

Process optimisation for anion exchange monolithic chromatography of 4.2 kbp plasmid vaccine (pcDNA3F)

Abstract

Anion exchange monolithic chromatography is increasingly becoming a prominent tool for plasmid DNA purification but no generic protocol is available to purify all types of plasmid DNA. In this work, we established a simple framework and used it to specifically purify a plasmid DNA model from a clarified alkaline-lysed plasmid-containing cell lysate. The framework involved optimising ligand functionalisation temperature (30–80 °C), mobile phase flow rate (0.1–1.8 mL/min), monolith pore size (done by changing the porogen content in the polymerisation reaction by 50–80%), buffer pH (6–10), ionic strength of binding buffer (0.3–0.7 M) and buffer gradient elution slope (1–10% buffer B/min). We concluded that preferential pcDNA3F adsorption and optimum resolution could be achieved within the tested conditions by loading the clarified cell lysate into 400 nm pore size of monolith in 0.7 M NaCl (pH 6) of binding buffer followed by increasing the NaCl concentration to 1.0 M at 3%B/min