The Use of Aqueous Two-Phase Separation for the Recovery and Purification of pDNA

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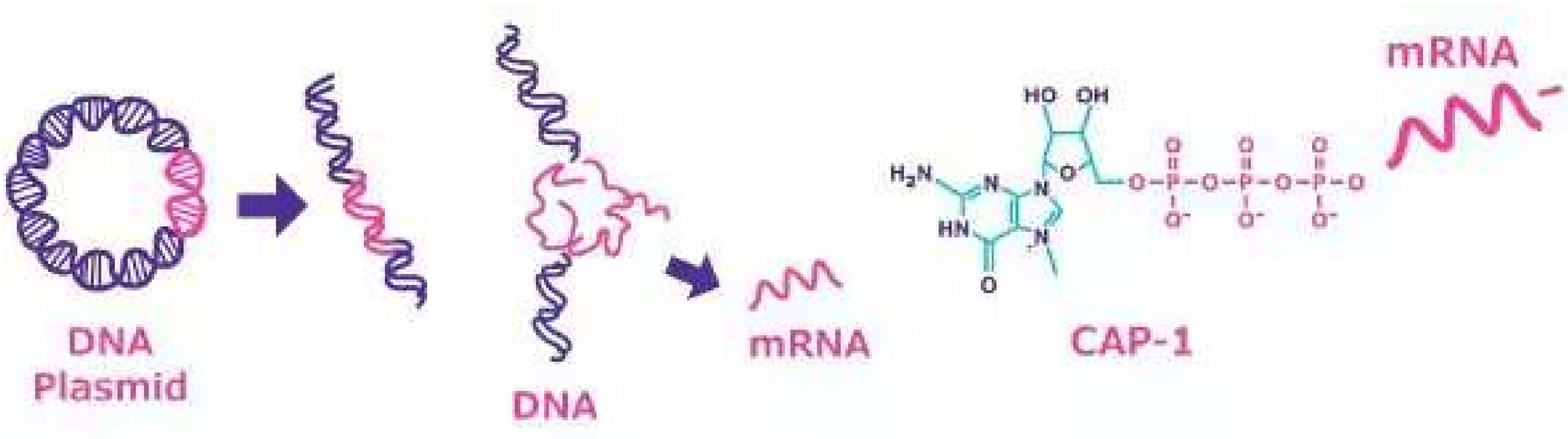
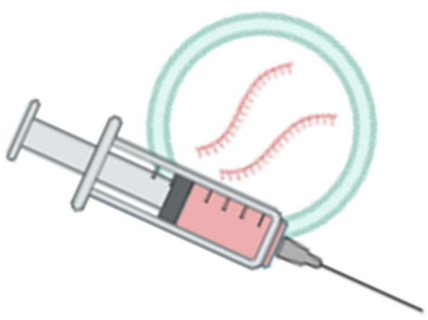


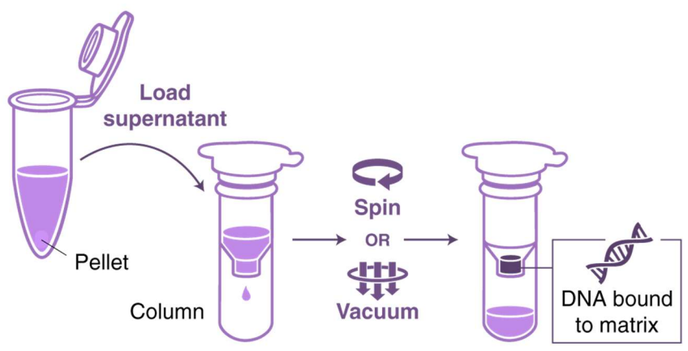
The use of plasmid DNA for mRNA vaccine production. Adapted from Sigma Aldrich

Background

- South Africa remains unable to manufacture its own vaccines and relies on international suppliers
- This results in high costs and delays

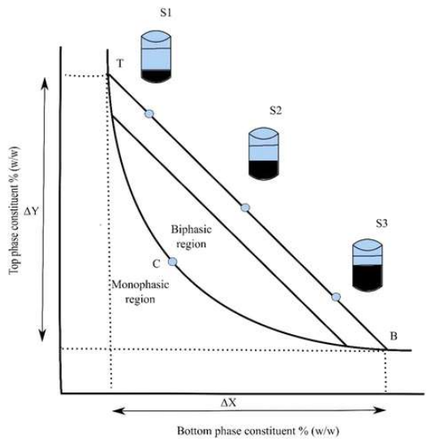


- Local manufacture of vaccines would benefit the South African pharmaceutical industry, as well as the population at large



Current method for plasmid purification. New England Biolabs

- Current methods for biomolecule purification are expensive, experience technical difficulty, and have limited scaling abilities
- Aqueous two-phase separation (ATPS) poses as an attractive alternative to mitigate these limitations



Schematic representation of a phase diagram of ATPS. Iqbal et al. 2016

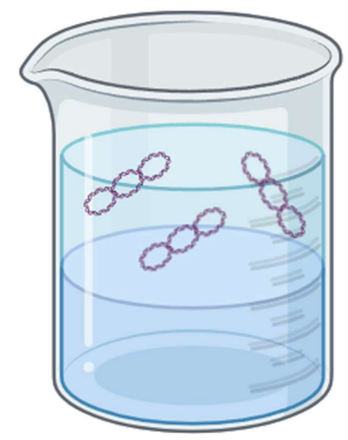
Aims and objectives

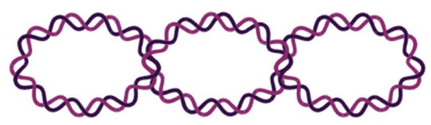
This project aims to develop a method using preferable ATPS conditions for the recovery and purification of plasmid DNA from crude bacterial lysate.

1. Identify, test, and select ATPS conditions where preferential recovery of pDNA purified using current methods is observed.
2. Demonstrate recovery of pDNA using ATPS on crude bacterial lysate.

Methods

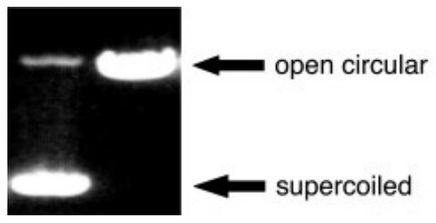
- Polymer/salt & alcohol/salt ATPS systems to be tested
- Different polyethylene glycol molecular weights and pH values 5-9 to be tested (where DNA is stable)
- PEG mw and pH influences partitioning behaviour of pDNA





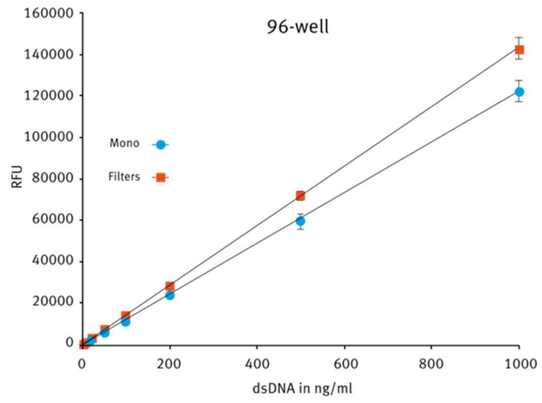
Supercoiled plasmid

- Supercoiled plasmids need to be isolated as this is the most stable and useful conformation
- Gel electrophoresis is used to monitor pDNA conformation
- Fluorescence spectrophotometry to be used for pDNA quantification in each phase



Zakharova et al. 2002

- Higher pDNA presence in a phase indicates preferable partitioning
- pDNA will thereafter be recovered and purified



Picogreen assay comparing filters

References

1. Saied, A. A. (2023). Building Africa's first mRNA vaccine facility. The Lancet, 402(10398), 287–288. https://doi.org/10.1016/s0140-6736(23)01119-4
2. Home. (2024, May 21). Health Justice Initiative (HJI). http://healthjusticeinitiative.org.za
3. Svetlana S. Zakharova, Wim Jesse, Claude Backendorf, Stefan U. Egelhaaf, Alain Lapp, Johan R.C. van der Maarel, Dimensions of Plectonemically Supercoiled DNA, Biophysical Journal, Volume 83, Issue 2, 200, https://doi.org/10.1016/S0006-3495(02)75234-X
4. Iqbal, M., Tao, Y., Xie, S. et al. Aqueous two-phase system (ATPS): an overview and advances in its applications. Biol Proced Online 18, 18 (2016). https://doi.org/10.1186/s12575-016-0048-8