CHROMOSOME WALKING

(NOTES)

Chromosome walking is a method of positional cloning used to find, isolate, and clone a particular allele in a **genomic library**.

> ***Genomic Library:*** *A genomic library is the term for collections of DNA fragments that have been cloned randomly from the genomes of organisms. They are cloned as inserts in plasmids or in the phages (parasitic virus that feeds on bacteria).*
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> *When cloned as plasmids, the collection is of the host cells, containing the target DNA insert. Thus, a genomic library is a collection of tens of thousands of bacteria colonies, each modulated with a fragment of DNA from the organism that contains a gene of special interest. When genomic libraries contain a collection of all the genetic information on an organism, they are considered to be a representational genomic library.*
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> *Phage genomic libraries are made up of what are called phage lysates, which are residues of destroyed cells with a DNA fragment inserted.*

Chromosome walking is conducted to isolate a particular allele (say **gene B**) for a genetically transmitted disease in the vicinity of a previously mapped sequence (say **gene A**).

_____**A**_____**B**_____

An **allele** is a gene for a particular genetic trait passed on from adults to their offspring, such as the allele for brown eyes in a gene for eye colour.

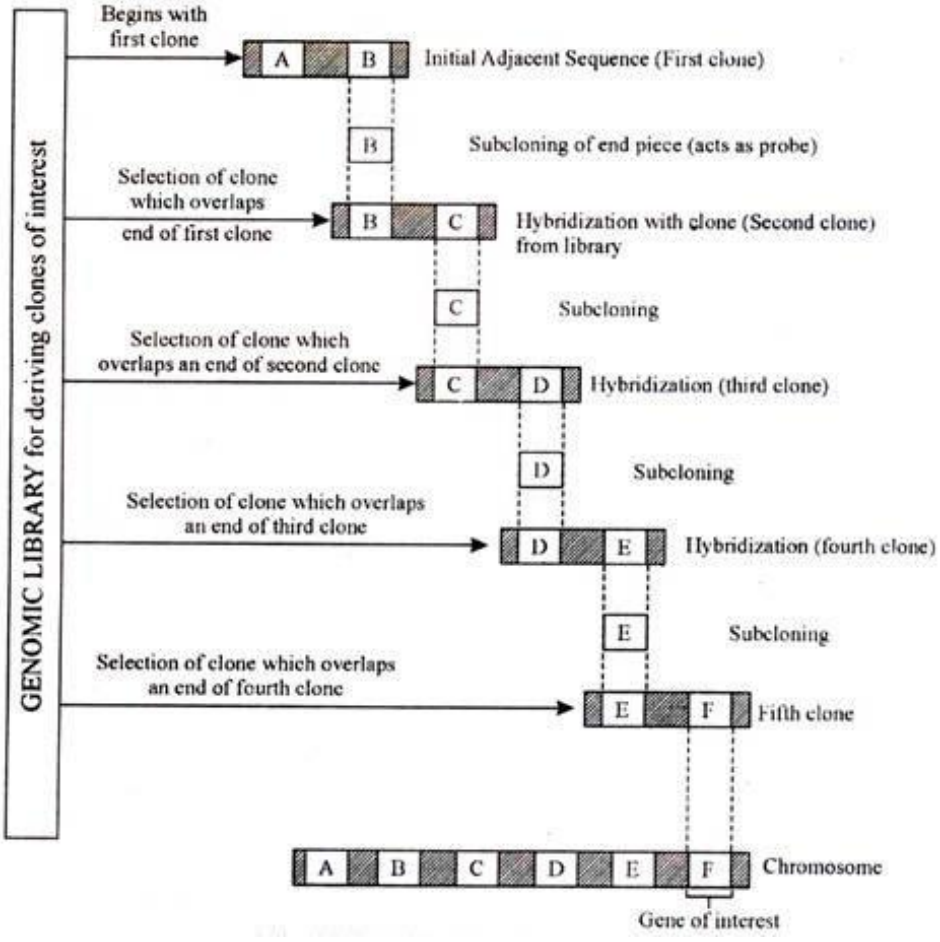
To locate a particular disease gene (say gene B), the walking starts at the closest gene that has already been identified (say gene A), known as a marker gene. Each successive gene in the sequence is tested repeatedly for what are known as overlap restrictions and mapped for their precise location in the sequence. Eventually, walking through the genes reaches the mutant gene (gene B) in an unmapped sequence that binds to a fragment of a gene of that particular disease. Once the gene is cloned, its function can be fully identified. Throughout this process, tests are done to fully identify the properties of each successive clone, to map their locations for future use.

Chromosome walking is a method used to clone in an orderly fashion the DNA segments along the chromosome starting at any point for which we have a probe

(gene A). To build up a series of overlapping cloned DNA fragments, it begins with one clone from genomic library, and then a second clone is identified whose insert overlaps with the insert in the first clone. This was the first method, called **chromosome walking**. But there is a **limitation** to **the speed of chromosome walking** and that is because of the small size of the fragments that are to be cloned, another limitation is the difficulty of walking through the **repeated sequence** that are scattered through the gene. A more straightforward approach thus is to use the insert-DNA from the starting clone as a **hybridization probe** to screen all the other clones in the library. Positive hybridization signals that are given by clones, whose inserts overlap with the probe, are used as new probes to continue the walk. The insert from one of the selected clones is then used as a hybridization probe with all other clones in the library.

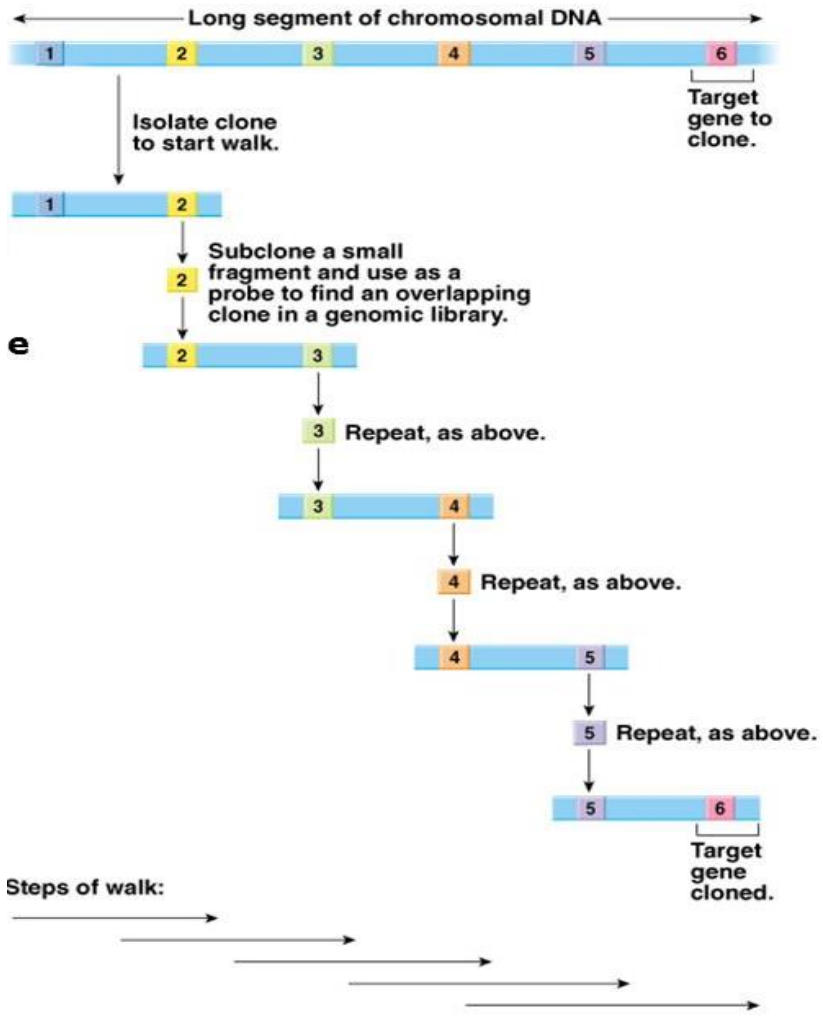
This procedure is well suited for **positional cloning** as the objective here is to walk from a mapped mark to an inserting gene.

Steps in chromosome walking



Chromosome walking is a technique used for characterizing large regions of chromosomes. Generally, a **cosmic library** is used for this technique. Each clone in this library has a DNA insert of 50 KB. It begins with a DNA fragment that contains a known gene/ genetic 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 fragment. Now the non-overlapping end is used as probe. In this way, one continues to move step-by-step toward a gene of interest located 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.

Steps in chromosome walking



Chromosome walking is a technique to clone a gene (e.g., a disease gene) from its known closest genetic marker/gene. It is a method used to clone (in an orderly fashion) the DNA segments along the chromosome, starting at any point for which

we have a probe. To build up a series of overlapping cloned DNA fragments, it begins with one clone from a library, and then identifies a second clone whose insert overlaps with the insert in the first clone. This process is repeated several times to walk across the chromosome and reach the gene of interest. A restriction fragment isolated from the end of the positive clones is used to reprobe the genomic library for overlapping clones.

1. The technique of chromosome walking involves the following steps:
2. From genomic library, a clone of interest (identified by a probe) is selected and a small fragment is sub-cloned from one end of the clone (there is a technique available to sub-clone a fragment from the end)
3. The sub-cloned fragment of the selected clone may be hybridized with other clones in the library and a second clone hybridizing with the sub-clone is identified due to presence of overlapping region.
4. The end of the second clone is then sub-cloned and used for hybridization with other clones in the library to identify the third clone having overlapping region with the sub-clone end of the second clone.
5. The third clone identified as above is also sub-cloned and hybridized with clones in the same manner and the procedure may be continued.

- Restriction maps of the selected overlapping clones may be prepared and compared to know the regions of overlapping so that identification of few overlapping restriction sites will amount to walking along the chromosome or along a long chromosome segment.
- Regions of chromosomes approaching 1000 kb have been mapped following the above technique. Restriction maps of entire chromosome can be prepared in this manner following the technique of chromosome walking.

Restriction Mapping

Restriction mapping is the process of obtaining structural information on a piece of DNA by the use of restriction enzymes, which are enzymes that cut DNA at specific recognition sequences called "Restriction Sites."

Restriction mapping involves digesting the DNA with a series of restriction enzymes and then separating the resultant DNA fragments by 'Agarose Gel Electrophoresis'. The distance between restriction enzyme sites can be determined by the patterns of

fragments that are produced by the restriction enzyme digestion. In this way, information about the structure of an unknown piece of DNA can be obtained. An example of how this works is shown below.

Uses of Restriction Mapping

Restriction map information is important for many techniques used to manipulate DNA. One application is to cut a large piece of DNA into smaller fragments to allow it to be sequenced. Genes and cDNAs can be thousands of kilobases long (megabases - Mb); however, they can only be sequenced 400 bases at a time. DNA must be chopped up into smaller pieces and subcloned to perform the sequencing. Also, restriction mapping is an easy way to compare DNA fragments without having any information of their nucleotide sequence.