by partial digestion.

③ complete digestion is avoided because it genrates fragments that are too heterogenous in size.

④ The fragments produced in partial digest with enzyme having 4-base recognition sites are more likely to be in appropriate size for cloning

⑤ Single or mixed digestions with the enzymes AluI (AG↓CT), HaeIII (GG↓CC) or Sau3A(↓GATC) have been used for construction of genomic libraries.

⑥ Use of restriction enzyme has advantage that the same sets of fragments are obtained from a DNA, each time specific enzyme is used.

⑦ The partial digests of genomic DNA are subjected to agrose gel electrophoresis or sucrose gradient centrifugation for separation.

⑧ Then mixture of the ~~size selected~~ fragments inserted into a suitable vectors.

⑨ The shotgun approch to gene cloning

⑩ Any vector most commonly used for DNA insert of 23-25kb in λ vector and cosmid. or high capacity vectors like BAC, PAC, YACs can be used.

(11) Recombinant genomic DNA libraries construction is used for transfection/transformation and multipled in the host field plaques or clones. (3)

(12) which are then stored in the amplified genomic library.

* cDNA library :-

(1) cDNA library is a population of bacterial transformants or phage lysate in which each mRNA isolated from an organism inserted in a plasmid or a phage vector.

(2) For this, the total mRNA extracted from a suitable organism/tissue.

(3) chromatography, on poly U-sepharose, enriches the preparations with mRNA of all kinds.

(4) Density gradient centrifugation is also used.

(5) protein produced by gene purified by specific antibodies.

→ Preparation of cDNA library :-

(1) cDNA is complementary DNA produced by using mRNA as a template.

(2) copies is produced with the help of enzyme reverse transcriptase.

(3) Eukaryotic mRNA used as template, a poly T-oligonucleotide is used as primer.

(4) These mRNA has poly(A) tail @ 3' end.

⑤ A poly(A) tail may added ⑥ 3'end by enzyme (4)
poly(A) polymerase
⑥ Reverse transcriptase extends 3'ends of mRNA.
⑦ Appropriate oligonucleotide primer is annealed with mRNA
⑧ This primer ~~using~~ will be base paired by using mRNA molecule as a template. This produces DNA·RNA hybrid molecules
⑨ RNA Strand is digested by RNaseH or alkaline hydrolysis
⑩ cDNA cloned in phage vector, afford high efficiency packaging in vitro as a result, gives large nos. of cDNA clones.
⑪ 10^5-10^6 cDNA clones are sufficient for isolation.

Properties of cDNA & cDNA library :-

1] Eukaryotic cDNA are free from intron sequences.
2] cDNA are smaller in size than corresponding genes.
3] A comparison of cDNA sequence with the corresponding genome sequence permits delineation of intron/exon boundries.
4] cDNA library from a single organism will vary widely depending on developmental stage & cell type.
5] cDNA library will be enriched for abundant mRNA, but may contain only a few or no clones representing rare mRNAs

* Application of cDNA library :- (5)

Use of cDNA absolutely essential when the expression of an eukaryotic gene is required in a prokaryotes.

1. Detection of clone
2. polypeptide products may be the primary objective of cloning.
3. Eukaryotic genes have intron, must be removed from transcript to yield mature mRNA
4. Bacteria do not possesses intron.

* Screening of Recombinants :-

When Recombinants DNA is constructed and used for transformation of E. coli cells.

1. Majority of the cells are non-transformed.
2. a proportion of the transformed cells. unaltered vector.
3. Remindes cells have recombinant DNA

A mixture of cells plated on suitable medium.

A clone containing a recombinant DNA molecules is called recombinant clones.

The two steps direct selection methods are

1. Reporter gene
2. Elimination of Non-transformed cells.

Reporter gene:-

- A marker gene or Reporter gene produces a phenotypes. which permits easy selection or quick identification of cells.
- Marker gene is either selectable or scorable
- A selectable marker governs a features, which enables only such cells that possesses it to survive under the selective conditions
- eg. genes confering resistance to antimetabolites like kanamycine – are good selectable marker. those cells have resistant gene survive & forms Colonies.
- Scorable Markers:- produce distinct phenotypes which allows easy identification of the cells. Such as gus (β-galactouronidase – produce blue color in the presence of appropriate substrate)
  lux (luciferase produce fluorscence)
  ocs (octopine synthase)
  nos (nopaline synthase! Both ocs & nos produce specific nitrogen compd called opines)
  GFP (green fluorscence proteins – green fluorscence on illumination).
- Scorable markers do not allow selective multiplication of cells having them.
- they only enable their easy identification.

Identification of Recombinant DNAs:-

Two steps consist of identification and isolation of those clones transformed by recombinant DNA this may be achieved by several ways:

(1) Vector with 2 selectable marker eg. pBR322 DNA insert placed in one of the amp^r gene. tet^r are used for elimination of non-transformed cells.

(2) A vector contain gene or a part of gene complements a function missing in their host cells. eg. gene lacZα – PUC vector such as lacZ$^-$ E.coli strains in which lacZα is deleted.

(3) ~~Recombinant~~ Insert DNA disrupts funct^n of lacZα in the recombinant DNA.

(4) DNA insert code for a gene product – defective in auxotrophic hosts cells. – a direct selection for recombinant vector is possible.
Hosts cells grow – on medium lacking auxotrophic host.
recombinant vector containing colonies grow only.

(5) selection by suppression of nonsense mutation present in the host – also permit direct selection.

(6) λ – retain lysogenic function as well eg. λgt10 DNA insert placed in λ lysis repressor gene CI – recombinant become CI$^-$.

Selection of clone containing a specific DNA insert!-

Steps to identify clones - has DNA insert. identification has to be highly precise and extremly sensitive to allow accurate detection.

library Screening Strategies - ① sequence dependent Screening - Nucleic acid in colony hybridization / PCR / chromosome walking / Jumping.

② protein based screening - differential electrophoretic mobility.

③ Immunological Screening

In hybridization probing:- suitably labelled DNA/RNA probe used for hybridization with recombinant clones in genomic library.

~~Result: Hence we find A^2, A^3, A^4, A^5 for the matrix~~

```
>> A^5

ans =

    21041    29102    37163
    30282    41883    53484
    48764    67445    86126
```

Sequence dependent Screening :-

① Colony hybridization :-

efficient & rapid Strategies For identification of a clones having desired insert we technique of colony hybridization.

(A) Practical No. 04

Aim: To find A^2, A^3, A^4, A^5 for the matrix

$$A = \begin{bmatrix} 1 & 2 & 3 \\ 2 & 3 & 4 \\ 4 & 5 & 6 \end{bmatrix}$$

MATLABProgramme:

```
>> A=[1 2 3;2 3 4;4 5 6]

A =

     1     2     3
     2     3     4
     4     5     6

>> A^2

ans =

    17    23    29
    24    33    42
    38    53    68

>> A^3

ans =

   179   248   317
   258   357   456
   416   575   734

>> A^4

ans =

  1943  2687  3431
  2796  3867  4938
  4502  6227  7952
```

Blotting:

E.M. Southern.

DNA-DNA-hybridization.--That forms its basis.

Sample of DNA with different size is subjected to electrophoresis

DNA may be subjected to restriction digestion in order to genrate the fragments.

Agarose gel is useful in separating DNA fragments of few hundred to 20kb in size.

Very large fragments 1000-2000kb separated in agarose gel electrophoresis.

- Restriction fragments of DNA in agarose gel denatured into single stranded form by alkali treatment.
- then transfered onto nitrocellulose filter membrane done by placing the gel on top of buffer saturated filter paper,

laying nitrocellulose filter membrane on the top of gel.

finally some dry filter papers on the top of memb.

buffer moves due to capillary action.

DNA become trapped in nitrocellulose paper. as buffer passes through it.

relative position of nitrocellulose is not disturbed.

DNA on nitrocellulose paper is immobilised by backing it at 80°C. in vacuo.

- ssDNA has high affinity for nitrocellulose memb.

(A) Practical No. 05

Aim: To find AB and BA for the given matrices.

$$A=\begin{bmatrix} 4 & 1 & 3 \\ 2 & 1 & 5 \\ 2 & 2 & 7 \end{bmatrix} \quad B=\begin{bmatrix} 1 & 6 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

MATLABProgramme:

```
A =

     4     1     3
     2     1     5
     2     2     7

>> B=[1 6 0;0 1 0;0 0 1]

B =

     1     6     0
     0     1     0
     0     0     1

>> A*B

ans =

     4    25     3
     2    13     5
     2    14     7

>> B*A

ans =

    16     7    33
     2     1     5
     2     2     7

>>
```

Result: AB≠BA

DNA fingerprinting:-

Repoat assay :-

S1 Mapping :-

1. Method used to locate 5'end of a transcript in a mixture of RNA wing nuclease S1
2. S1 Nuclease mapping is a nuclease protection assay wing Nuclease S1.
3. This technique is used to quantify and map RNA transcripts.
4. It is capable to identify individual RNAs in a mixture of RNA sample of known sequence.
5. It can particularly map introns and 5' and 3' ends of transcribed gene regions.
6. As it is capable of quantifying the amount of mRNA, it can therefore identify the level of transcription of the gene in the cells.
7. Apart from this, it can also determine RNA interference since it can detect the presence of double stranded RNA.
8. The disadvantage though with S1 nuclease mapping is its limitation of the size of initial probe because the destruction of the non-hybridized RNA during nuclease digestion.

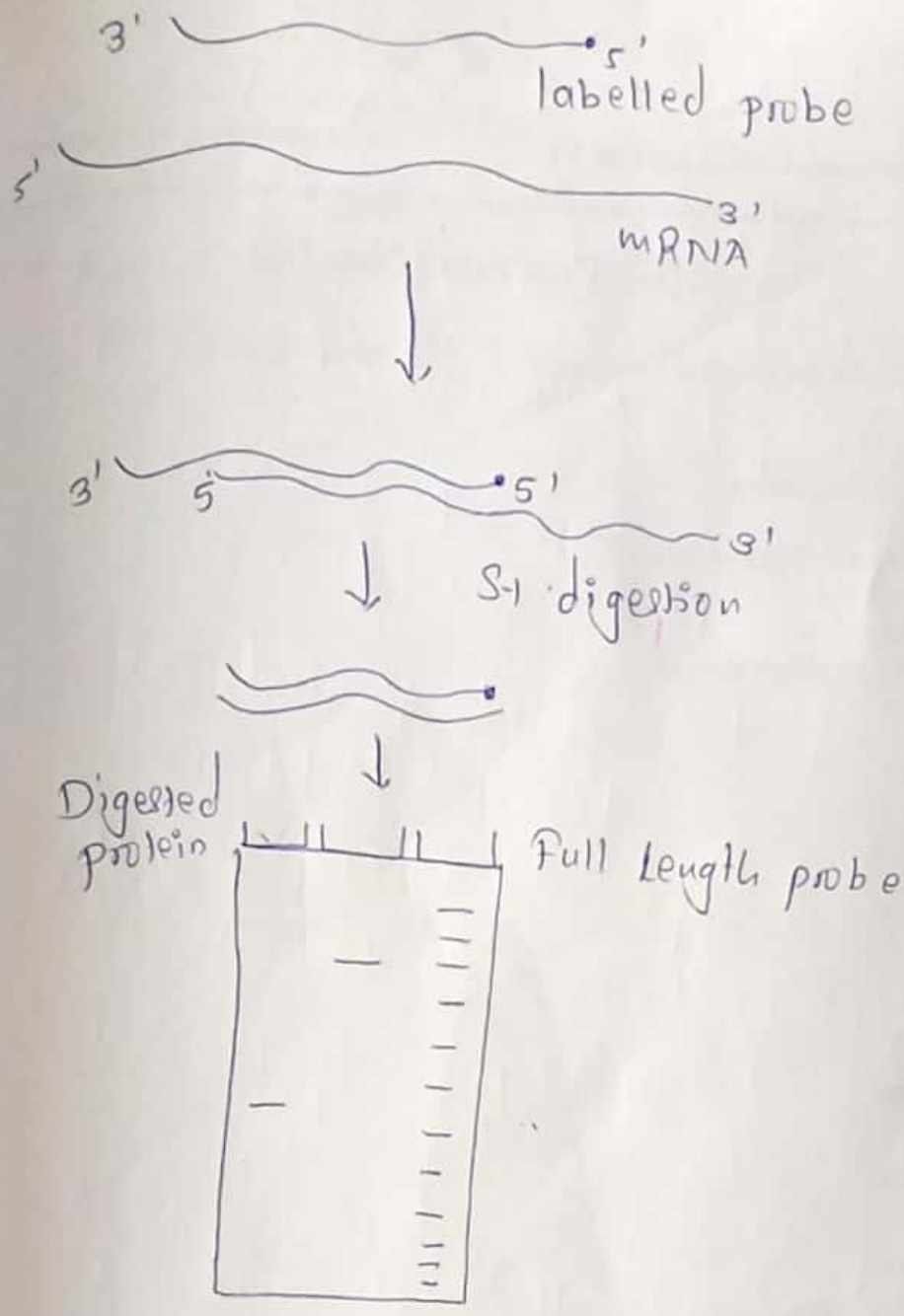


Fig:- S-1 mapping.

people were working on structure of eukaryotic genes searched for splicing of cellular RNAs. Within a very short time after the discovery of adenovirus splicing, the coding sequence of β-globin, Ovalbumin, and immunoglobulin genes were also interrupted by non-coding DNA.

Chromosomal genes were spliced come initially electron microscopy.

The size and location of these introns were then estimated by a technique called S1 nuclease mapping.

Nucleic Acid:→

* In the mid 1960s - sanger had stopped sequencing of protein procedure and turned his attention to working out fast, simple procedures for sequencing long stretches of RNA.

Advent of method allowed sequencing of fragments of DNA of from 100-500bps

Sanger devised first of these direct DNA sequencing methods.

It is based on elongation of DNA chain with DNA polymerase.

With this technique 5386bps sequence of the small DNA phage ϕX174 was quickly determined.

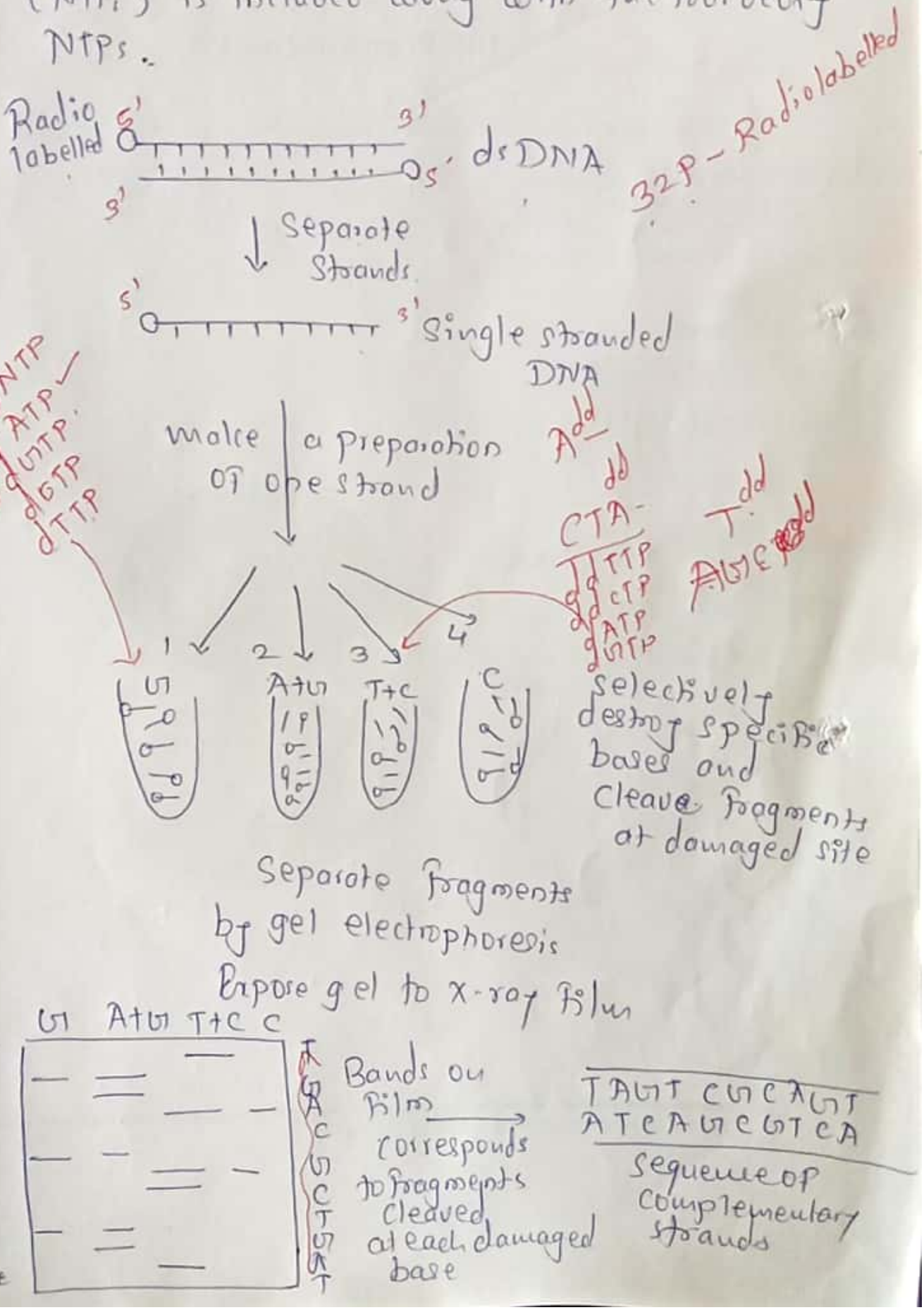
He devised second method for sequencing DNA and again, he used enzymatic rather than chemical, technique.

Specific terminators of DNA chain elongation 2'3' dideoxynucleoside triphosphate - were synthesized.

These ddNTPs incorporated normally into growing chain of DNA through their 5'-triphosphate groups.

Next incoming deoxynucleotide triphosphate (dNTP)

When small amount of a specific dideoxy (NTP$_i$) is included along with the four deoxy NTPs.



The maxam and Wilbert DNA Sequencing procedure

Segements of DNA is labelled at one end with ^{32}P.

The labelled DNA is divided into four samples and each sample is treated with chemical that specifically destroys one or more of the four bases in the DNA.

The conditions of the reaction are controlled so that only a few sites are nicked in any one DNA molecule.

When these nicked molecules are treated with piperidine, the DNA backbone is broken at site at which the base had been destroyed.

This generates the series of labelled fragments the lengths of which depend on the distance of the destroyed base from the labelled.