Quadrant II – Notes

Programme: Bachelor of Science (Third Year)

Subject: Botany

Course Code: BOC 109

Course Title: Molecular Biology and Genetic Engineering

Unit: 09 (Gene Cloning)

Module Name: Construction of genomic and cDNA libraries; Screening of DNA libraries

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Genomic Library

A genomic library is a collection of plasmid clones or phage lysates containing recombinant DNA molecules so that the sum total of DNA inserts in this collection, represents the entire genome of the concerned organism.

Construction of A Genomic Library

For preparation of a genomic library, the total genomic DNA of an organism is extracted. The DNA is broken into fragments of appropriate size either by mechanical shearing (this generates blunt-ended fragments), sonication or by using a suitable restriction endonuclease for partial digestion of the DNA. Complete digestion is avoided since it generates fragments that are too heterogeneous in size.

For partial digestion, restriction enzymes having 4-base (tetrameric) recognition sequences are employed in preference to those having 6-base (hexameric) target sites. This is because a given 4-base recognition site is expected to occur every 4^4 (256) base pairs in a DNA molecule, while a 6 base target site would occur only after every 4^6 (= 4096) base pairs. (It is assumed here that the arrangement of the 4 bases in DNA molecules is random). Therefore, the fragments produced in partial digests with enzymes having 4 base recognition sites are more likely to be of appropriate size for cloning than those generated by enzymes having 6 base recognition sites.

Single or mixed digestions with the enzymes *Alu*I, *Hae*III or *Sau*3A have been used for constructing genomic libraries. The use of enzymes has the advantage that the same set of fragments are obtained from a DNA each time a specific enzyme is used, and many of the enzymes produce cohesive ends.

The partial digests of genomic DNA are subjected to agarose gel electrophoresis or sucrose gradient centrifugation for separation from the mixture of fragment of appropriate size. These fragments are then inserted into a suitable vector for cloning. This constitutes the shotgun approach to gene cloning. Any vector can be used, but 2 vectors and cosmids have been the most commonly used since DNA inserts of upto 23-25 kb can be cloned in these vectors. The vectors containing the inserts are cloned in a suitable bacterial host.

cDNA Library

A cDNA library is a population of bacterial transformants or phage lysates in which each mRNA isolated from an organism or tissue is represented as its cDNA insertion in a plasmid or a phage vector. The frequency of a specific cDNA in such a library would ordinarily depend on the frequency of the concerned mRNA in the tissue/organism in question.

Preparation of cDNA.

cDNA is the copy or complementary DNA produced by usually using mRNA as a template. DNA copy of an RNA molecule produced by the enzyme reverse transcriptase generally obtained from avian mycloblastosis virus (AMV). This enzyme performs similar reactions as DNA polymerase and has an absolute requirement for a primer with a free 3'-OH.

When eukaryotic mRNA is used as a template, a poly-T oligonucleotide (more specifically, oligodeoxynucleotide) is conveniently used as the primer since these mRNAs have a poly-A tail at their 3' ends. But special tricks are required to utilize primers for other RNAs, e.g., prokaryotic mRNA, rRNA, RNA virus genomes, etc. For example, a poly-A tail may be added to 3' end of the RNA to

make it analogous to eukaryotic mRNA (oligo-T is now used as primer), this reaction is catalyzed by the enzyme poly-A polymerase.

The appropriate oligonucleotide primer (oligo-T for eukaryotic mRNA) is annealed with the mRNA; this primer will base-pair to the 3'-end of mRNA. Reverse transcriptase extends the 3'-end of the primer using mRNA molecule as a template. This produces a RNA-DNA hybrid molecule, the DNA strand being the cDNA. The RNA strand is digested either by RNase H or alkaline hydrolysis; this frees the single-stranded cDNA. Curiously, the B'-end of this cDNA serves as its own primer and provides the free 3'-OH required for the synthesis of its complementary strand; therefore, a primer is not required for this step. The complementary strand of cDNA single strand is synthesized by either the reverse transcriptase itself or by *E. coli* DNA polymerase I. This generates a hairpin loop in the cDNA. The hairpin loop is cleaved by a single strand-specific nuclease to yield a regular DNA duplex.

Screening of DNA Libraries

The process of identifying one particular clone containing the gene of interest from among the very large number of others in the gene library is called screening. Screening to isolate one particular clone from a gene library routinely involves using a nucleic acid probe for hybridization. The probe will bind to its complementary sequence allowing the required clone to be identified. The most common ways to make DNA probes for library screening are PCR probes and colony hybridization.