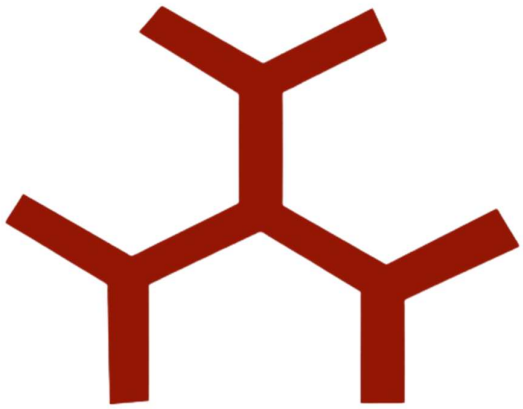
INTERNSHIP REPORT

Summer Research Internship Programme 2023

National Institute of Immunology



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Title: Bacterial cell transformation, plasmid isolation, and recombinant protein extraction and purification

BONAFIDE CERTIFICATE

This is to certify that the project entitled "**Bacterial cell transformation, plasmid isolation, and recombinant protein extraction and purification**" is the bonafide work of **Nabeela Ansari** who carried out the work under my supervision.

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Nabeela Ansari

INTRODUCTION

Bacterial transformation is an important technique in the field of biotechnology and molecular biology which is used to insert plasmid vectors with our gene of interest into a competent bacterial cell such as E.coli (Hanahan et al.). Transformation can be done for plasmid amplification as well as protein expression based on different strains of competent cells used. The protein expressed consequently can be extracted from cell culture and purified through subsequent purification steps which majorly involve Ni-NTA chromatography.

Bacterial Transformation

E. coli competent cells are prepared through a chemical method (Calcium chloride) to make them readily take up plasmid from their surroundings. The calcium ions coat the negatively charged bacterial cell membrane with positive charges which increases its interaction with the negatively charged plasmid. Subjecting the cells to heat shock or electroporation causes pores to form in the bacterial membrane through which the plasmid enters the cell.

XL 1 blue strain of cells is generally used for cloning the plasmid vector. These cells can be used in blue-white screening and give high-quality plasmid.

On the other hand, BL21 cells are used for protein expression since they have a higher plasmid copy number than XL 1 blue strain. BL21(DE3) cells have the T7 RNA polymerase gene under the control of the lacUV5 promoter. This can be used to express proteins whose gene is controlled by the T7 promoter which requires T7 RNA polymerase for transcription. IPTG (Isopropyl β-D-1 thiogalactopyranoside) is a molecular analog of allolactose, which binds to the lac repressor to subsequently induce protein expression in the cell.

Plasmid vectors

A small circular plasmid DNA in which our gene of interest is inserted is used for bacterial transformation (Wang et al.). This plasmid has various components which are as follows:

- The gene of interest- for expression of the desired protein
- Multiple cloning sites- regions where the gene of interest can be inserted by treatment of the plasmid with restriction endonucleases
- Antibiotic resistance gene- which provides resistance against a specific antibiotic and can be used to selectively grow only the transformed cells in a medium containing that antibiotic.
- an origin of replication where DNA replication is initiated.
- Promoter region- the DNA sequence that drives protein expression in cloned DNA by facilitating gene transcription. It is where RNA polymerase binds.