Course: M.Sc. Biotechnology

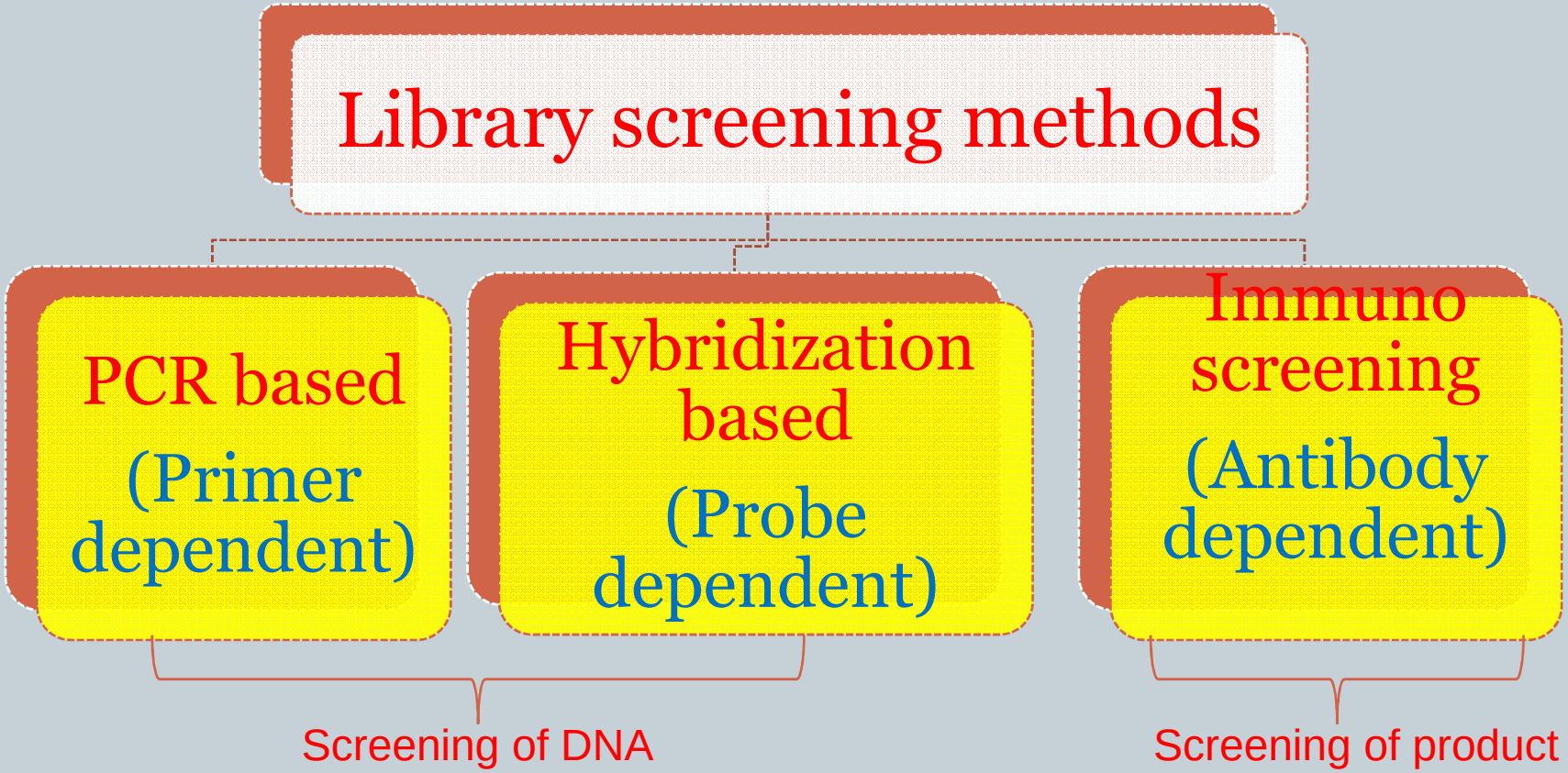
Paper: BIOT4009: Genetic Engineering and Gene Therapy

UNIT – IV
Gene library-2

BRIJESH PANDEY
DEPARTMENT OF BIOTECHNOLOGY
MAHATMA GANDHI CENTRAL UNIVERSITY, BIHAR

Gene library screening

Screening: Fishing the gene of interest from library



PCR based screening of library

- It can be used to screen all libraries
- It has same versatility as of hybridization based methods
- Instead of spreading on solid plate, libraries are maintained in pool of diluting subsets
- Pools are maintained in multiwell plates
- Gene specific primers (homo/ heterologous/ degenerate) are used for in situ amplification
- Positive wells are identified

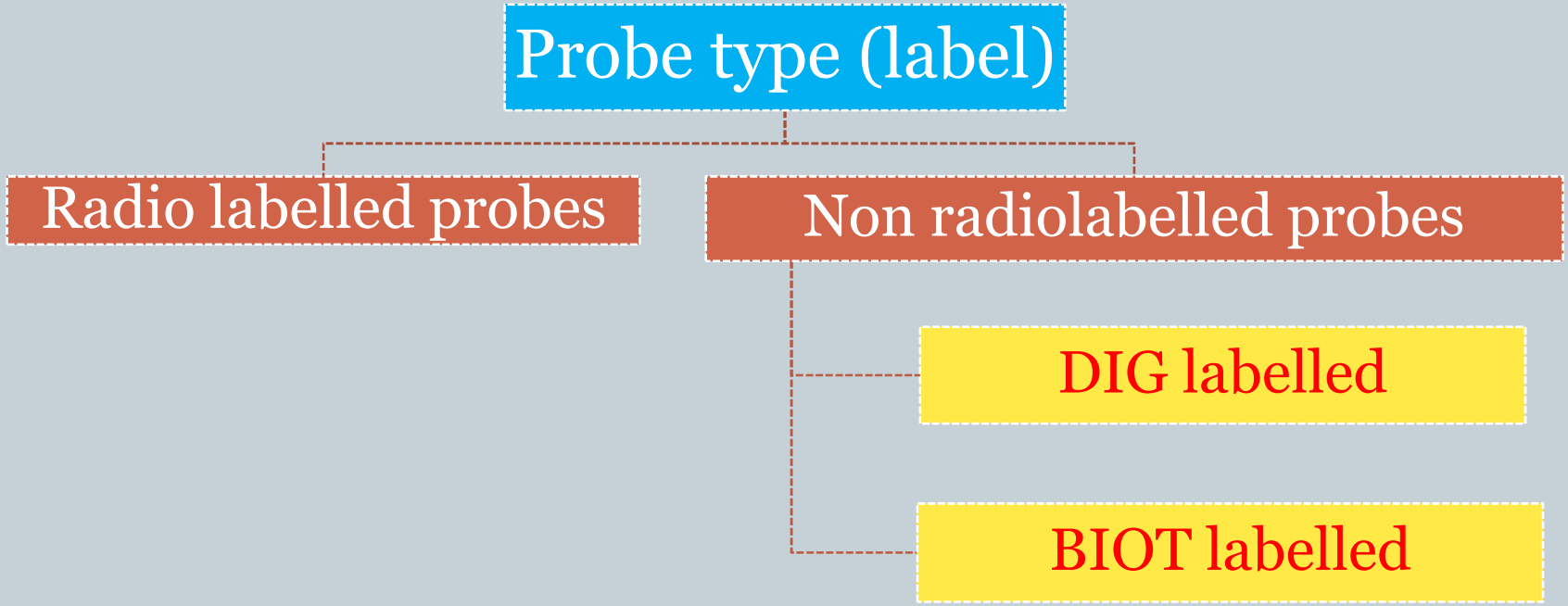
PCR based screening of library contd.

- Clones of positive wells are separately spread on solid plate
- Process continues till wells with homogeneous clones are identified

Hybridization based screening

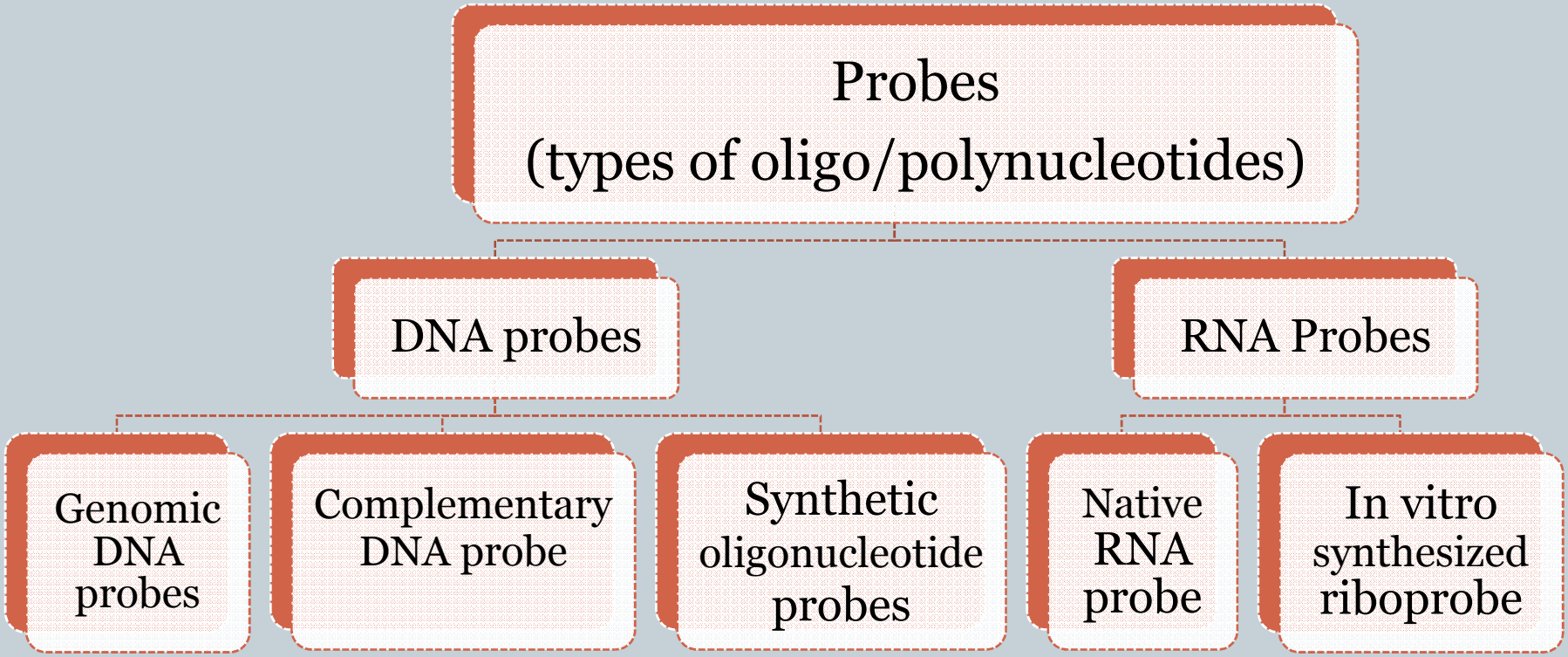
It is used for all kind of libraries and requires ‘PROBE’

PROBE-labelled oligo/ polynucleotide complementary to gene

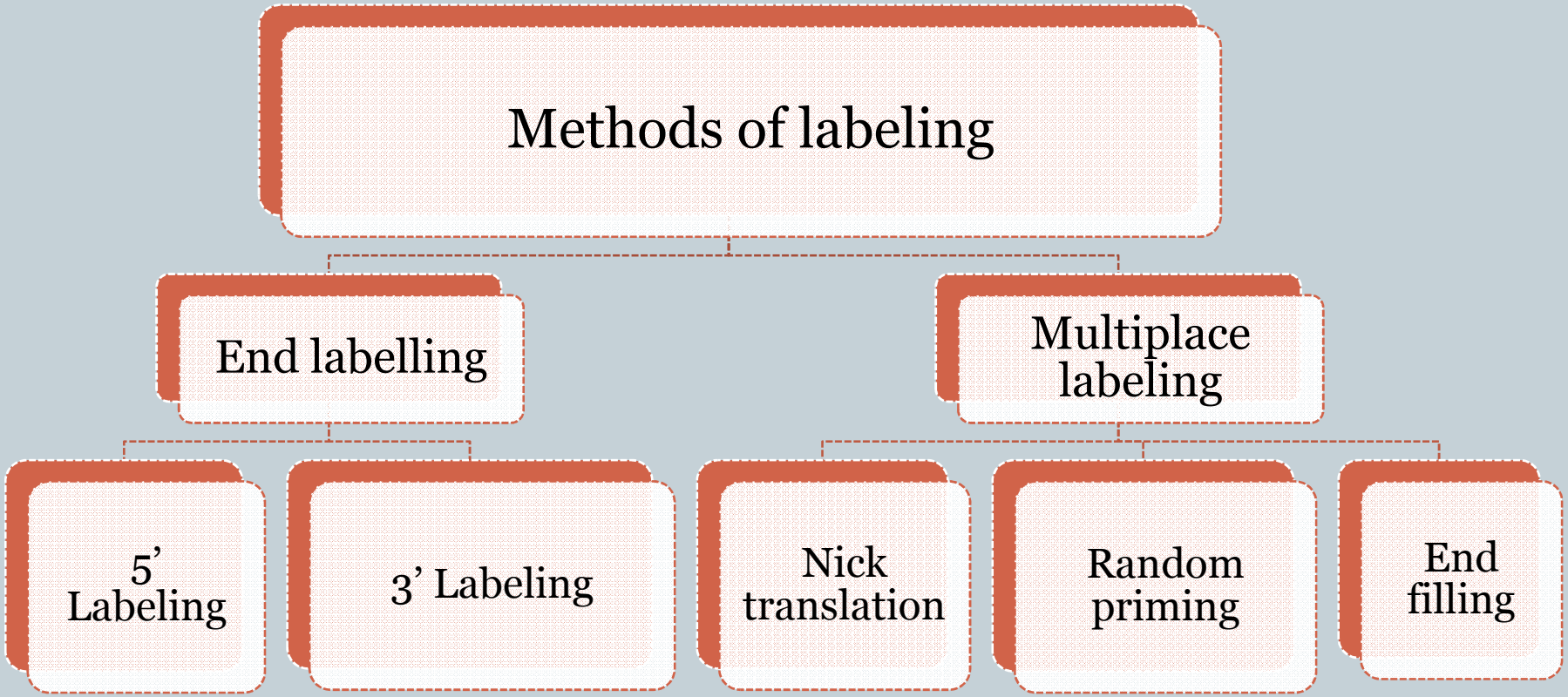


For labeling P^{32}, DIG, BIOT labeled NTPs are used in different labeling methods

Probe types

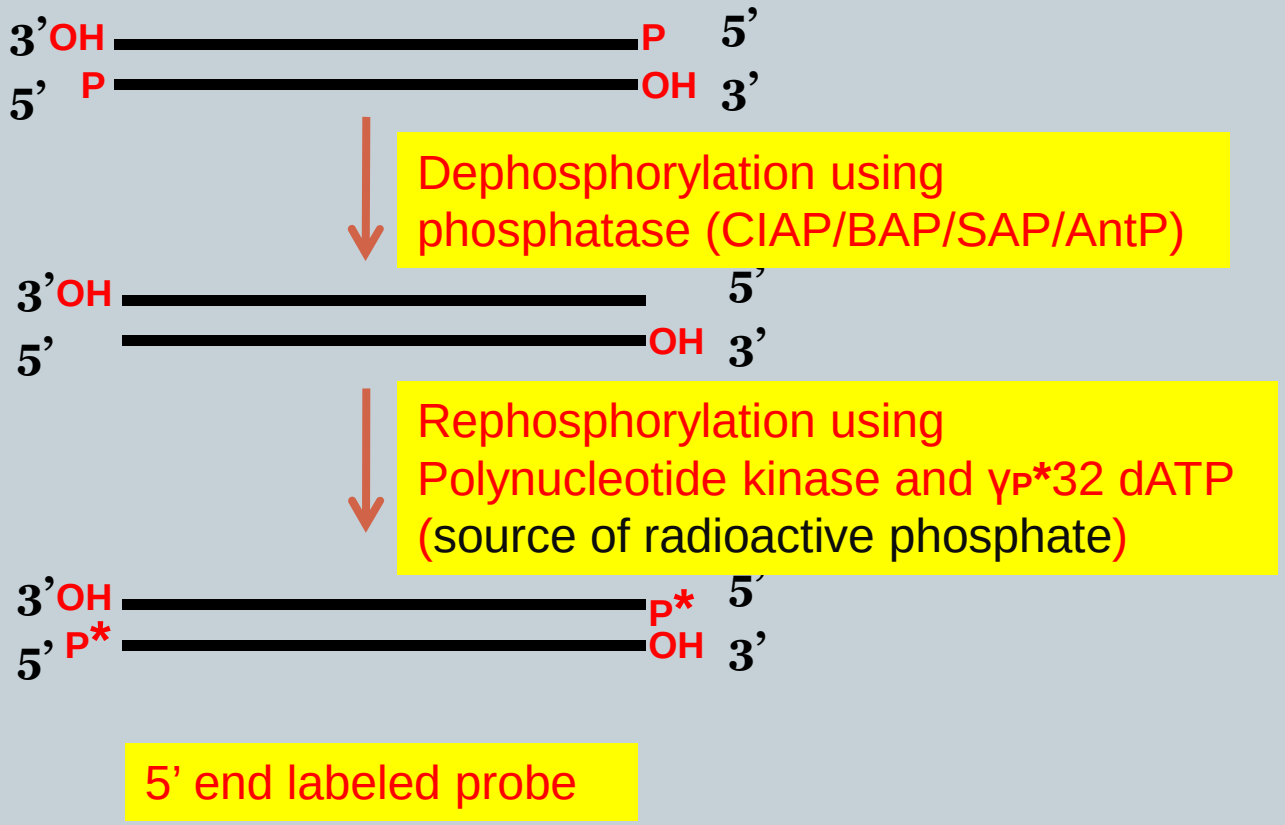

Probes
(types of oligo/polynucleotides)
DNA probes
RNA Probes
Genomic DNA probes
Complementary DNA probe
Synthetic oligonucleotide probes
Native RNA probe
In vitro synthesized riboprobe

Labeling methods



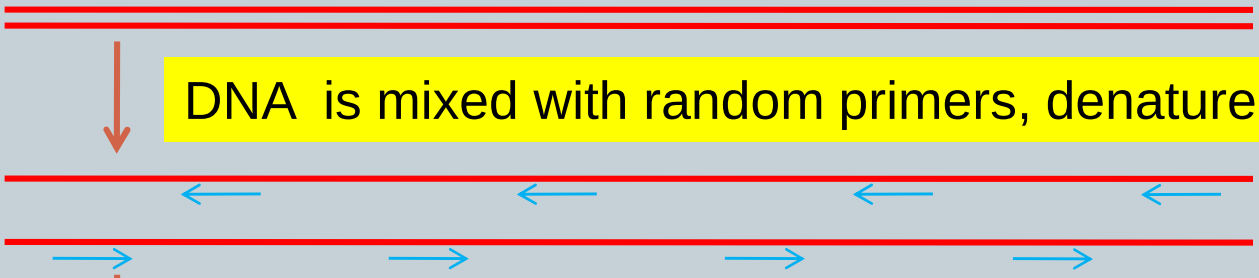
End labeling of probes

dsDNA is labeled at the end only



Random priming

Random primers are used to synthesize complementary strand in presence of radioactive nucleotide precursor and polymerase



DNA is mixed with random primers, denatured and renatured

Strands of template DNA with many primer binding sites on the two

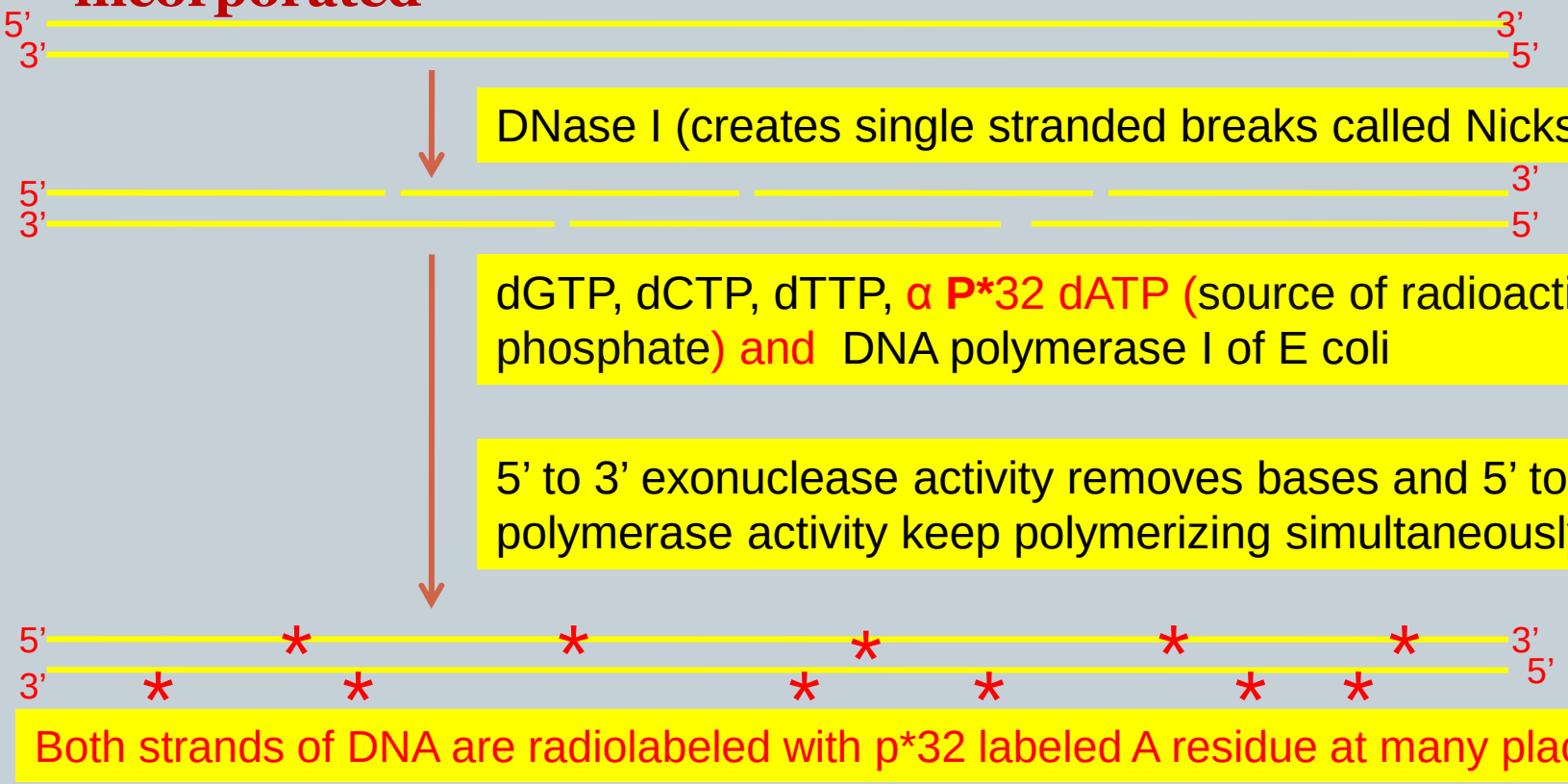
E coli DNA Polymerase 1, dGTP, dCTP, dTTP and α **P*** 32 dATP (source of radioactive phosphate), Buffer

Strand synthesized incorporate radioactive phosphate when Nucleotide A residue is incorporated

Both newly synthesized strands are labeled with P*32 at many places

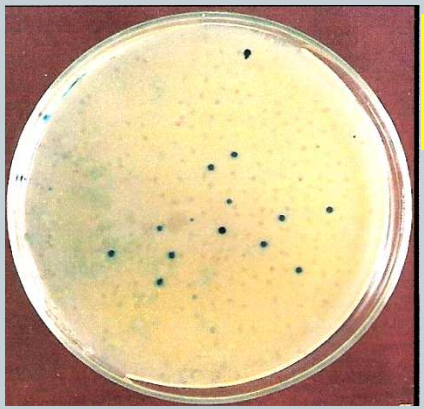
Nick Translation

Nicks are created in DNA and in presence of polymerase nick is translated and radiolabeled nucleotide residues are incorporated



Both strands of DNA are radiolabeled with p*32 labeled A residue at many places

Hybridization based screening

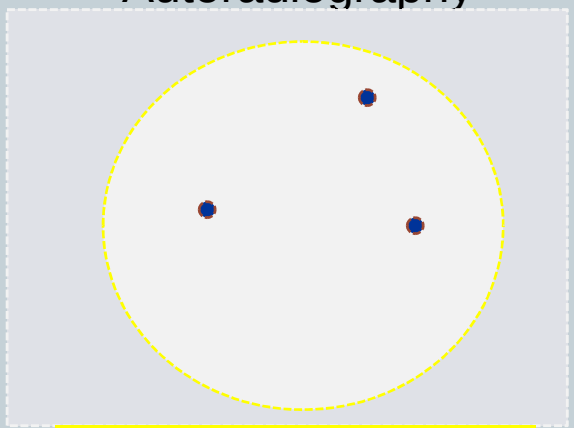


Library spread on agar plate

Hybridize with labeled denatured probe

Stringency washing

Autoradiography



Spots on X-ray film

Lyse cells, denature protein and DNA, immobilize on membrane

Block membrane with prehybridization buffer

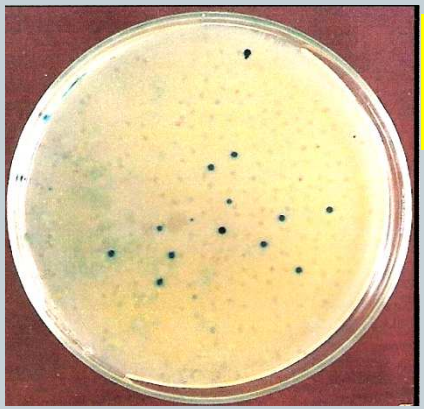
Lift plaques on Nitrocellulose paper

Spots showing positive signals are used to pick corresponding plaques/ clones from master plate (Library plate)

Immunological screening

- In is used to screen library where cloned genes are expressed
- Works well with cDNA library
- Antibodies are raised against proteins coded by target genes
- Antibodies are used to identify proteins expressed from target gene when library is spread
- Secondary antibody conjugated to enzyme is used to identify specific primary antibody
- Reactions catalyzed by conjugated enzyme are used to locate specific clone

Immunological screening



Library spread on agar plate

Lift plaques on Nitrocellulose paper

Block membrane with blocking agent like BSA/ Casein in PBS

Lyse cells, denature protein and DNA, immobilize on membrane

allowed to react with specific primary antibody

Washing allowed to react with secondary antibody enzyme conjugate

Washing and detection by reaction catalysis

Spots of product formation on membrane

Spots showing positive signals are used to pick corresponding plaques/ clones from master plate (Library plate)

References

- Primerose, Twyman and Old. Principles of gene manipulations (6th edition), Blackwell science.
- Maniates et al., Molecular Cloning vol 1-3
- Instruction manual, Stratagene Lamdba ZAP Express II cDNA Library construction kit

Thanks

PLEASE CONSULT MOLECULAR CLONING BY SAMBROOK ET AL.,

AND SUPPLEMENTARY STUDY MATERIAL PROVIDED FOR MORE DETAILS