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
 - 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
- sale.co.uk 2. Restriction of Lambda Phage DNA
 - es or fragments a. Visualisation of NAm

M fragment sizes

- 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