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Abstract
This study is aimed to develop a simple and rapid method for purification of ultrapure supercoiled plasmid DNA with high yields from bacterial cultures. Nanosized superparamagnetic nanoparticles (Fe_3O_4) were prepared by chemical precipitation method using Fe^{2+}, Fe^{3+} salt, and ammonium hydroxide under a nitrogen atmosphere. The surface of Fe_3O_4 nanoparticles was modified by coating with a multivalent cationic agent, polyethylenimine (PEI). The nanoparticles were characterized by transmission electron microscopy, X-ray diffraction, Fourier transformation infrared spectroscopy and superconducting quantum interference device magnetometer. The PEI-modified magnetic nanobeads were employed to simplify the purification of plasmid DNA from bacterial cells. We demonstrated a useful plasmid, pRSETB-EGFP, encoding the green fluorescent protein with T7 promoter, was amplified in DE3 strain of E. coli. The loaded nanobeads are recovered by magnetically driven separation and regenerated by exposure to the elution buffer with optimal ionic strength (1.25 M) and pH (9.0). Up to approximately 800 U+B5g of high-purity (A260/A280 ratio=1.857) plasmid DNA was isolated from 100 ml of overnight bacterial culture. EGFP expression was detected by fluorescent microscopy in the transformed E. coli cells, indicating the biological activities of DNA fragments were retained after the purified from magnetic nanobeads. The protocol, starting from the preparation of bacterial lysate and ending with purified plasmids takes less than 30 minutes. The purified pDNA can be used to confirm the restriction enzyme, ligation, gene cloning, E.coli transform, cell transfection .
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