Summary

Primer extension is a technique whereby the 5' ends of RNA can be mapped. It is another technique used to analyze RNA structure and expression. In this method, an oligonucleotide primer is annealed to RNA and extended to a cDNA copy by reverse transcriptase in the presence of labeled dNTPs. Alternately, the primer is labelled and no label is included in the extension reaction. If the RNA of interest is present, extended products will appear on a denaturing gel. Furthermore, the size of the extended product will indicate the position of the 5' end of the RNA, and, if an excess of primer is used, the amount of cDNA produced will reflect the amount of target RNA in the sample. Primer extension provides the same type of information as S1 mapping. However, primer extension is unaffected by splice sites. In cases where only a genomic probe is available and an intervening splice site prevents S1 mapping of the start site, primer extension offers a useful alternative. Primer extension offers additional advantages over S-1 mapping. A genomic clone of the target RNA is not required; only 30 - 50 bases of sequence need be known to generate the primer. Additionally, probe preparation is easier, because the primer is single stranded. This means that no elaborate procedures are needed prior to labelling. Site-directed mutagenesis is a molecular biology method that is used to make specific and intentional changes to the DNA sequence of a gene and any gene products. Also called site-specific mutagenesis or oligonucleotide directed mutagenesis, it is used for investigating the structure and biological activity of DNA, RNA, and protein molecules, and for protein engineering. Site-directed mutagenesis is one of the most important techniques in laboratory for introducing a mutation into a DNA sequence. However, with decreasing costs of oligonucleotide synthesis, artificial gene synthesis is now occasionally used as an alternative to site-directed mutagenesis.

Mutagenesis in the laboratory is an important technique whereby DNA mutations are deliberately engineered to produce mutant genes, proteins, strains of bacteria, or other genetically modified organisms. Various constituents of a gene, such as its control elements and its gene product, may be mutated so that the functioning of a gene or protein can be examined in detail. The mutation may also produce mutant proteins with interesting properties, or enhanced or novel functions that may be of commercial use. Mutants strains may also be produced that have practical application or allow the molecular basis of particular cell function to be investigated.

Early approaches to mutagenesis rely on methods which are entirely random in the mutations produced. Cells or organisms may be exposed to mutagens such as UV radiation or mutagenic chemicals, and mutants with desired characteristics are then selected. Hermann Muller discovered that x-rays can cause genetic mutations in fruit flies (published in 1927), and went on to use the *Drosophila* mutants created for his studies on genetics. For *Escherichia coli*, mutants may be selected first by exposure to UV radiation, then plated onto agar medium. The colonies formed are then replica-plated, one in rich medium, another in minimal medium, and mutants that have specific nutritional requirements can then be identified by their inability to grow in minimal medium. Similar procedures may be repeated with other types of cells and with different media for selection. Although the concept that gene sequences could be altered *via* synthetic nucleotides was put forward in the early 1970's, it was only in the last years of that decade that Michael Smith and colleagues demonstrated that the approach could be successfully implemented. Within three years, several reports showed that oligonucleotide-directed mutagenesis could be used to introduce specific changes in the primary structure of b-lactamase, tyrosyl-tRNA synthetase and prolipoprotein opening the way to the field of research that has become subsequently known as Protein Engineering. For his pioneering work in this field British-born Canadian Michael Smith was awarded the Nobel Prize for Chemistry in 1993. Subtilisin is a non-specific protease (a protein digesting enzyme) initially obtained from *Bacillus subtilis*. Subtilisins belong to subtilases, a group of serine proteases that like all serine proteases initiate the nucleophilic attack on the peptide (amide) bond through a serine residue at the active site. Subtilisins typically have molecular weights of about 20,000 to 45,000 dalton. Alpha-1 Antitrypsin or α_1-antitrypsin (A1AT) is a protease inhibitor belonging to the serpin superfamily. It is generally known as serum trypsin inhibitor. Alpha 1-antitrypsin is also referred to as alpha-1 proteinase inhibitor (A1PI) because it inhibits a wide variety of proteases. It protects tissues from enzymes of inflammatory cells, especially neutrophil elastase, and has a reference range in blood of 1.5 - 3.5 gram/liter, but the concentration can rise manyfold upon acute inflammation. In its absence (such as in alpha 1-antitrypsin deficiency), neutrophil elastase is free to break down elastin, which contributes to the elasticity of the lungs, resulting in respiratory complications such as emphysema, or COPD (chronic obstructive pulmonary disease) in adults and cirrhosis in adults or children. A1AT is a 52-kDa serpin and, in medicine, it is considered the most

prominent serpin*;* the terms *α1-antitrypsin* and *protease inhibitor* (*Pi*) are often used interchangeably.