

Chemical Engineering Thesis Defense

Methylated and Unmethylated pDNA Delivery Comparison in Mammalian Cells

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abstract

In this study, the differences in delivery of methylated and unmethylated prokaryotic DNA in mammalian cells was investigated. 3 plasmids, DH5- α , ER2925 and GM272 were extracted and transformed from E. coli bacteria. DH5- α is the regular methylated plasmid, however, ER2925 and GM272 lack Dam and Dcm enzymes which methylate adenine and internal cytosine in prokaryotes respectively, hence they are unmethylated. The 3 plasmids were delivered via different delivery vectors in two cell lines, UMUC3 and MDA-MB-231 which are human bladder cancer cell line and human triple negative breast cancer cell line, respectively. Luciferase and BCA assay were carried out to quantify transgene expression to compare the efficacy of gene delivery in three aforementioned plasmids. In addition, hydrodynamic diameter and zeta potential was measured for all delivery vectors, to correlate with other transgene expression data. The results show that methylated plasmid has significantly higher transgene expression in mammalian cell lines. This can be either a result of smaller size and more positive zeta potential that the methylated plasmid had, or a result of having Dam and Dcm enzymes which enhance binding of DNA and transcription factors and enhance gene expression. Having smaller size and more positive zeta potential was proven to be the case for the methylated plasmid in this study. However the latter hypothesis should be investigated furthermore.