INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
34100 TRIESTE (ITALY) - P.O.B. 586 - MIRAMARE - STRADA COSTIERA 11 - TELEPHONES: 224281/2/3/4/5/6
CABLE: CENTRATOM - TELEX 460392-1

SMR/111 - 18

SECOND SUMMER COLLEGE IN BIOPHYSICS

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Recombinant DNA Technology

A. NORDHEIM
Zentrum für Molekularbiologie
Universität Heidelberg
Im Neuenheimer Feld 364
6900 Heidelberg
Fed. Rep. Germany

Lecture Series: Recombinant DNA Technology

Lecture 1: Concepts and Fundamentals of Gene Cloning

The revolution in genetics which was brought about by the advent of in vitro recombinant DNA technology in the early 1970s was made possible by:

- the discovery of bacterial plasmids, i.e. extrachromosomal genetic elements that could be used as vehicles (vectors) for the propagation of DNA segments.
- the discovery of restriction endonucleases for defined fragmentation of DNA
- the development of biochemical methods to covalently link pieces of DNA
- the development of bacterial transformation as a way to reintroduce genetically manipulated DNA into bacteria
- screening methods, that allow identification and selection for specific recombinant DNA molecules.

Main topics to be discussed in this lecture will include:

- Plasmid biology: autonomous replication, copy number, relaxed replication (chloramphenicol treatment).
- Microbiology: bacterial growth, growth curves, antibiotic resistance, bacterial pathogenicity.
- Plasmid DNA Purification
- Bacterial Transformation: Ca++ Technique,
- Restriction Endonucleases: recognition sequences, palindromes
- Generation of a Restriction Map: concept and example
- Concept of a Cloning Experiment: Ligation of vector and DNA fragment, transformation of hybrid DNA into E. coli, selection of E. coli that carry recombinant molecules by the presence of antibiotic resistance and the loss of antibiotic resistance due to insertional inactivation.
- Cloning allows for specific isolation of DNA Sequences: Gel electrophoresis
- DNA Sequencing

Lecture 2: Biochemistry of Creating Recombinant Molecules

Enzymes: Restriction Endonucleases
T4 DNA Ligase (sticky/blunt end ligation)
Terminal Deoxynucleotidyl Transferase
Reverse Transcriptase
Kinase
DNA Polymerase (Klenow fragment)
S1 Nuclease
Bal 31 Nucleases

Tools in Cloning: DNA Linkers
Polylinker fragments
Double digestion (to direct orientation)
Regeneration of Restriction enzyme sites

In vitro Mutagenesis: Deletions
Insertions
Substitutions
Integration of oligonucleotides

Lecture 3: The Isolation and Identification of Genes

- Concepts for the Isolation of a Gene
- Construction of a Genomic DNA Library (Gene Bank)
- Selection for an active gene (in procaryotes)
- Construction of a cDNA Library
- Identification of a gene: -colony hybridisation
  hybrid arrest
  hybridisation using specific oligonucleotid
- Cosmid cloning

Lecture 4: Expression of Genes

- Procaryotic Expression Vectors
- Eucaryotic Expression Vectors
- Getting DNA into eucaryotic cells
- Expression of Oncogenes
- Industrial Applications of Gene Cloning

Lecture 5: DNA Cloning to Study DNA Conformation

- Conformational Flexibility of the DNA double helix
- Left-handed Z-DNA
- DNA Supercoiling (Definitions)
- Effect of torsional stress on DNA structure
- Cloning potential Z-DNA segments
- Antibodies specific for Z-DNA
- Two-dimensional gel electrophoresis