CO29. Purification of Plasmids using Aqueous Two-Phase Systems with Amino Affinity Ligands

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for their wide application large amounts of plasmid DNA (pDNA) are required with a stringent clearance of impurities. This prompted the development of new, efficient and cost-effective large-scale processes for the production and purification of pDNA. Most of the purification processes described are based on chromatography but dispite their high resolution, frequently they are difficult to scale-up, have low capacity and present low yields. In order to overcome these disadvantages other methodologies are also being developed.

Aqueous two-phase systems (ATPS) are one of the most promising approaches for pDNA purification given their several advantages like easy scale-up, high capacity and the possibility of continuous operation. Despite their great potential ATPS have low selectivity, which limits the purification outcome. The addition of certain molecules with affinity for the target molecules (pDNA in this case) may increase their selectivity.

In this work it was studied the possibility of using amino ligands for the affinity purification of pDNA from bacterial alkaline lysates. Two free amino acids, lysine and arginine, their respective Polyethylene glycol (PEG) conjugates, PEG-lysine and PEGarginine, and PEG-amine were tested. The system used was composed of 16,2% (w/w) PEG 600 and 17,4% (w/w) dextran 100 (DEX) and it was evaluate the ability of each ligand to steer the pDNA to the phase where less impurities are accumulated (PEG rich phase). The results show that free amino acids did not have any effect on pDNA partitioning but the PEG conjugates were able to steer the pDNA to the PEG phase, at low concentrations. With the addition of 0,2% of PEG-lysine, or 0,5% of PEG-arginine or 4% of PEG-amine in relation to the total PEG, all the pDNA is recovered in the PEG phase. However it presents some RNA contamination, that could be removed by re-extracting with a new phase containing 30% of ammonium sulphate (NH4)2SO4. The purified pDNA is obtained in the bottom phase of this new system with no measurable presence of RNA or proteins.