cDNA AND GENOMIC LIBRARIES

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INTRODUCTION

- A DNA library is a collection of DNA clones ,gathered together as a source of DNA for sequencing, gene discovery , or gene function studies .

- There are two types of DNA libraries:

    1. DNA
    2. Genomic

cDNA LIBRARY

- A cDNA library is a set of cDNA clones prepared from the mRNAs isolates from a particular type of tissue.
- The cDNA library contains only complementry DNA molecules synthesized from mRNA molecules in a cell.
- This molecules represents all the gene that are expressed in the cell at different stage of its development.

No cDNAwas made from prokaryotic mRNA

- Prokaryotic mRNA is very unstable.

- Genomic libraries of prokaryotes are easier to make and contain all the genome sequences.

cDNA Libraries Are Very Useful For Eukaryotic Gene Analysis

- Condensed protein encoded gene libraries,Have much less junk sequences
- cDNAs have no introns⟶genes can be expressed in E. coli directly
- Are very useful to identify new genes
- Tissue or cell type specific(differential expression of genes)

Synthesis of Cdna:

- FIRST STAND SYNTHESIS: materials as reverse transcriptase, primer(oligo(dT) or hexanucleotides) and dNTPs
- SECOUND STRAND SYNTHESIS: best way of making full-length cDNA is to ‘tail’ the 3’- end of the first strand and then use a complementary primer to make the secound.

CONSTRUCTION

- cDNA libraries are constructed by synthesizing cDNA from purified cellular mRNA via oligo(dT)-cellulose chromatography.

- This is done to recover the poly(A) mRNA so as to anneal with the oligo(dT) chains .

SCREENING

- A prope is a piece of DNA or RNA used to detect specific nucleic acid sequence by hybridization (binding of two nucleic acid chains by base pairing). Oligonucleotide can be used as a probe.

- They are radioactivity labeled so that the hybridized nucleic acid can be identified by autoratiography.

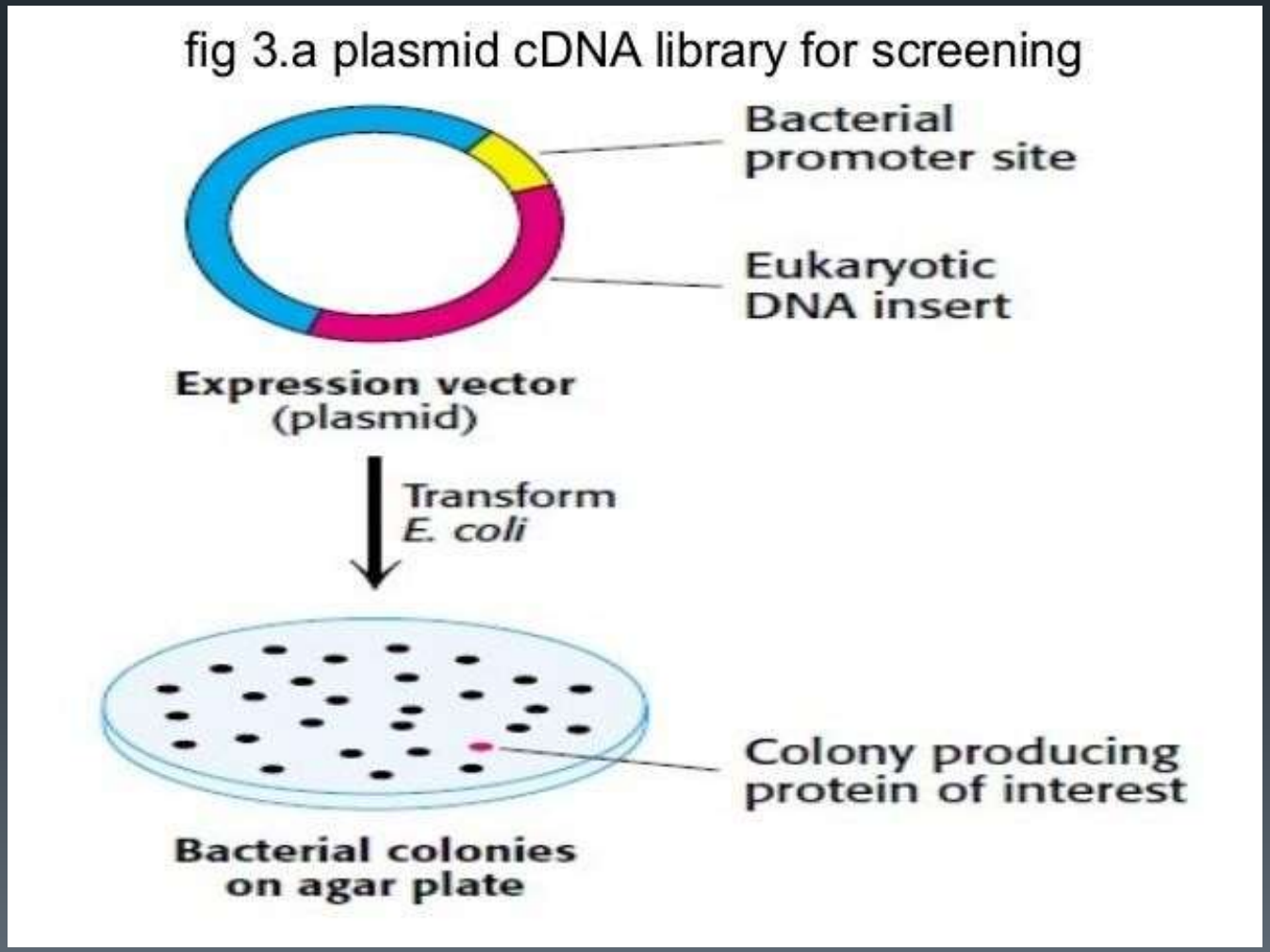
Two general approaches are avilable for screening libraries to

identify clones carrying a gene or other DNA region of interest.

Detection with oligonucleotide probes that bind to the clone of interest .

Detection based on expression of the encoded protein.

A PLASMID cDNA LIBRARY FOR SCREENING



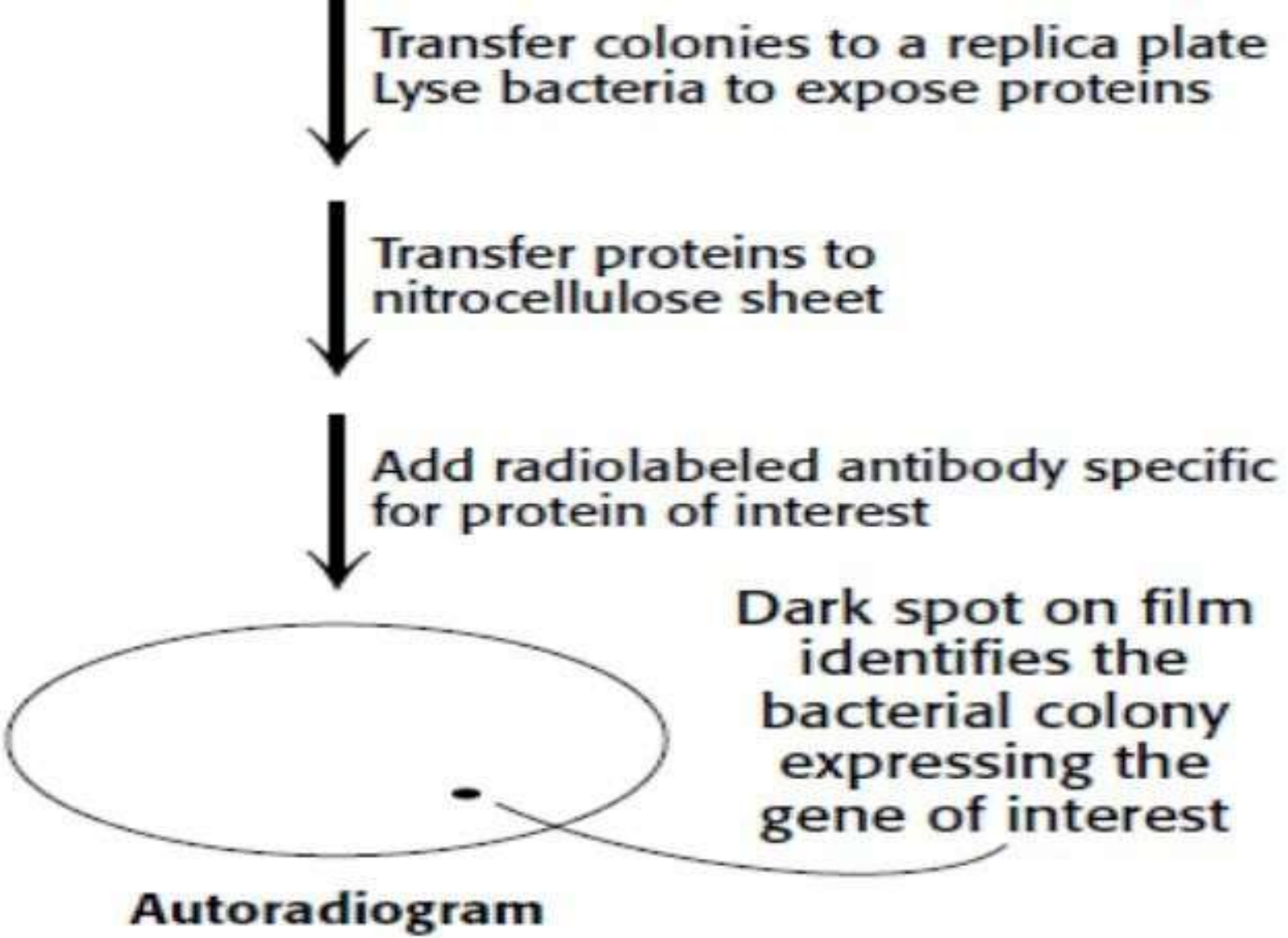
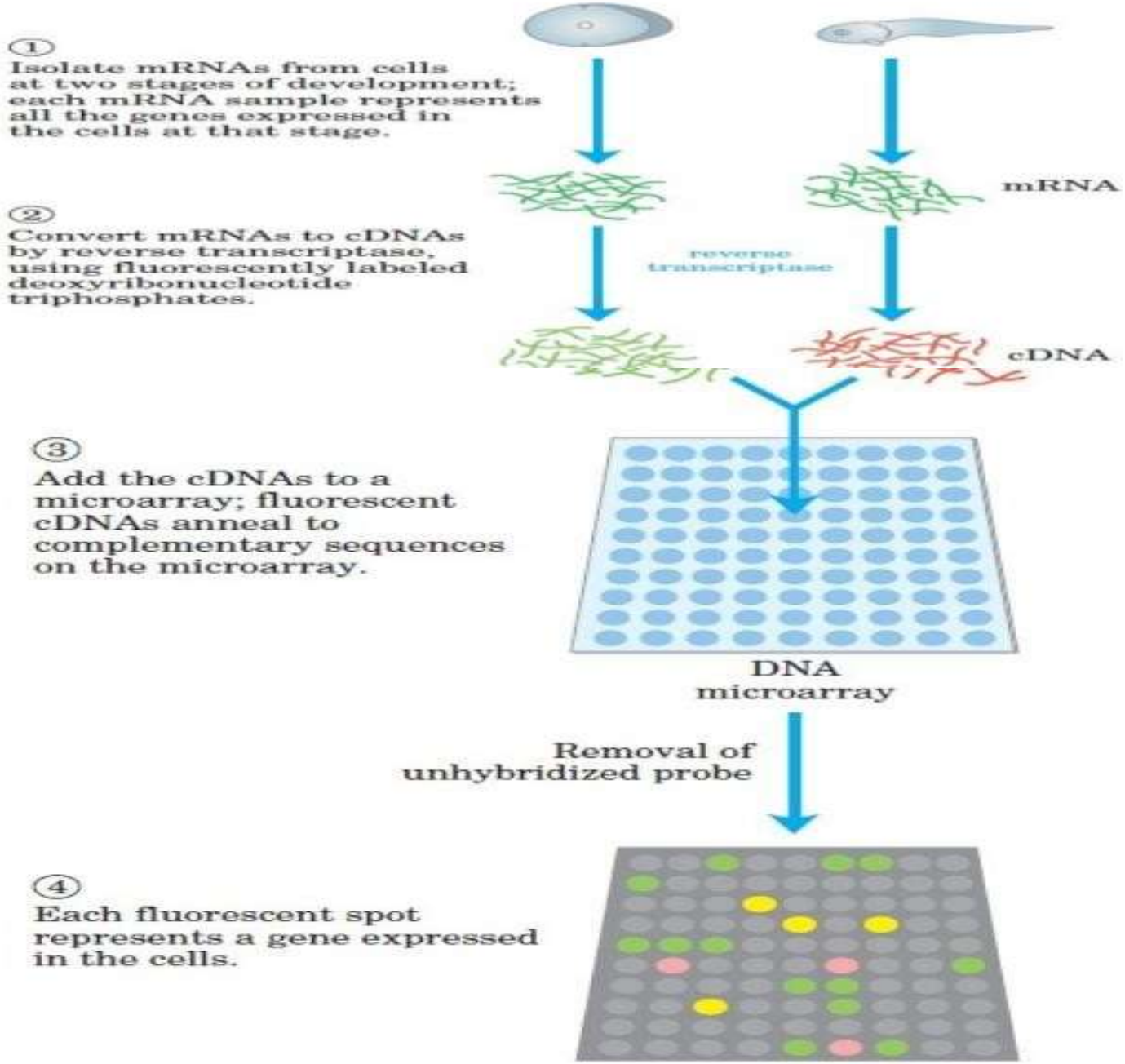

Transfer colonies to a replica plate
Lyse bacteria to expose proteins
Transfer proteins to nitrocellulose sheet
Add radiolabeled antibody specific for protein of interest
Dark spot on film identifies the bacterial colony expressing the gene of interest
Autoradiogram

FIG 4.To detect the expression of encoded protein



GENOMIC LIBRARY

- Is the largest type of library which consist of the complete genome of a complete genome of a particular organism which is cleaved into thousands of fragments, are all the fragments are cloned by insertion into a cloning vector.

- A collection of clones that collectively represent all the DNA sequences in the genome of a particular organism.

CONSTRUCTION

- The first step in preparing a genomic library is partial digestion of the DNA by restriction endonucleases, such that any given sequences will appear in fragments of a range of sizes and is represented in the library.
- Secondly,the cloning vector ,such as a BAC or YAC plasmid ,is cleaved with the same restriction endonuclease and ligated to the genomic DNA fragment.

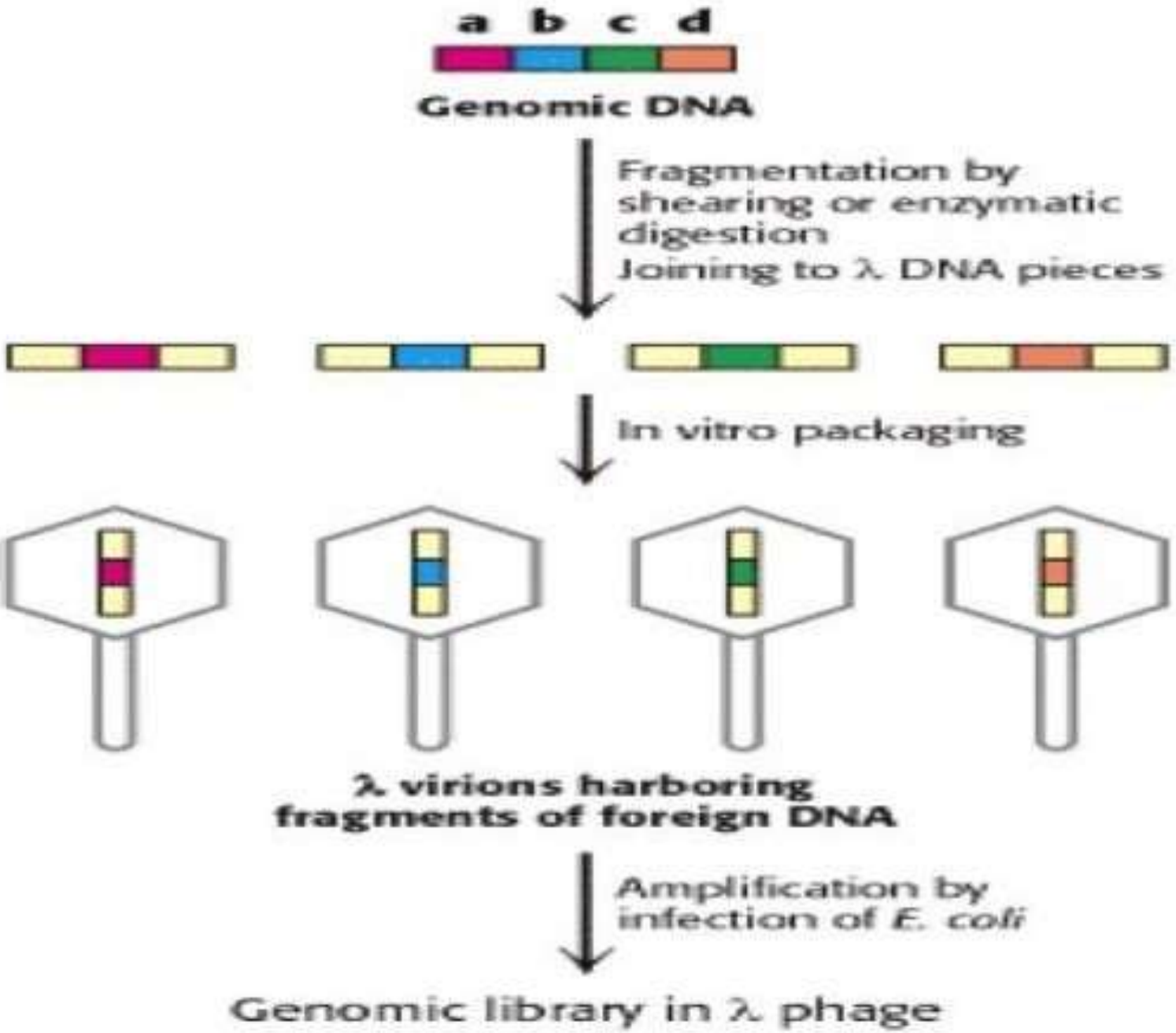
- Thereafter ligated DNA mixture is then used to transform bacterial or yeast cells to produce a library of cell types,each type harboring a different recombinant DNA molecule.
- Each transformed bacterium or yeast cell grows into a colony,or “clone”,of identical cells,each cell bearing the same recombinant plasmid.
- The ability to clone such large DNA fragments raise the possibility of the Genomic library,but it has been found that there is a problem as to what number of clones are required to construct a genomic library.

- A solution has been provided with thw use of a fornuker:

  $N=\ln(1-P)/\ln(1-a/b)$

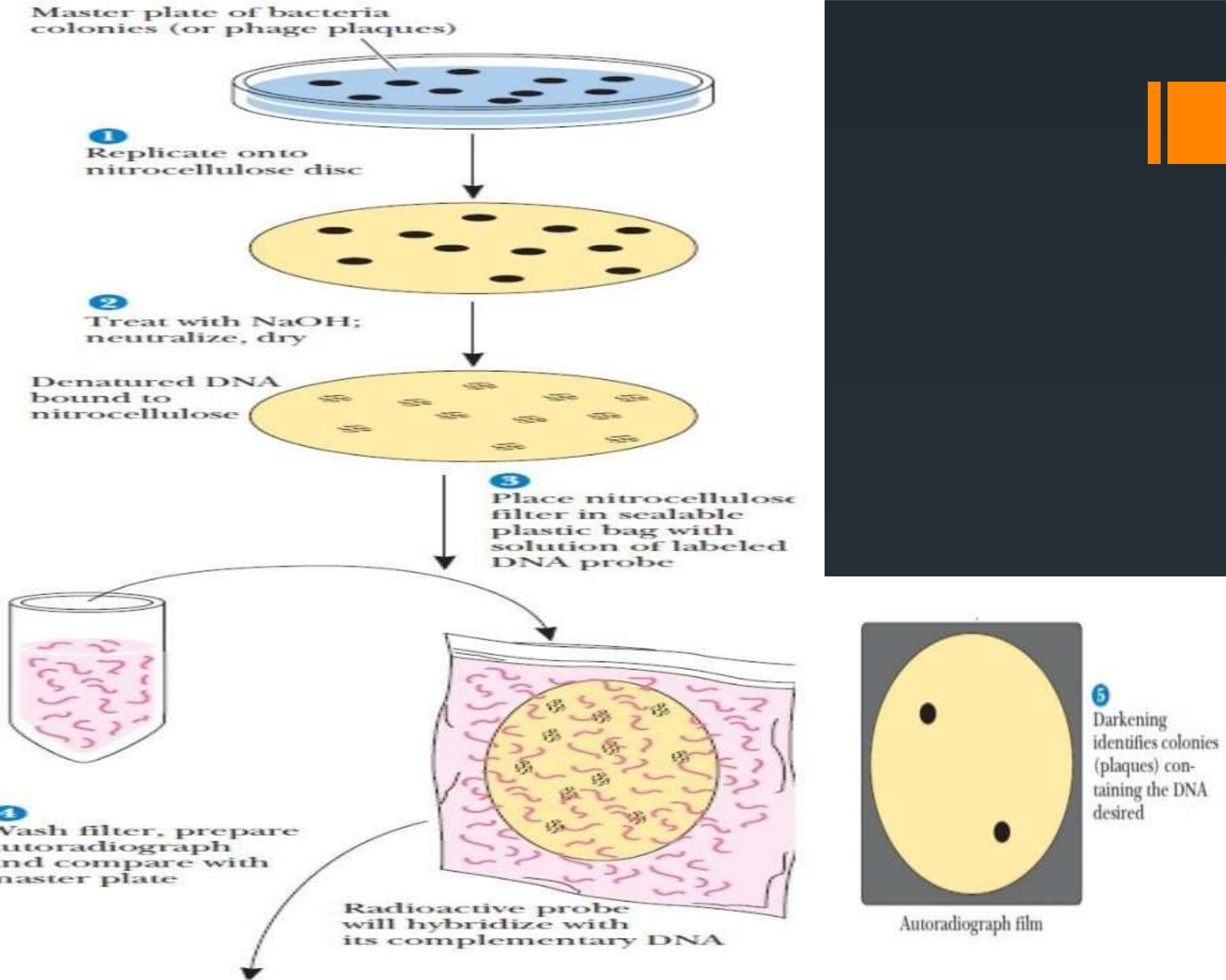
- Which enchances the capacity of constructing a genomic library,and also ease the problem of screening.(i.e..the higher the fragments,the smaller the number of clones).

Fig 6.showing the genomic library construction



SCREENING

- A common method of screening is the plasmid- based genomic libraries which is to carry out a colony hybridization experiment. Host bacteria containing either a plasmid based or bacteriophage-based library are plated out on petri dish and allowed to grow overnight to form colonies.


Master plate of bacteria colonies (or phage plaques)
1 Replicate onto nitrocellulose disc
2 Treat with NaOH; neutralize, dry
Denatured DNA bound to nitrocellulose
3 Place nitrocellulose filter in sealable plastic bag with solution of labeled DNA probe
4 Wash filter, prepare autoradiograph and compare with master plate
Radioactive probe will hybridize with its complementary DNA
5 Darkening identifies colonies (plaques) containing the DNA desired
Autoradiograph film

CONCLUSION

- In conclusion, genomic DNA segments can be organized in libraries known as genomic libraries and cDNA libraries with a wide range of designs and purposes.

THANK YOU