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MOLECULAR GENETIC TECHNIQUES

REVIEW THE CONCEPTS

1. A recessive mutation must be present in both alleles of a diploid organism in order for the mutant phenotype to be observed; that is, the individual must be homozygous for the mutation to be expressed. Recessive alleles usually result from a mutation that inactivates the affected gene, leading to loss of function. If the inactivated gene is an essential gene, loss of function would be lethal in homozygotes with both mutated alleles. Lethal recessive mutations can be maintained in heterozygotes. In contrast, a dominant mutation produces a mutant phenotype even in the presence of one mutant and one wild-type allele. Dominant alleles often result from a mutation that causes some kind of gain of function. Dominant alleles that affect the function of essential genes can be lethal even in heterozygotes. Thus, researchers may use conditional mutations such as temperature sensitive mutations to study the effects of dominant lethal alleles.

2. A temperature sensitive mutation is one where the gene is non-functional at a given temperature, usually a lower or higher than normal growth temperature. These are useful for the study of essential genes, as it is not possible to create a viable cell that lacks an essential gene.

3. Complementation analysis can be used to determine whether two recessive mutations are present in the same or different genes. If a heterozygous organism containing both mutations shows the mutant phenotype, then the two mutations are in the same gene because neither allele provides a functional copy of the gene. In contrast, if a heterozygous organism shows a wild-type phenotype, then the two mutations are in

different genes because a wild-type allele of each gene is present. Dominant mutations cannot be tested by complementation analysis because they will display a mutant phenotype even in the presence of a wild-type allele of the gene.

4. Bacteria that synthesize restriction enzymes also synthesize a DNA modifying enzyme to protect its own DNA. The modifying enzyme is a methylase, which methylates the host DNA. Methylated DNA is no longer a substrate for the encoded restriction enzyme. Restriction enzyme sites commonly consist of 4–8 base pair, palindromic sequences. After being cut with a restriction enzyme, the ends of the cut DNA molecule can exist as single-stranded tails (sticky ends) with either 5′ or 3′ overhangs, or as blunt (flush) ends. DNA ligase is an enzyme that catalyzes the reformation of the phosphodiester bond between nucleotides in the presence of ATP.

5. A plasmid is a circular, extrachromosomal DNA molecule that contains an origin of replication, a marker gene that permits selection, and a region into which foreign DNA can be inserted (cloning site). A plasmid is useful for cloning DNA fragments up to approximately 20 kb. Specialized plasmid vectors such as BACs (bacterial artifi cial chromosomes) have been developed that can accommodate DNA fragments as large as several million nucleotides.

6. DNA libraries are collections of randomly cloned DNA fragments. A cDNA (complementary DNA) library is a collection of DNA molecules that are copied from messenger RNA molecules using the enzyme reverse transcriptase. A cDNA library does not contain every gene, only those that are expressed as mRNA at the time of RNA isolation. In contrast, a genomic DNA library consists of random fragments of the total genome. This would include not only genes but also areas of the genome that do not encode for genes. You could use either of the genomic libraries or the cDNA library from neurons to clone gene X. You could not use the cDNA skin library because cDNA libraries are based on the cell's mRNA. If the skin cell does not express gene X, that cDNA will not be present in the library.

7. The PCR reaction is performed as multiple cycles of a three-step process. The first step involves heat denaturation of a target DNA molecule. The second step involves cooling the DNA solution to allow annealing of short single-stranded oligonucleotide primers that are complementary to the target DNA molecule. In the final step, the hybridized oligonucleotides serve as primers for DNA synthesis. The resultant double-stranded DNA molecules are then subjected to further rounds of denaturation, annealing, and DNA synthesis (extension). A thermo stable DNA polymerase was essential for automation of the PCR process. A nonthermostable DNA polymerase would be inactivated by heat denaturation during each cycle of the PCR process and would necessitate the addition of new enzyme prior to each DNA synthesis step.

8. In order to express a foreign gene, a recombinant plasmid would require a promoter for efficient transcription of the foreign gene. A promoter that is inducible would provide even higher expression levels of the foreign gene product. To facilitate purifi cation of the foreign protein, a molecular tag can be added to the recombinant protein. An example of this type of molecular tag is a short sequence of histidine residues (a polyhistidine sequence). The resultant His-tagged protein will bind specifically to a bead that has bound nickel atoms. Other proteins can be washed out and the His-tagged protein can be released from the nickel atoms by lowering the pH of the solution. It is better to express a (non-bacterial) proteins in mammalian cells since post-translational modification largely does not occur in bacterial cells. Post-translational modification may be essential for the structure and activity of a protein.

9. RT-PCR is useful for analyzing fewer genes because it relies on analyzing each gene in a separate reaction. It is less feasible to process a large quantity of genes in this manner.. Studying a large number of genes (for example an entire chromosome) is most easily accomplished with a microarray, which uses a chip that can hybridize to hundreds or thou-sands of genes based on complementary base pairing.

10. Paralogous genes are genes that have diverged as a result of a gene duplication (i.e., two genes in an organism that have different functions but very similar nucleotide sequences). Orthologous genes are genes that arose because of speciation (i.e., genes found in different species that have very similar nucleotide sequences and functions). Because of alternative splicing, a gene can give rise to numerous protein products. Thus, a small increase in gene number could result in a very large increase in protein number. Thus, the number of proteins and protein-protein interactions could be much greater in the organism with the larger genome. Also, complexity among multicellular organisms arises largely from organizing a larger number of cells into more complex groups of interacting cells. This requires evolution of control regions that regulate transcription and cell division as well as the evolution of new proteins.

11. The expression of mRNA in individual cells can be determined by in situ hybridization in whole cells or tissue sections. Fixed cells are exposed to labeled DNA probes that are complementary to the mRNA of interest. After washing to remove excess probe, the cells can be examined microscopically to detect the locations of labeled mRNA. This process can also be used to identify mRNA locations in embryos.

12. Single-nucleotide polymorphisms (SNPs) are changes in a single nucleotide between two individuals. Short tandem repeats (STRs), also known as microsatellites, consist of a variable number of repeating one-, two-, or three-base sequences. The number of these repeat units at a specifi c genetic locus varies between individuals. Both types of polymorphisms can be used as molecular markers for mapping studies. The recombination frequency between two polymorphisms can be determined and can serve as the basis for development of a genetic map. In general, the farther two markers are separated on a chromosome, the greater the recombination frequency between those two markers, and vice versa.

13. Linkage disequilibrium mapping can sometimes be used in cases where a genetic disease commonly found in a particular population results from a single mutation that occurred many generations in the past. In such cases, most of the individuals with the disease would have inherited the disease from the same ancestral chromosome. The closer genetic markers are to each other, the less likely they will be recombined by crossing over during meiosis. Thus, individuals inherit sections of DNA from their parents, not just individual genes. DNA polymorphisms on part of a chromosome that are inherited together are called haplotypes. If geneticists can identify a haplotype common to all the affected individuals in a particular population, DNA markers associated with the disease haplotype might help localize the disease-associated gene to a relatively small chromosomal region.

14. Once a gene is roughly located along a chromosome by genetic linkage studies, further analysis is required to identify the "disease" gene. One strategy for identifying a disease gene involves gene expression analysis. Comparison of gene expression in tissues from normal and affected individuals by in situ hybridization, microarrays, or RNAseq can help identify the disease gene. Sometimes expression levels do not alter between the normal and the disease states. In this case, DNA sequencing of a potential disease gene from tissues of a normal and a disease state could reveal a single nucleotide change that results in the disease phenotype.

15. To generate a knockout mouse, mouse embryonic stem cells are fi rst transfected with a disrupted allele of the target gene. Through a process known as homologous recombination the disrupted allele replaces the functional homologous gene in the chromosome, resulting in a nonfunctional chromosomal gene. The ES cells, which now contain a mutant gene, are injected into a blastocyst. The blastocyst is transferred into a recipient mouse. Pups that are born will be chimeras. The *loxP*-Cre system can be used to conditionally knock out a gene. Using the above technology, *loxP* sites can be engineered to fl ank the gene of interest. Expression of the recombinase, Cre, in a specifi c tissue will result in loss of the fl anked gene in that tissue. Knockout mice serve as models for human diseases. For example, if a human disease is known to result from a mutation in gene *X*, a knockout mouse can be generated that lacks gene *X*.

16. A dominant negative mutation is a mutation that produces a mutant phenotype even in cells carrying a wild-type copy of the gene. This type of mutation produces a loss of function phenotype. RNA interference (RNAi) is a method of inactivating gene expression by selectively destroying RNA. In this method, a short double-stranded RNA molecule is introduced into cells. This double-stranded RNA base pairs with its target mRNA, promoting degradation of the mRNA by specifi c nucleases.