-stranded RNA formation during IVT reaction

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Following the COVID-19 pandemic, messenger RNA (mRNA) has transformed conventional vaccine production, leading to a surge in RNA-based therapeutics and novel scientific understandings. A key research area focuses on the formation of double-stranded RNA (dsRNA) during in vitro transcription (IVT), a significant impurity triggering cellular immune responses. Hence, there's growing emphasis on refining purification processes to eliminate dsRNA. While efforts traditionally concentrated on post-IVT mRNA purification via chromatographic methods, the impact of linearized plasmid quality remains underexplored. Plasmid production entails intricate steps like bacterial culture growth, harvesting, and filtration, often resulting in inconsistent batches with limited control over dsRNA by-products. This study delves into how purifying linearized plasmids affects dsRNA formation. Various techniques, including resin filtration and chromatographic separations, were explored. Optimizing a chromatographic method using monolithic columns with C4 chemistry proved effective, yielding homogeneous linearized plasmids, and reducing dsRNA levels in mRNA batches during IVT. This underscores that dsRNA formation is influenced not just by RNA polymerase and IVT conditions, but also by linearized template quality. Plasmid impurities could contribute to dsRNA production by providing additional templates that anneal with mRNA molecules. Therefore, the quality of plasmid purification is crucial in dsRNA generation during transcription. Further research is required to fully grasp the mechanisms and implications of plasmid-derived dsRNA, potentially reshaping mRNA vaccine development.