Quadrant II – Transcript and Related Material

Programme: Bachelor of Science (Third Year)

Subject: Microbiology

Course Code: MIC 110

Course Title: Genetic Engineering

Unit: 2: Methods in molecular cloning

Module Name: Chromosome walking and Chromosome jumping

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Notes:

Chromosome walking and chromosome jumping are two technical tools used in molecular biology for locating genes on the chromosomes and physical mapping of the genomes.

Chromosome walking: It is a method of analyzing long stretches of DNA by small overlapping fragments from the reconstructed genomic library. In other words, it is a technique for cloning a specific gene (B), based on its position in the genetic map in relation to another gene or marker (A).

In chromosome walking, a part of a known gene is used as a probe and continued with characterizing the full length of the chromosome to be mapped or sequenced. The ends of each overlapping fragments are used for hybridization to identify the next sequence. The probes are prepared from the end pieces of cloned DNA and they are sub cloned; further used to find the next overlapping fragment. All these overlapping sequences are used to construct the genetic map of the chromosome and locate the target gene.

Steps in Chromosome Walking Technique

- Isolation of a DNA fragment which contains the known gene or marker (A) near target gene (B).
- Subcloning of a small fragment from the end of 'A'
- Probing of the subcloned fragment against the entire library

Wherever it hybridizes, that clone contains an overlapping region which will be the adjacent region on the chromosome .This “walk” is continued until ‘B’ is reached.

Chromosomal Jumping: It is a technique used in molecular biology for physical mapping of genomes of the organisms. It is a rapid method compared to chromosomal walking and enables bypassing of the repetitive DNA sequences which cannot be cloned during chromosomal walking. Therefore, chromosome jumping technique can be considered as a special version of chromosomal walking which overcomes the breakpoints of chromosomal walking.

Chromosomal jumping narrows the gap between the target gene and the available known markers for genome mapping.

Chromosome jumping tool starts with the cutting of a specific DNA with special restriction endonucleases and ligation of the fragments into circularized loops. Then, a primer designed from a known sequence is used to sequence the circularized loops. This primer enables jumping and sequencing in an alternative manner. Hence, it can bypass the repetitive DNA sequences and rapidly walk through the chromosome for the search of the target gene. The discovery of the gene encoding for cystic fibrosis disease was done using the chromosomal jumping tool. Combined together, chromosomal jumping and walking can enhance the genome mapping process.

Chromosome Walking versus Chromosome Jumping: Chromosomal walking is frequently applied when it is known that a particular gene is located near a previously cloned gene in a chromosome and it is possible to identify it with repeated isolation of adjacent genomic clones from the genomic library. However, when repetitive DNA regions are found during the chromosomal walking technique, the process cannot be continued. Hence, the technique breaks from that point. Chromosomal jumping is a molecular biological tool which overcomes this limitation for mapping genomes. It bypasses these repetitive DNA regions which are difficult to clone and helps in physical mapping of genomes.

Chromosomal walking can only sequence and map small lengths of chromosomes while chromosomal jumping enables sequencing of large parts of chromosomes.

Applications of Chromosome Walking and Jumping:

These techniques are important in:

- ✓ Identification of defective genes that cause inherited disorders such as cystic fibrosis muscular dystrophy, breast cancer, etc .
- ✓ To study mutations in relevant genes.
- ✓ Building up a large genome sequence by assembling smaller fragments.
- ✓ Gene mapping and positional cloning.

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