RECOMBINATION

SECTION OVERVIEW:

I. Introduction

A. Gene Cloning
B. The importance of Gene Cloning

II. Construction of recombinant DNA molecules

A. Types of DNA needed in preparation
    1. Preparation of total cell DNA
        a. Preparation of cell extract
            i. Removal of contaminants
            ii. Concentration of DNA
    2. Plasmid Purification
B. Cleavage and Insertion
    1. Cleavage of the Vector
    2. Cleavage of the DNA to be cloned
C. Restriction Enzymes
    1. Blunt Ends
    2. Cohesive (sticky) ends
    3. Complimentary sticky ends
D. The importance of the site of cleavage
    1. Intramolecular association
    2. Restriction of Lambda Phage DNA
        a. Visualisation of DNA molecules or fragments
            i. Electrophoresis
            ii. Gel electrophoresis
        b. Estimation of DNA fragment sizes
E. Joining DNA molecules
    1. Ligation
    2. Products of Ligation
F. Transformation
    1. Process
    2. Inefficiency
    3. Ensuring transformation
G. Function of Cloning
    1. Increasing the quantity of available starting material
    2. Purification of recombinant DNA

III. Cloning Vectors

A. Plasmid Vectors
    1. Properties of an ideal plasmid vector
        a. pBR322
            i. insertional inactivation
        b. PUC plasmids
            i. β-galactosidase
                - α-complementation