Chromosome walking, Chromosome jumping, Transposone tagging, Map based cloning

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Chromosome Walking

•This technique is used for characterising large regions of chromoosmes. Using the conventional cloning techniques to isolate fragments, one may be able to characterise about a 100 kb chromosome region per month.

•Generally a cosmid library is used for chromosome walking; each clone in a such a library may be expected to have a DNA insert of, on an average, 40 kb.

• In chromosome walking, one begins with a DNA fragment that contains a known gene/genetic marker, e.g., an RFLP marker.

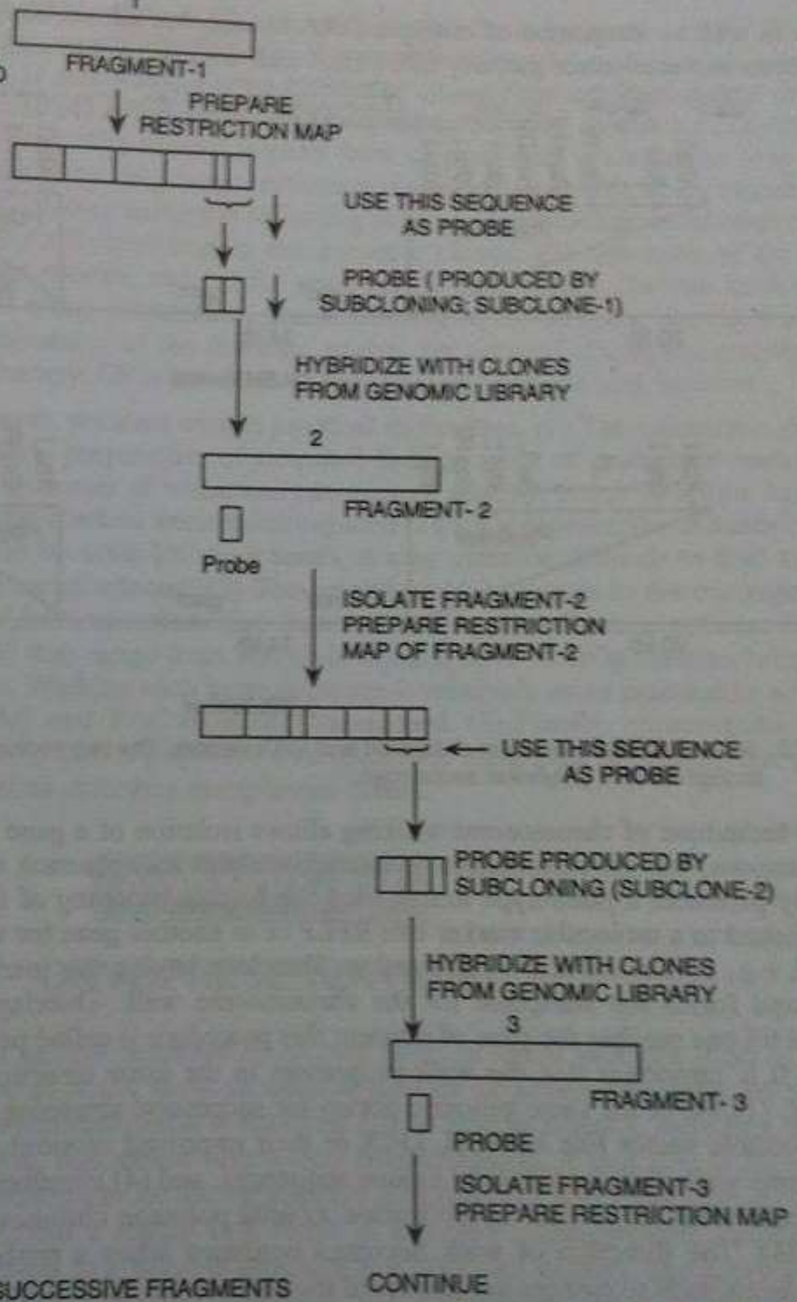
•The sequence located at one end of this fragment is used to identify a clone that has such a DNA insert, which partly overlaps the first segment.

•Now the other (non-overlapping) end of this new DNA fragment is used as a probe.

- In this way, one continues to move step-by-step toward a gene of interestlocated close to the known gene/genetic marker.

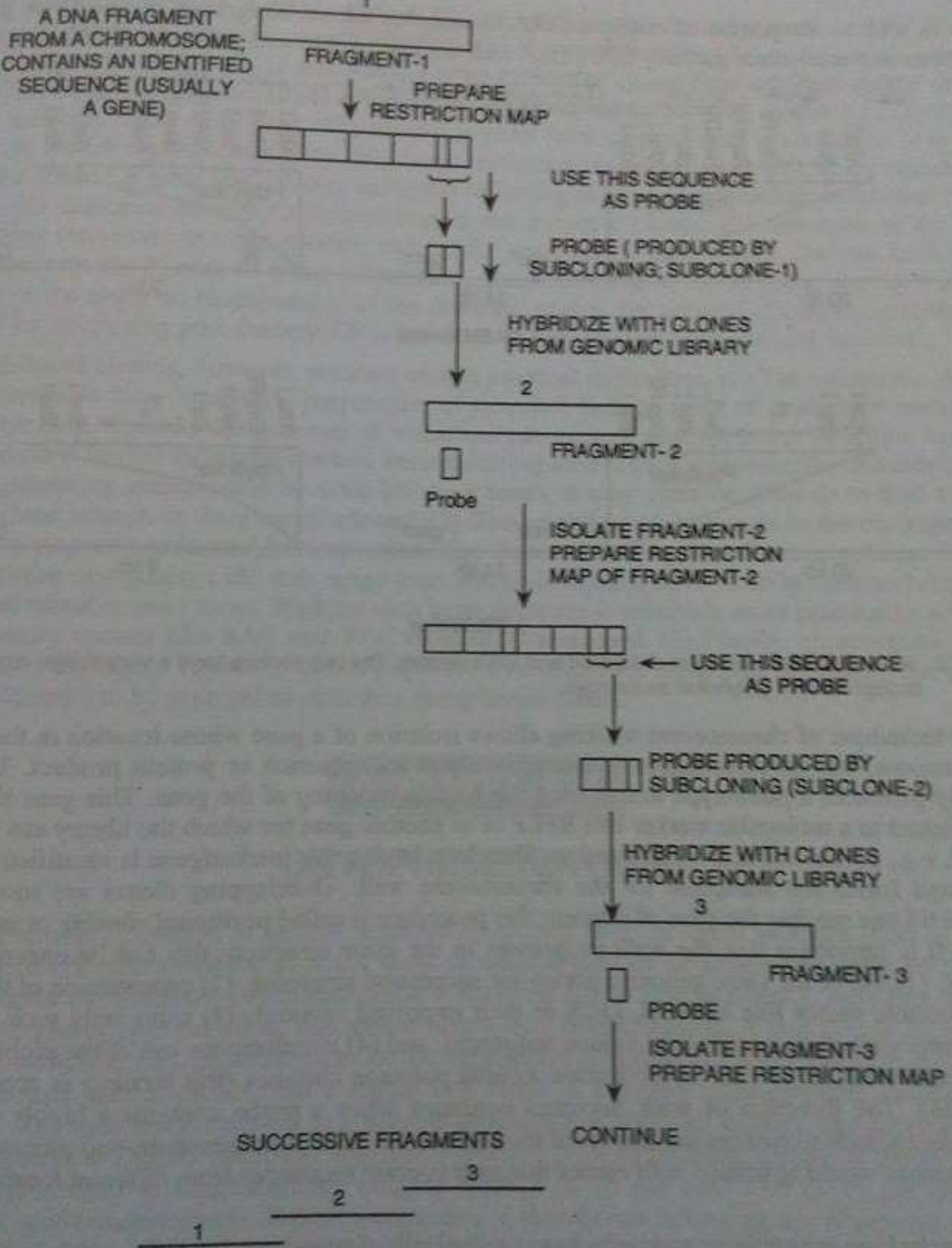
- This technique, therefore, is called chromosome walking because each clone takes the researcher one step closer to the gene of interest.

- A generalised procedure for chromosome walking is as follow:



- The first step in 'walking' a chromosome consists of the isolation of a DNA fragment (fragment-1) containing a known gene or marker located near some region of interest in the given chromosome.

This fragment provides the starting point for the 'chromosome walk'; it also provides a point of reference in the genetic map.



A restriction map is prepared. A small segment representing one end of this original fragment (fragment 1) is isolated and cloned; this is called subcloning. The subcloned sequence need not to be the end segment of the fragment; it should, however be located close to the end.

This subclone is now used as a probe for the identification of such clone(s) in the genomic library that overlap fragment 1.

A DNA FRAGMENT FROM A CHROMOSOME; CONTAINS AN IDENTIFIED SEQUENCE (USUALLY A GENE)

1

FRAGMENT-1

PREPARE RESTRICTION MAP

USE THIS SEQUENCE AS PROBE

PROBE (PRODUCED BY SUBCLONING; SUBCLONE-1)

HYBRIDIZE WITH CLONES FROM GENOMIC LIBRARY

2

FRAGMENT- 2

Probe

ISOLATE FRAGMENT-2 PREPARE RESTRICTION MAP OF FRAGMENT-2

USE THIS SEQUENCE AS PROBE

PROBE PRODUCED BY SUBCLONING (SUBCLONE-2)

HYBRIDIZE WITH CLONES FROM GENOMIC LIBRARY

3

FRAGMENT- 3

PROBE

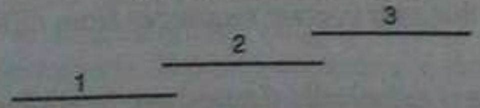
ISOLATE FRAGMENT-3 PREPARE RESTRICTION MAP

CONTINUE

SUCCESSIVE FRAGMENTS

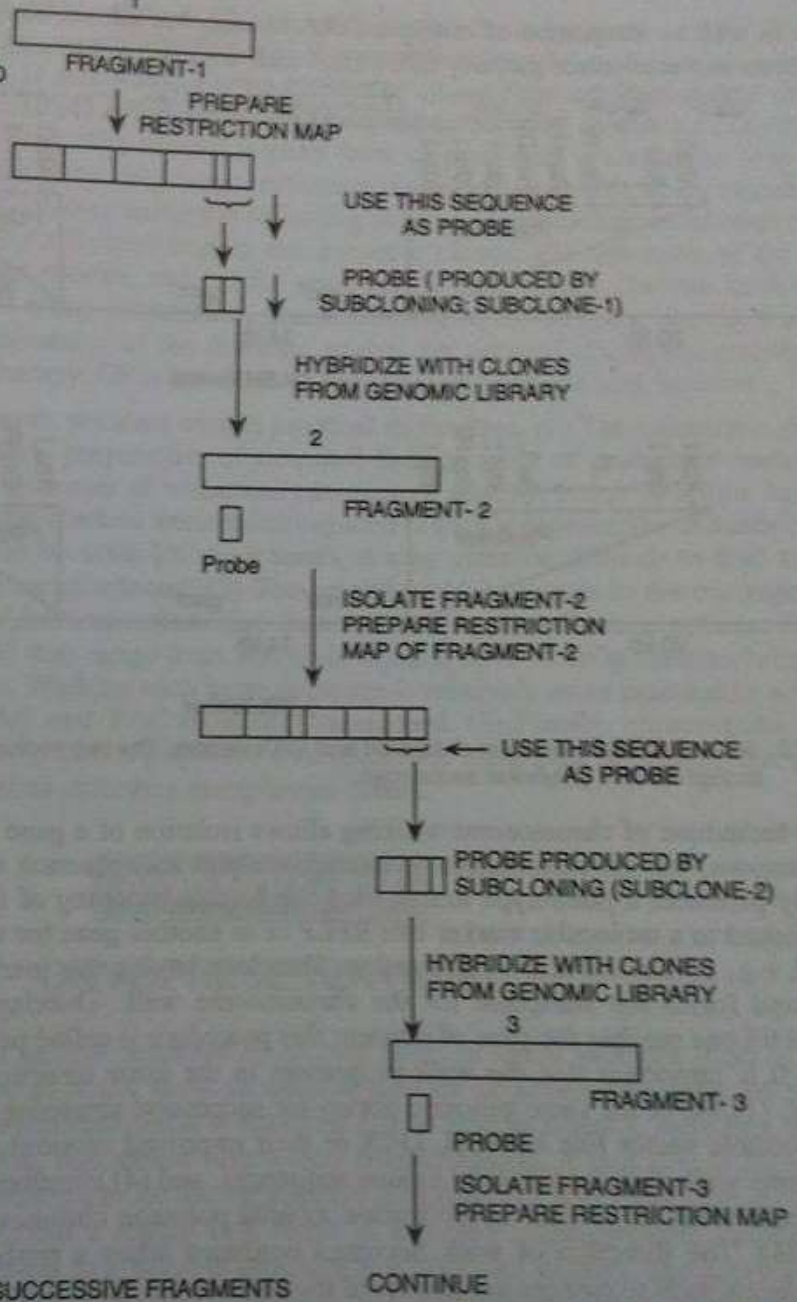
3

2



The clone(s) identified in this way will contain such a DNA insert that overlaps fragment-1 preferably at one end; the new genomic fragment may be referred to as fragment 2.

A restriction map of fragment 2 is prepared, and the sequence at the other end of this fragment is now used as a probe to identify clones having DNA inserts overlapping with fragment 2.



The DNA inserts obtained from such clones will be overlapping with fragment 2 preferably at one end; this new genomic fragment may be called fragment 3.

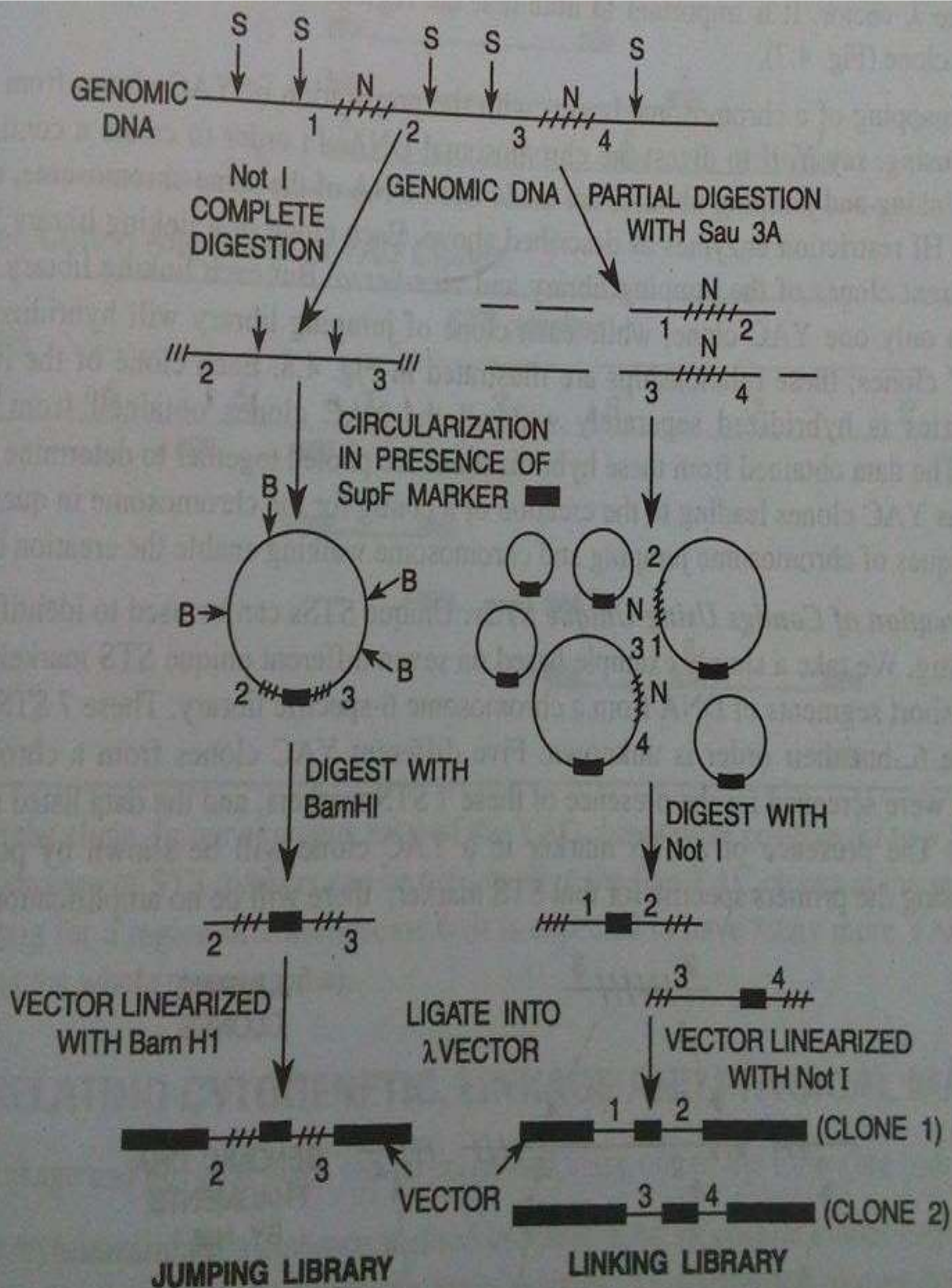
This process of step 3 is repeated till we reach one end of the chromosome.

Chromosome Jumping

•The techniques of chromosome jumping and chromosome walking are used for the construction of restriction maps of genomes.

•Chromosome jumping is used for rather large DNA fragments, usually of several hundred kb in size, while chromosome walking is applicable to much smaller DNA fragments.

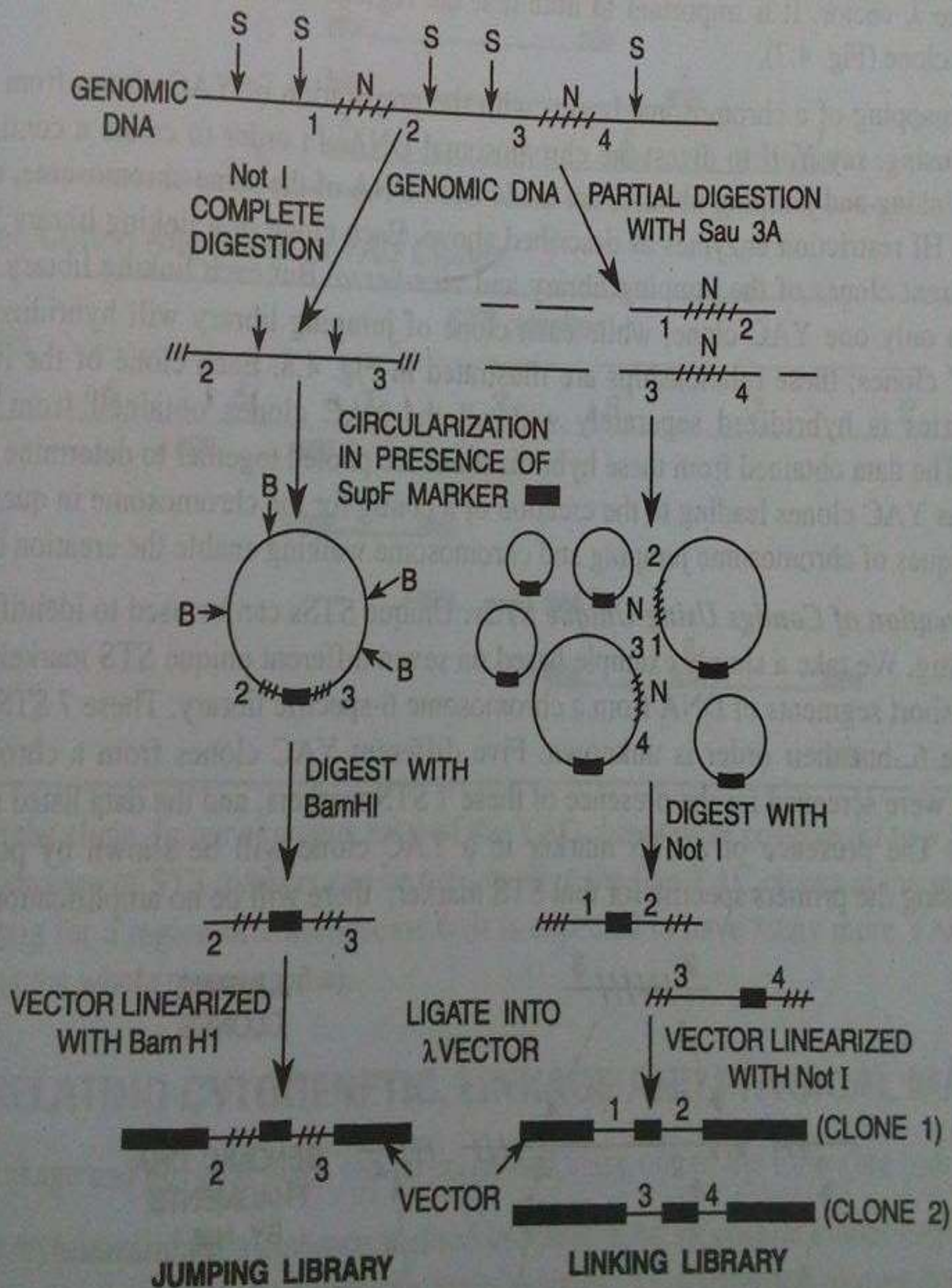
•A simple approach to chromosome jumping uses ‘jumping’ and ‘linking’ libraries generated by a rare cutting enzyme, e.g. Not I for humans.



•Each clone in a jumping library contains the DNA sequences on one side each, e.g. sequences 2 and 3, of two neighbouring cutting sites of enzyme Not I, while a clone in linking library has the DNA sequence located on either side of a single Not I site, e.g. regions 1 and 2 in clone 1 and regions 3 and 4 in clone 2.

The circularized fragments are digested with a frequent cutting enzyme like BamHI to delete bulk of the DNA leaving only a small DNA sequence on either side of the supF marker. The resulting fragments are ligated in a suitable λ vector and cloned.

•The procedure for construction of a Not I jumping library is, in simple terms, as follows. Genomic DNA is completely digested with Not I, and the resulting fragments are circularized in the presence of supF marker



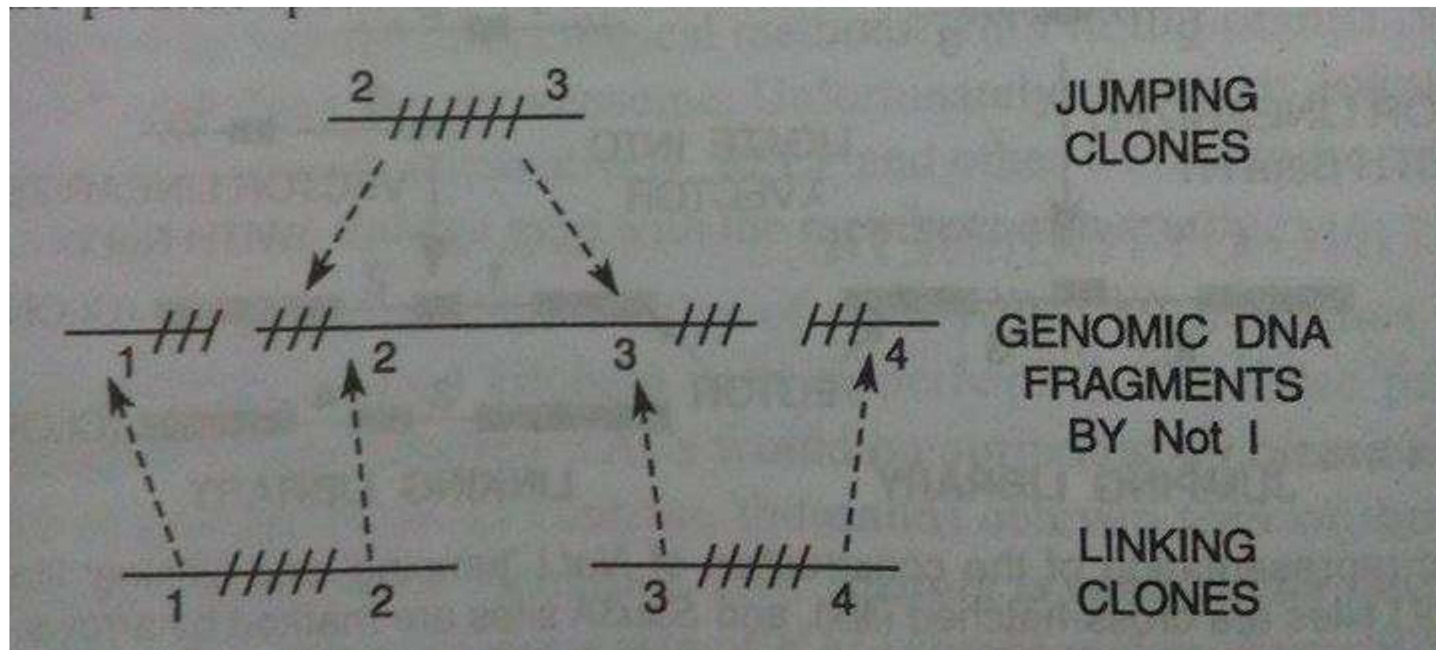
- Similarly, the linking library clones are prepared. The genomic DNA is partially digested with a frequent cutting enzyme like Sau 3A and the fragments are circularized in the presence of supF marker.

- The circularized fragments are cut open with the enzyme Not I, and the linear fragments are integrated in a suitable λ vector. Only those circles that have a Not I site will become linear and hence, integrate in the λ vector. It is important to note that the regions surrounding a single Not I site are present in one clone.

- Physical mapping of a chromosome begins with the preparation of a YAC clones from the target chromosome, using, say NotI to digest the chromosomal DNA.

- In order to create a contig of these YAC clones, linking and jumping libraries are made from DNA of the same chromosome, using, say, NotI and BamHI restriction enzymes as described above.

- Each clone of a linking library hybridizes with two different clones of jumping library and vice versa.



- Each clone of the linking and jumping libraries is hybridized separately with all the YAC clones obtained from the target chromosome.

- The data obtained from these hybridizations are pooled together to determine the correct order of various clones leading to the creation of a contig for the chromosome in question.

- Thus both the techniques of chromosome jumping and walking enable the creation of contigs.

Transposon Tagging

•Transposons are mobile genetic elements that move (transpose) from one region of the genome to another.

•Transposons are used as tools for gene cloning because insertion of a transposon into a gene disrupts its function, often producing a visible mutant phenotype.

•When the DNA sequence of the transposon is known, it is possible to clone the disrupted gene by using the transposable element as a "tag" to identify the segment of DNA harboring the element.

• Transposon tagging involves inducing transposition, screening for mutations caused by transposon insertion, identifying the element causing the mutation, and cloning the tagged gene.

•Transposons are found in almost all organisms where they have been looked for, including bacteria, yeast, plants, and mammals.

• Transposons from one organism often also transpose in heterologous systems. Therefore it is possible to use transposon-tagging methods to clone genes in a wide variety of organisms, including those where there are few other tools available for gene isolation.

Characteristics of Transposons Relevant to Tagging

•There are many families of transposons**,** and they are generally divided into two classes.

• Class I is the retrotransposons, and Class II is the DNA-based transposons. Class I transposons are used for tagging in mammals and yeast, and class II transposons are those used most frequently in tagging schemes for bacteria, plants, Drosophila, and Caenorhabditis elegans.

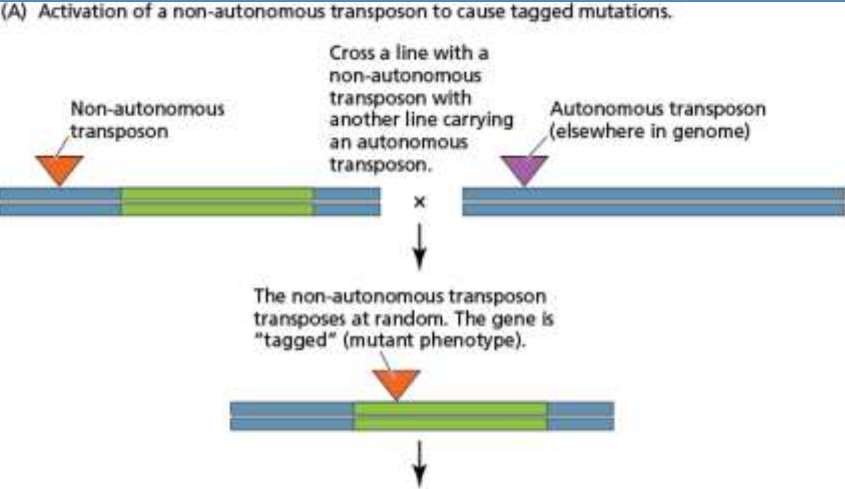
• Class II elements have inverted repeats at their ends and produce the products needed for their own excision and insertion, termed transposase.

- Transposable elements can change their genomic location upon activation of their transposase, causing transposition of the element.

- If the activated transposon inserts inside the coding or regulatory sequence of a gene, disruption of the reading frame can lead to a loss of gene function.

- Transposons can therefore be mutagenic. Genes that are disrupted with a transposon of known sequence are much easier to map than are mutants that arise from just a single nucleotide change.

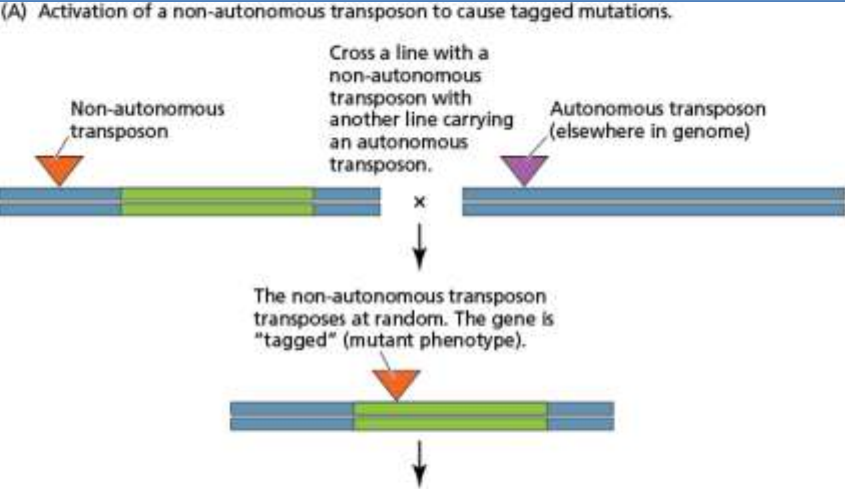
- To systematically use transposons in a mutagenesis experiment, genetic lines with inactive or **non-autonomous transposons** are crossed with lines containing an active or **autonomous transposon**.



- The offspring carrying an autonomous transposon that is transcribing transposase can mobilize the **non-autonomous transposon**.

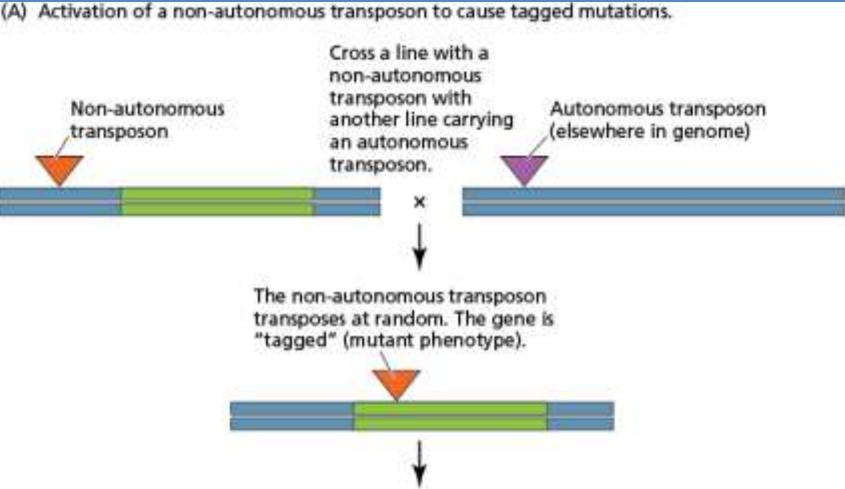
- The reactivated transposon is now free to insert randomly into new sequences within the genome, causing mutations in the offspring.

- If the mutation is caused by the **non-autonomous** transposon copy, the mutation will be stable in subsequent generations if the **autonomou**s element can be segregated away.



- Since the transposon is found on only one copy of a chromosome (one could say it is hemizygous), one can select for offspring that do not carry the active transposon, yet have sustained a mutation from the non-autonomous element.

- Hundreds or even thousands of individuals from the next generation can then be screened for a new mutation of interest.



- What makes the tagging approach so powerful is the relative ease with which the newly transposon-disrupted gene can be identified in the screening population.

- Since the sequence of the transposon used for the tagging approach is known, primers can be synthesized matching the transposon. Using **inverse PCR**, the regions adjacent to the mutation-inducing tranposon can be identified.

Map Based Cloning

•The first step of map-based or positional cloning is to identify a molecular marker that lies close to you gene of interest.

•This procedure typically is done by first finding a marker in the vicinity of the gene (several cM away).

•For the initial screening smaller population sizes are used (60-150 individuals). The next step is to saturate the region around that original molecular marker with other markers.

•At this point you are looking for a one that rarely shows recombination with your gene. At this stage, the population size could increase to 300-600 individuals.

•The next step is to screen a large insert genomic library (BAC or YAC) with your marker to isolate clones that hybridize to your molecular marker.

•Once you identify the initial markers that map are near (or better yet) flank your gene and found a clone to which the markers hybridize, you are on your way to determining where that gene resides.

• The steps that follow are termed **chromosomal walking**.

•This procedure involves creating new markers (usually sequences at the end of the clone) and screening your segregating population with these new markers.

• Often this population is large (1000-3000 individuals). The goal is to find a set of markers that co-segregate (no recombination) with your gene of interest.

•**Co-segregation** means that whenever one allele of your gene is expressed, the markers associated with that allele are also present. In other words, recombination is not seen between your gene and the markers.

- If these markers do not co-segregate, you select new large insert clones and repeat the process until you have a clone whose markers co-segregates with your gene.

- To speed the cloning process, it is best to begin with a marker that is tightly linked to the gene with which your are working. Therefore you will not have to do a lot of additional screening.

- Because you have your gene flanked on a single clone between two markers, you now know that the gene must be between those two markers.

- DNA fragments between the flanking markers are cloned and introduced into a genotype mutant for your gene by a genetic engineering technique called **plant transformation**.

- If **transgenic plant** expresses the wild type phenotype, you then know the gene of interest is on that fragment. At this point you must sequence the fragment to find a potential open reading frame (ORF), sequences that most likely will encode a gene product.

- In the best situation, only a single ORF is found, but this often is not the case.

- Usually several possible ORFs are found and new transgenic plants are created by transforming with a single ORF.

- Once this ORF is shown to rescue the mutant phenotype, you then perform an in-depth molecular and biochemical analysis of newly cloned gene.

These steps can be summarized as follows:

•Identify a marker tightly linked to your gene in a "large" mapping population

•Find a YAC or BAC clone to which the marker probe hybridizes

•Create new markers from the large-insert clone and determine if they co-segregate with your gene

•If necessary, re-screen the large-insert genomic library for other clones and search for co-segregating markers

•Identify a candidate gene from large-insert clone whose markers co-segregate with the gene

•Perform genetic complementation (transformation) to rescue the wild-type phenotype

•Sequence the gene and determine if the function is known

•**Example of Map-based Cloning is the Cloning of the tomato Pto gene which is the first example of map-based cloning in plants.**

Thank You