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اسم المادة: التقنيات الاحيائية

عنوان المحاضرة : Biotechnology Concept and its History

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construction of gene library

Introduction

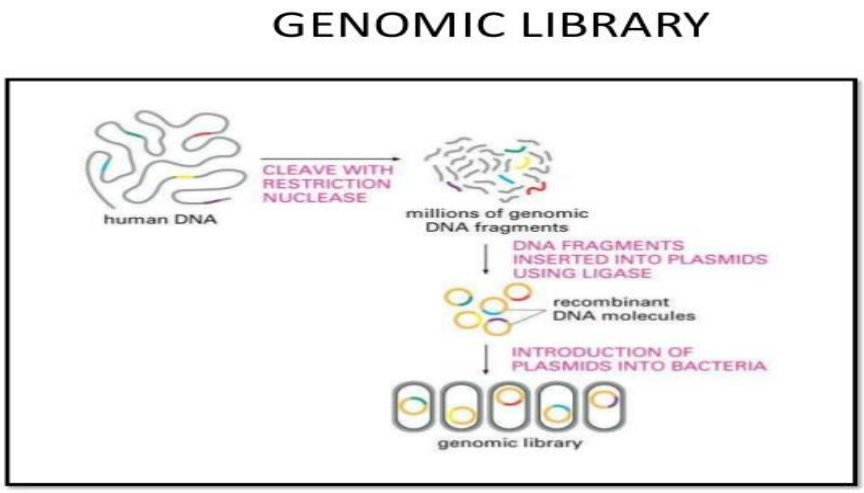
- In humans, approximately 25,000 genes exit among the 3 billion base pairs of DNA in the genome.
- To study anyone of these genes, a researcher first isolates it from all of the other genes in an organisms DNA.
- One isolation method has a relatively long history and involves the construction of a DNA library
- When a gene is identified and copied, it is said to have been “clone

What is genomic library?

A genomic library is a collection of bacteria which have been genetically engineered to hold the entire DNA of an organism

- A genomic library is a collection of genes or DNA sequences created using molecular cloning.
- These libraries are constructed using clones of bacteria or yeast that contain vectors into which fragments of partially digested DNA have been inserted.
- These bacteria and yeast are subsequently grown in culture and when these microorganisms replicate their genome, they also replicate the vector genome contained within them, that is, they replicat
- DNA fragments that had been inserted in vectors producing clones of the orignal genome.
- This collection of clones, in theory, contains all sequences found in the original source, including the sequence of interest.

- Genomic libraries can be constructed using various hosts like plasmids, bacteriophage lambdas , cosmids, YACs and many more



What are DNA libraries?

A DNA library is a collection of clones of DNA designed so that there is a high probility of finding any particular piece of the source DNA in the collection.

What are the types of DNA libraries?

The two types o DNA libraries are:

1) GENOMIC LIBRARY:

The genomic library contains DNA fragments representing entire genome of an organism.

2) cDNA LIBRARY:

The cDNA library contains only complementry DNA molecules synthesized from mRNA molecules in a cell.

For the construction of DNA Library we have to know

- Size of the gene
- Capacity of the vector
- Molecular tools
- Vectors

Steps of Genomic Library construction

- Isolation of DNA from cells
- Digestion into small fragments
- Introduction into suitable vectors
- Insertion into bacteria
- DNA isolation
- Collection of Genomic DNA library

Collection of Genomic DNA library

Isolation of DNA (purification)

- ✓ Eukaryotes : Prepare cell nuclei, remove proteins, lipids and other unwanted macromolecules by protease digestion and phase extraction.
- ✓ Prokaryotes : Extracted DNA directly from cells

Digestion into small fragments

- ➢ Physical shearing : Pipetting, mixing
- ➢ Restriction enzyme digestion : Partial digestion is preferred to get a greater lengths of DNA fragments.

Introduction into suitable vectors

- Each fragment is different and have a unique DNA sequence

- Inserted into suitable vectors including plasmids and bacteriophage vectors.
- Vectors are digested with the same Restriction Enzymes and sealed to Human DNA using DNA Ligase enzyme .
- The resulting molecules are recombinant.

Insertion into Bacteria

- Inserted into host bacteria (E.coli)
- The microbes are grown in culture.
- They are made to take up the DNA.
- They replicate their genome along with the vector genome contained with them.
- Produce clones of the original genome. This collection of clones which contains all the sequences found in the original source, including the sequence of interest forms the genomic library

Multiplication and Production of clones

- Independent plasmid replication
- Host cell replication

What are the steps involved in the construction of genomic library?

1) To create a human genome library, a researcher begins by extracting and purifying DNA from human cell.

2) The purified DNA consist of extremely long strands.To begin working with DNA, the strands must first be cut into manageable sizes.

3) The DNA therefore is digested with restriction enzymes which cut the DNA at specific sequences. The restriction enzyme cut the DNA into

1000s of smaller fragments, each of which may contain one or more gene.

4) The DNA therefore is digested with restriction enzymes which cut the DNA at specific sequences. The restriction enzyme cut the DNA into 1000s of smaller fragments, each of which may contain one or more gene
5) Each fragment is different and have unique DNA sequence. To create the library,each fragment must be inserted into loops of DNA called plasmids. A plasmid is a type of vector that allows the DNA to be shuttled in bacteria
6) The plasmids are digested with the restriction enzymes and then sealed to human DNA using DNA ligase enzymethe resulting molecules are "recombinanat".
7) The recombinant DNA molecule are added to bacteria,and the bacteria are made to take up the DNA. When bacteria have taken up the DNA, the entire collection of cells and DNA represents a human genome library. The recombinant plasmids remain separate from the bacteria's own DNA, making it easy to extract again

What are the uses of Genomic Library?

a) Researchers can explore the genome of an organism to learn more about genomic structure and function
b) They can map the genome, identifying the locations of specific genes.
c) Helps to develop tests which can be used to locate genetic variations including mutations
d) Useful in Recombinant DNA Technology, helps to genetically modify organisms and produce clones of desired types.

e) Genomic library construction is the first step in any DNA sequencing projects.

f) Genomic library helps in identification of the novel pharmaceutically important genes.

References

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