ESSENTIAL REPLICATION FUNCTIONS OF THE F PLASMID

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TABLE OF CONTENTS

CHAPTER II MATERIALS AND METHODS

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ABSTRACT

The work in this thesis concerns the analysis of essential replication genes of the F plasmid of *Escherichia coli*. The techniques principally employed were the cloning of restriction fragments, transposition mutagenesis, maxicell analysis of proteins, and DNA sequencing. This work led to the following findings.

1. A 1.15kb region of f5 was shown to contain all the functions required for initiation of F replication. Insertions of a transposon within this 1.15kb region abolished mini-F replication.

2. A mini-F plasmid was constructed which constitutes the smallest F derivative so far reported. This plasmid has an elevated copy number, as a result of deletion of the *incC* region.

3. At least two proteins specified by the essential F replication region were identified in maxicells. The promoter fragments of two mini-F genes were fused to a β-galactosidase gene and demonstrated to have low but significant *in vivo* activities.