

Two-step process for initial capture of plasmid DNA and partial removal of RNA using aqueous two-phase systems.

ABSTRACT

In this paper, a two-step process for initial capture of plasmid DNA (pDNA) and partial removal of RNA using polyethylene glycol (PEG) and di-potassium hydrogen phosphate aqueous two-phase systems (ATPS) has been investigated. A Kühni-type ATPS extraction column was prepared with 50 ml (12% (w/w) PEG 1450, 12% (w/w) phosphate) of stationary phase and loaded with crude mobile phase (26% (w/w) PEG 1450, 4% (w/w) phosphate and 70% (w/w) lysate) at a flow rate of 6 ml min⁻¹ at an impeller speed of 200 rpm. The experiment was terminated after 100 min, and after complete resettling of the phases, 45 ml of stationary phase was harvested. During a subsequent second extraction step contained 18% (w/w) PEG 300 and 14% (w/w) phosphate, a proportion of RNA, which was also concentrated during the column process, was removed. It was demonstrated that the recovery of pDNA in the second bottom phase was 89.4%, which was similar to the initial recovery after column extraction (92.1%).

Keyword: Aqueous two-phase systems (ATPS); Column; Plasmid DNA (pDNA); Recovery; Two-step process.