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TOPIC: RDT- CONSTRUCTION & SCREENING OF DNA LIBRARIES – PART II

COURSE : M.Sc. ZOOLOGY

ELECTIVE PAPER : CELL & MOLECULAR BIOLOGY

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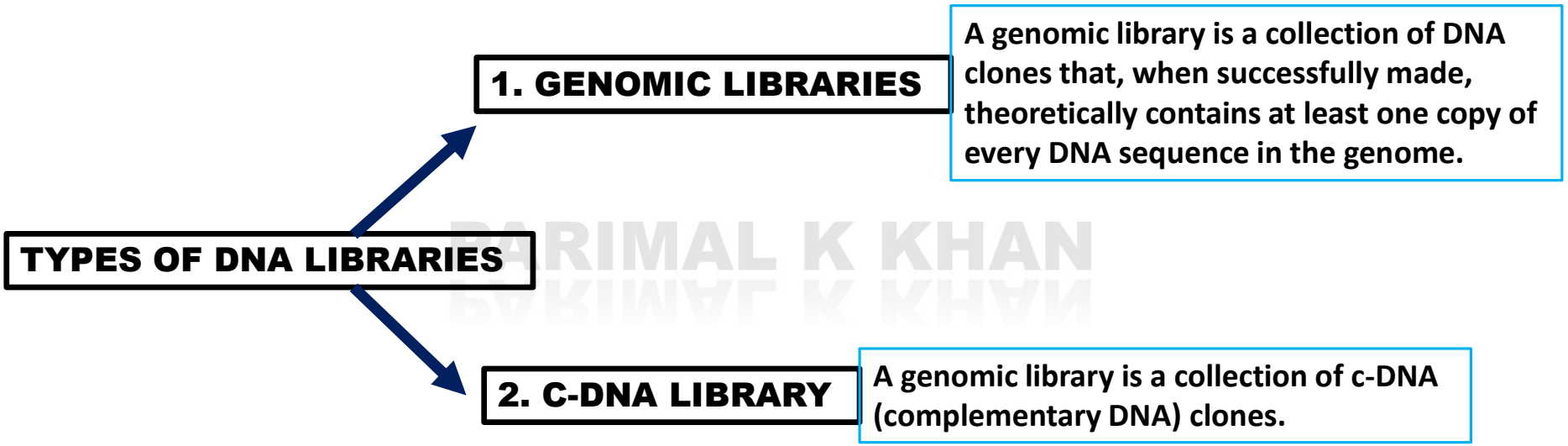
DNA LIBRARIES

A DNA Library is collection of DNA fragments cloned into vectors from a cell, tissue or organism.



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CONSTRUCTION OF GENOMIC LIBRARIES

- The genome of an organism is cut into pieces using Restriction Enzyme (RE) and the DNA fragments are inserted into suitable vectors, using the same RE along with DNA Ligase, forming large number of recombinant DNA molecule.

- Each vector, possessing a particular DNA fragment is introduced into host cell which divides and redivides to form a colony of identical cells each possesses clones recombinant DNA molecules.

- A genomic library is thus constructed of all such types possessing clones of different DNA fragments.

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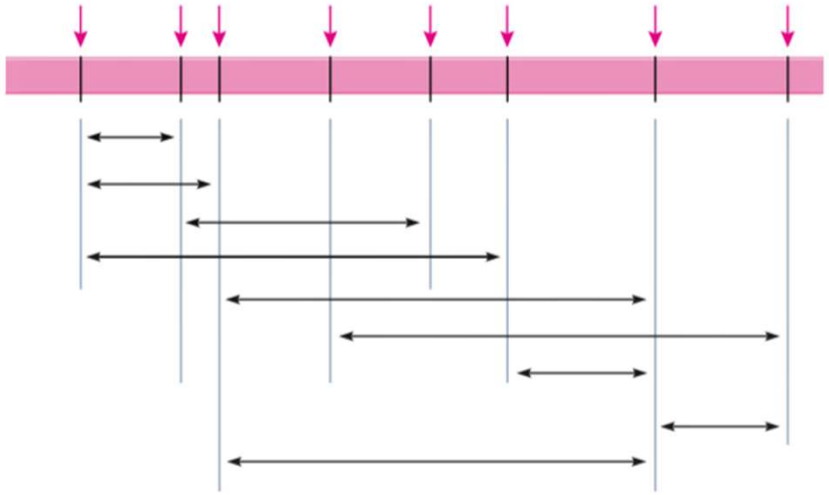
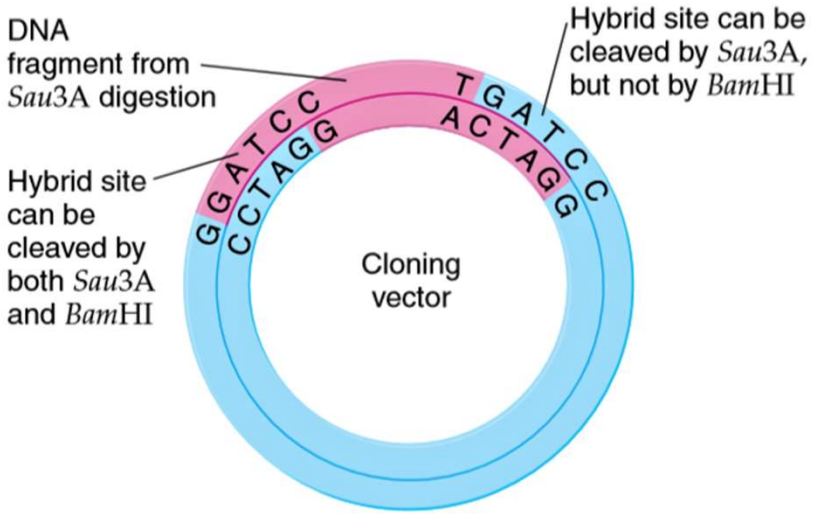
PARTIAL DIGESTION WITH RESTRICTION ENZYME TO PRODUCE OVERLAPING DNA FRAGMENTS OF APPROPRIATE SIZE FOR CONSTRUCTING A GENOMIC LIBRARY

a) Partial digestion of DNA by a restriction enzyme (for example *Sau*3A) generates a series of overlapping fragments, each with identical 5′ GATC sticky ends



b) Resulting fragments may be inserted into *Bam*HI site of cloning vector



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SIZE OF HUMAN GENOMIC LIBRARIES PREPARED IN DIFFERENT TYPES OF CLONING VECTORS

Types of Vector	Inserted Size (kb)	Number of Clones* P=95%	Number of Clones* P=99%
λ phage	18	532500	820000
Cosmid/ fosmid	40	240000	370000
F1	125	77000	118000
BAC/PAC	300	32000	50000
YAC	600	16000	24500
Mega-YAC	1400	6850	10500

*Calculated from the equation: $N=\ln(1-P)/\ln(1-f)$

where N= number of recombinant DNA molecules

P= probability desired

f= fractional proportion of the genome in a single recombinant DNA molecule

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CONSTRUCTION OF cDNA LIBRARY

- In genomic libraries, molecular clones posses both introns and exons. However, in c-DNA library, as a superior grade library, molecular clones posses only the coding sequences of genes of an organism.

- It is prepared by producing RNAs from the cells of an organism by RNase method which is then used for generating c-DNA clones followed by c-DNA library.

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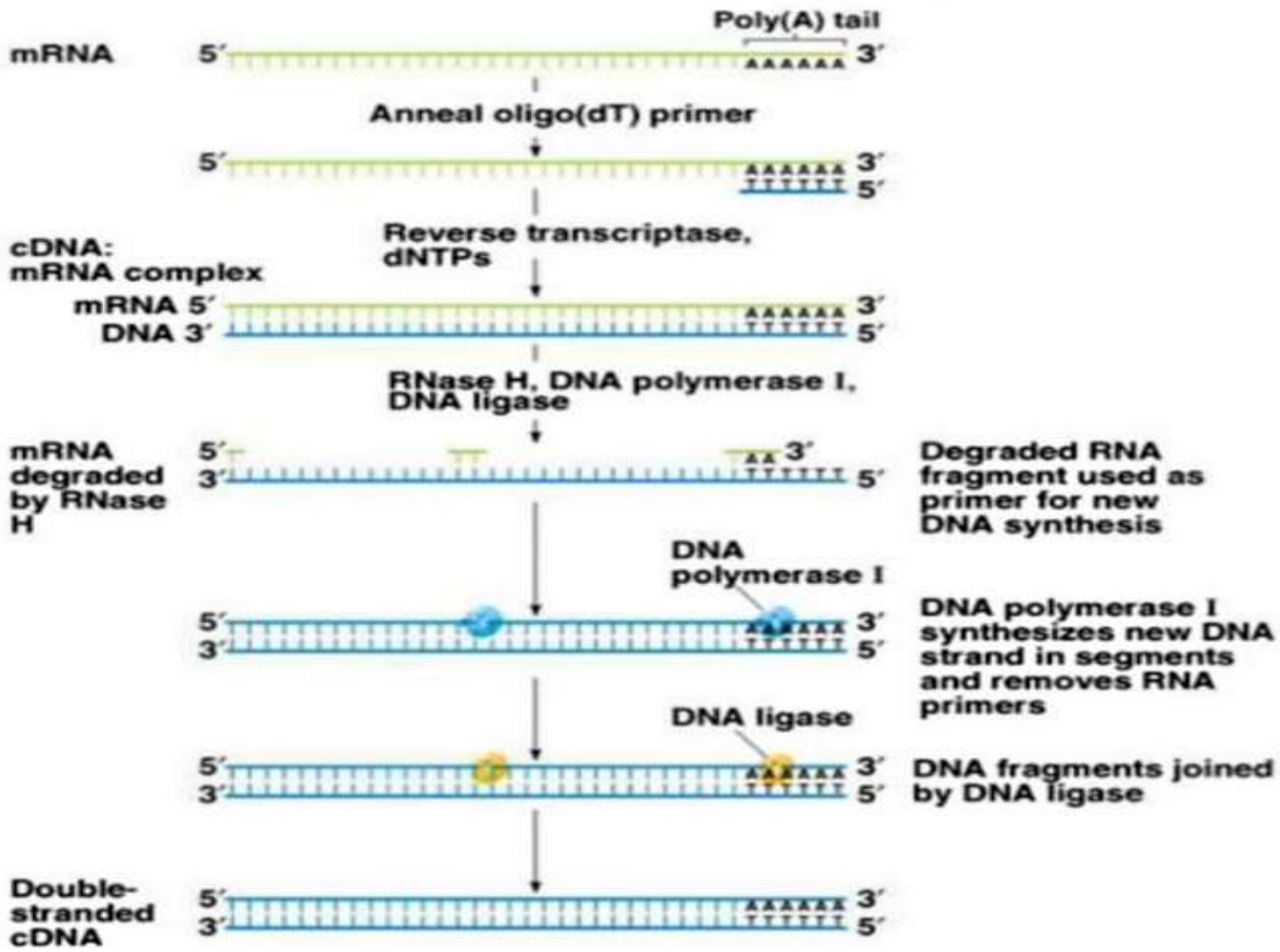
CONSTRUCTION OF c-DNA (COMPLEMENTRY DNA)



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SCREENING OF DNA LIBRARY

1. SCREENING BY GENETIC SELECTION

Genetic Selection is the most powerful screening procedure that searches for a DNA sequence in the library that can restore a wild type phenotype to a mutant organism.

2. MOLECULAR SCREENING

Radioactive c-DNA probes as Molecular Screening screens genomic DNA libraries by in situ colonies or plague hybridization.

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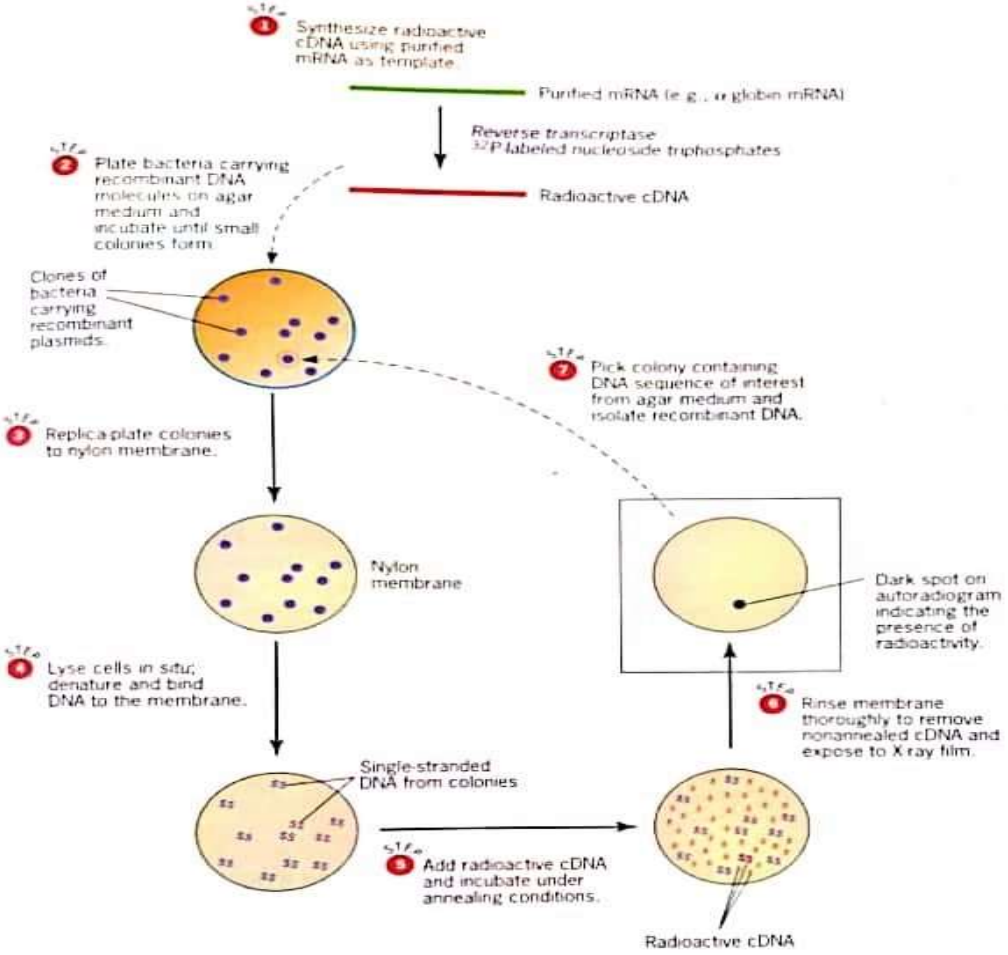
SCREENING OF DNA LIBRARIES BY COLONY HYBIDIZATION



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THANK YOU

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