Artificial Transformation of *E.coli* cells with pUC19 plasmid DNA by

CaCl2 Method

Principle

Bacterial transformation is a natural process in which cells take up foreign DNA from the environment at a low frequency. After transformation, the cells may express the acquired genetic information, which may serve as a source of genetic diversity and potentially provide benefits to the host (e.g., antibiotic resistance). The process of transformation was exploited and enhanced to introduce recombinant plasmid DNA into bacterial strains that were made "competent," or more permeable, for DNA uptake artificially. Artificial transformation can be performed by various methods -

1 Chemical Transformation: In 1970 Mandel and Higa first reported that bacterial cells upon treatment with Ca2+ and elevated temperatures, known as heat shock can uptake naked DNA. This method became the basis of chemical transformation which was later modified by various groups to increase the efficiency of transformation. The repulsion between foreign DNA and the bacterial cell, owing to negative charges on them both, are overcome by these divalent cations. This is applicable for linear DNA fragments as well as circular DNA molecules such as plasmids. It is thought that the divalent cations bind both to the cell and the DNA, thus neutralizing the charge altogether. The calcium bound to the DNA further helps the DNA to adsorb to the competent cell. Heat shock followed by cold shock lead to transient pore opening through which DNA enters the cell.

2. Electroporation: In this method an electric field instead of any chemical is used for uptake of DNA. In 1982, Neumann et al. first reported introduction of foreign DNA into mouse cells by short pulses of high-voltage electric fields.

Another very critical aspect of artificial transformation is the choice of host cell. The genotype of an E. coli strain determines whether the cells can be grown on specific media, whether they may be used for transformation with specific DNA types, and whether they are appropriate for certain cloning strategies. The commonly used host strains contain mutations in non-sepcific endonuclease gene (endA1, improves quality of isolated plasmid DNA), general recombination genes(recA1, increases the stability of cloned DNA) and restriction

and modification systems genes (hsdRMS, allows cloning of DNA from non–E. coli sources, such as PCR products, without cleavage by endogenous restriction endonucleases)

Reagents Required:

• E. coli cells- DH5□/XL1Blue/BL21

• 100 mM CaCl2

• Luria-Bertani Broth

• Luria Bertani agar with ampicillin at 100 ug/ml.

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