

Preface

Gene cloning is a common practice in molecular biology labs that is used by researchers to create copies of a particular gene for downstream applications, such as sequencing, mutagenesis, genotyping or heterologous expression of a protein. The traditional technique for gene cloning involves the transfer of a DNA fragment of interest from one organism to a self-replicating genetic element, such as a bacterial plasmid. This technique is commonly used today for isolating long or unstudied genes and protein expression. A more recent technique is the use of polymerase chain reaction (PCR) for amplifying a gene of interest. The advantage of using PCR over traditional gene cloning, as described above, is the decreased time needed for generating a pure sample of the gene of interest. However, gene isolation by PCR can only amplify genes with predetermined sequences. For this reason, many unstudied genes require initial gene cloning and sequencing before PCR can be performed for further analysis. Sequencing uses biochemical methods to determine the order of nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA oligonucleotide. Knowing the sequence of a particular gene will assist in further analysis to understand the function of the gene. PCR is used to amplify the gene of interest before sequencing can be performed. Many biotechnology companies offer sequencing instruments, however, these instruments can be expensive. As a result, many researchers usually perform PCR in-house and then send out their samples to sequencing labs.

Site-directed mutagenesis is a widely used procedure for the study of the structure and function of proteins by modifying the encoding DNA. By using this method, mutations can be created at any specific site in a gene whose wild-type sequence is already known. Many techniques are available for performing site-directed mutagenesis. A classic method for introducing mutations, either single base pairs or larger insertions, deletions, or substitutions into a DNA sequence, is the Kunkel method. The first step in any site-directed mutagenesis method is to clone the gene of interest. For the Kunkel method, the cloned plasmid is then transformed into a dut ung mutant

of *Escherichia coli*. This *E. coli* strain lacks dUTPase and uracil deglycosidase, which will ensure that the plasmid containing the gene of interest will be converted to DNA that lacks Ts and contains Us instead. The next step is to design a primer that contains the region of the gene which you wish to mutate, along with the mutation you want to introduce. PCR can then be used with the mutated primers to create hybrid plasmids; each plasmid will now contain one strand without the mutation and uracil bases, and another strand with the mutation and lacking uracil. The final step is to isolate this hybrid plasmid and transform it into a different strain that does contain the uracil-DNA glycosylase (ung) gene. The uracil deglycosidase will destroy the strands that contain uracil, leaving only the strands with your mutation. When the bacteria replicate, the resulting plasmids will contain the mutation on both strands.

Genotyping is the process of determining the DNA sequence specific to an individual's genotype. This process can be accomplished by several techniques, such as high resolution melt (HRM) analysis, or any other mutation detection technique. All of these techniques will provide an insight into the individual's genotype, which can help determine specific sequences that can be manipulated and cloned for further analysis. Heterologous protein expression uses gene cloning to express a protein of interest in a self-replicating genetic element, such as a bacterial plasmid. Heterologous expression is used to produce large amounts of a protein of interest for functional and biochemical analyses.

The book provides a clear, scientific and technical understanding of this subject. The information contained herein will equip the readers with latest and up-to-date knowledge.

—Editor