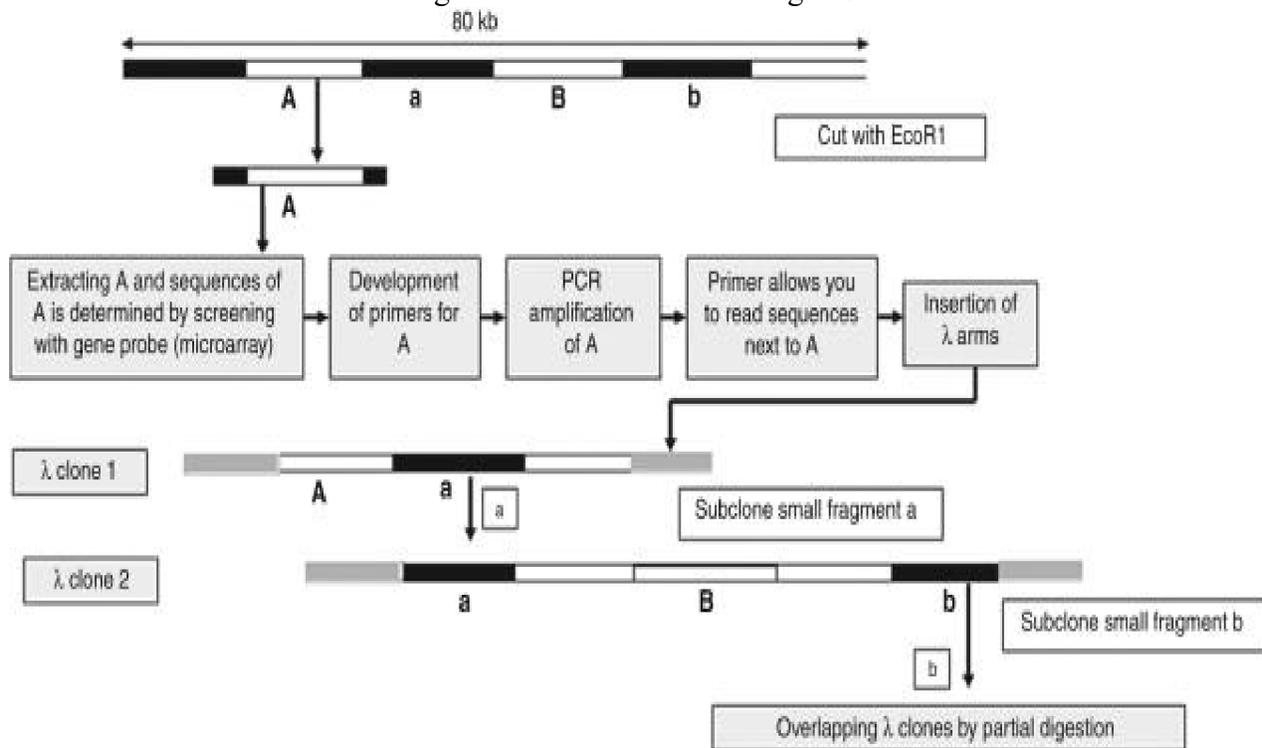
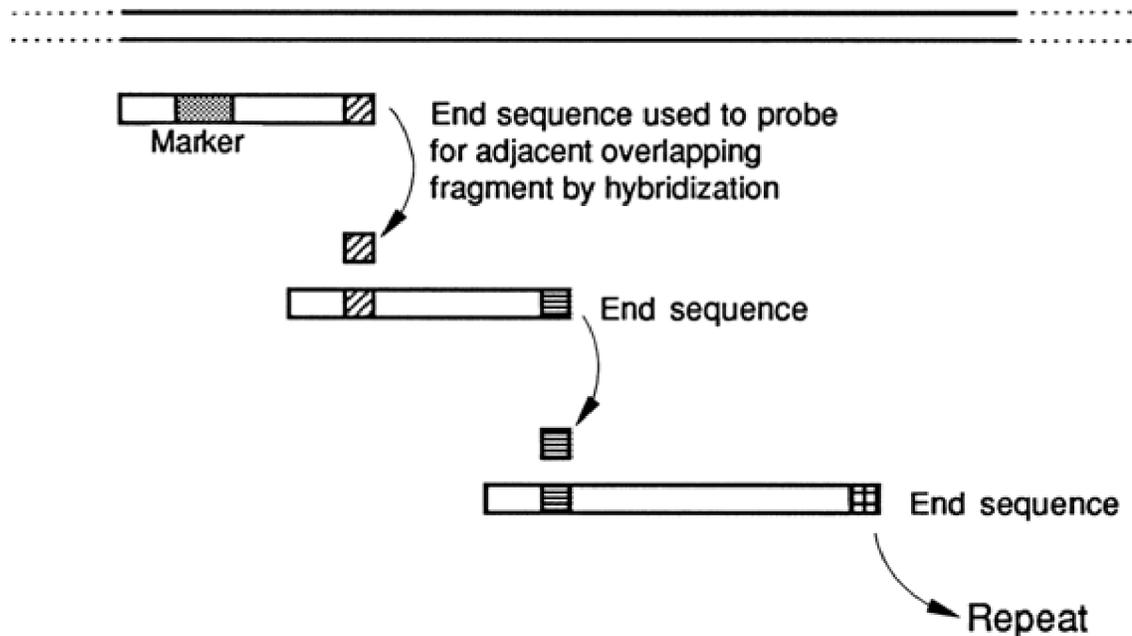


**Chromosome Walking:** Chromosome walking is a technique used to clone a gene (e.g., disease gene) from its known closest markers (e.g., known gene) and hence is used in moderate modifications in cloning and sequencing projects in plants, fungi, and animals. In another words, it is used to identify, isolate, and clone a particular sequence present in close vicinity of the gene to be mapped. Libraries of large fragments, mainly bacterial artificial chromosome libraries, are mostly used in genomic projects. Primer walking is a method to determine the sequence of DNA up to the 1.3–7.0 kb range whereas chromosome walking is used to produce the clones of already known sequences of the gene. To identify the desired colony and to select a particular clone the library is screened first with a desired probe. After screening, the clone is overlapped with the probe and overlapping fragments are mapped. These fragments are then used as a new probe (short DNA fragments obtained from the 3' or 5' ends of clones) to identify other clones. A library approximately consists of 96 clones and each clone contains a different insert. Probe one identifies  $\lambda 1$  and  $\lambda 2$  as it overlaps them (Fig. 4.5). Probe two derived from  $\lambda 2$  clones is used to identify  $\lambda 3$ , and so on. Orientation of the clones is determined by restriction mapping of the clones. Thus, new chromosomal regions present in the vicinity of a gene could be identified. Protocol for chromosomal walking of DNA is described in Fig. 4.7.



**Fig: Procedure for chromosome walking of DNA**

## (A) CHROMOSOME WALKING

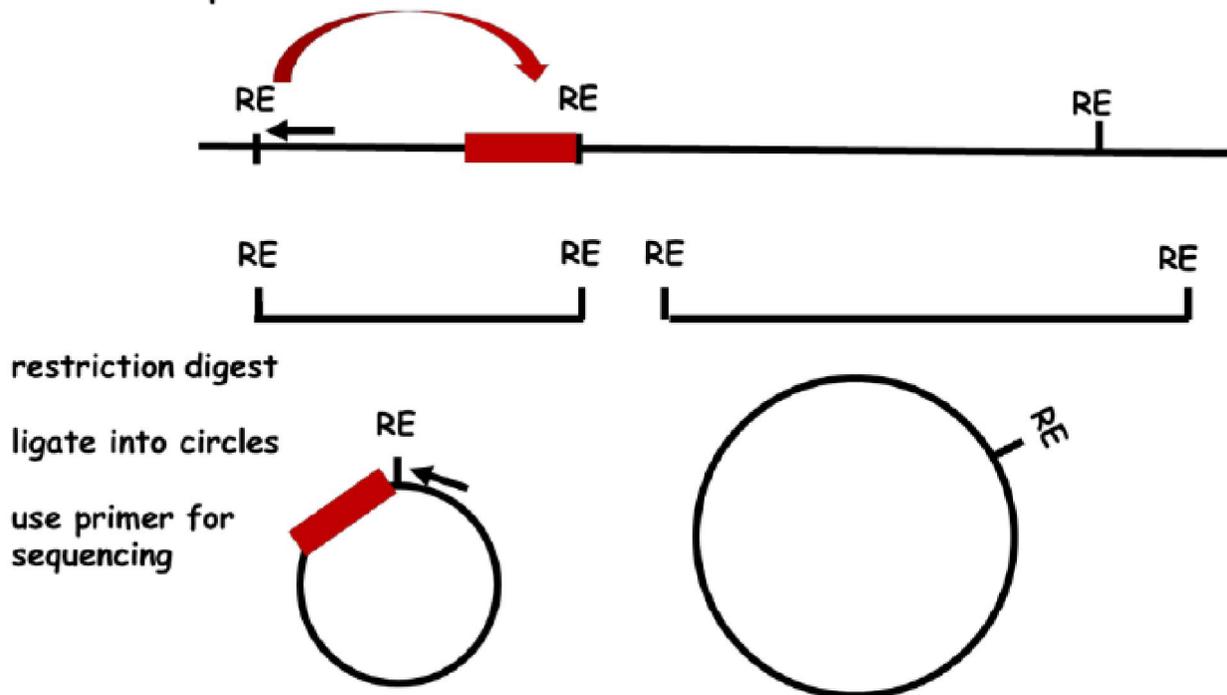


**Chromosome jumping** is a tool of molecular biology that is used in the physical mapping of genomes. It is related to several other tools used for the same purpose, including chromosome walking. Chromosome jumping is used to bypass regions difficult to clone, such as those containing repetitive DNA, that cannot be easily mapped by chromosome walking, and is useful in moving along a chromosome rapidly in search of a particular gene.

Chromosome jumping allows more rapid movement through the genome compared to other techniques, such as chromosome walking, and can be used to generate genomic markers with known chromosomal locations. Chromosome jumping enables two ends of a DNA sequence to be cloned without the middle section. Genomic DNA may be partially digested using restriction endonucleases and with the aid of DNA ligase, the fragments are circularized.<sup>[2]</sup> From a known sequence, a primer is designed to sequence across the circularised junction. This primer is used to jump 100 kb-300 kb intervals: a sequence 100 kb away would have come near the known sequence on circularisation. Thus, sequences not reachable by chromosome walking can be sequenced.

## Chromosome jumping

a VERY simplified version



**Artificial chromosome construction and its uses:** For many years, [plasmids](#) were used to clone [DNA fragments](#) of 1–30 kb. Larger DNA fragments (inserts) are difficult to clone. Then researchers began to use artificial chromosomes— a large [DNA vectors](#) such as P1 artificial chromosomes (PACs), [bacterial artificial chromosomes](#) (BACs) and [yeast artificial chromosomes](#) (YACs) listed in order of size from small to large. Similar to endogenous chromosomes, Artificial Chromosomes can replicate and segregate autonomously without integration into the host chromosome. ACs can broadly be classified into two groups: linear ACs (YACs) and circular ACs (BACs and PACs). However, YACs are linear and requires a number of *cis*-acting elements for successful maintenance *in vivo*. It consists of 1) yeast centromeric repeat, used for maintaining stability during the cell cycle and mitotic segregation 2) a [replication origin](#), to maintain faithful DNA replication 3) [telomere](#) sequence, to maintain linearity. YACs are able to maintain large fragments of DNA, hundreds of kilobases in size (100-1000 kb), making them useful for sequencing and mapping experiments. As a result, YACs were first used to create physical maps of human DNA, and were subsequently used as part of the Human Genome Project. While YACs have proven useful for mapping and sequencing projects, there are some limitations. For instance, there have been reports that a large fraction of YAC clones are often unstable, exhibiting chimerism, deletions, and rearrangements of inserts. Chimerism means a single clone may contain inserts from multiple genomic locations. As a result clones must be screened before they can be used, an expensive and time-consuming process. In addition, constructs must be grown in yeast, which are generally slower growing

than *E. coli*. Lastly, there is nothing preventing a cell from harboring multiple YACs, which could potentially complicate further studies.

As the Human Genome Project was beginning in the early 1990s, researchers were seeking an alternative to YACs for creating libraries. To accomplish this, Circular ACs were created with an origin of replication and an episomal maintenance element or a method of chromosome integration. The BAC was created based on the [F-factor](#) (the naturally occurring sex factor plasmids of *E. coli*) for episomal maintenance, which allows stable maintenance of 300 kb of DNA fragments in *E. coli*. Due to their presence in *E. coli*, BACs can be maintained in a circular form. Due to the F-factor, BACs can only be maintained as single copy, which, contrary to the YAC system, means that it is not possible to have different inserts in the same cell. The BAC system has been shown to be able to stably maintain DNA fragments of 150–350 kb and can be easily extracted from cells through an [alkaline lysis](#) protocol. As a result of their ease of production and purification, BACs quickly served as the go-to method for construction of genomic libraries, with libraries of [Arabidopsis](#), rice, and of complex organisms—including those representing the human and mouse genomes. It was found that BACs could be used to create transgenics through pronuclear injection in mice, and [microinjection](#) in zebrafish. In plants, BACs have also been used for transformation of large DNA fragments via [particle bombardment](#).

In contrast to YAC clones, P1 artificial chromosomes (PACs) and [bacterial artificial chromosomes](#) (BACs) are generally stable and not typically chimeric. Because average insert sizes range from 90 to 300 Kb, a typical gene and its flanking regulatory sequences are often encompassed within a single clone. The basic elements of the P1 [artificial chromosomes](#) PAC vectors are derived from P1, a temperate phage with a genome that is approximately 100 kb long. Both cloning systems (PACs and BACs) are relatively new.

### Uses of Artificial Chromosomes

- A. YACs and BACs were used to create physical maps of human DNA, and were subsequently used as for genome sequencing as a part of the Human Genome Project.
- B. BACs could be used to create transgenics in mice, zebrafish and plants.
- C. Sequencing the candidate gene to look for mutations or creating a targeted mutation in a candidate gene.
- D. Rescuing the phenotype by over-expressing the wild-type allele in a [transgenic mouse](#). For example, the use of a YAC or BAC carrying the entire *frataxin* gene has rescued *frataxin knock-out* mice from embryonic lethality.
- E. Artificial chromosomes are said to be a better candidates as vectors for gene therapy.
- F. To understand chromosome function.

