Script for DNA Sequence Determination - Chain-Terminator Method

Scene 1

Most modern DNA sequencing efforts use a method devised by Frederick Sanger known as the chain terminator or
dideoxy method. DNA polymerase is an enzyme that synthesizes the complementary copy of a strand of DNA, which is
therefore called the template strand. However, DNA polymerase can only extend an existing DNA strand, known as a
primer, which must be base paired to the template strand. The enzyme extends the primer by adding nucleotides, which
are derived from nucleoside triphosphates. The nucleotide is added to the 3' end of the growing strand to form a
phosphodiester bond that links the 3'-hydroxyl group at the end of the growing strand to the 5'-hydroxyl group on the
incoming nucleoside.

Scene 2

The sequence of the DNA is determined by tricking the DNA polymerase into prematurely terminating the chain
extension process. This is accomplished by introducing a small amount of a dideoxy nucleoside triphosphate, a
compound which resembles a normal nucleoside triphosphate but which lacks its 3'-hydroxyl group. Since DNA chains
are synthesized by successively attaching nucleotides to their 3' ends, the extension of a chain ceases once a dideoxy
nucleoside has been appended to its 3' end.

Scene 3

In order to determine the sequence of a template strand, four separate reaction are carried out. Each reaction contains
DNA polymerase, the template strand, all four nucleoside triphosphates, and a small amount of one of the four dideoxy
nucleotides, one with dideoxy-GTP, one with dideoxy-ATP, one with dideoxy-TTP, and one with dideoxy-CTP. The
concentration of the dideoxynucleoside triphosphate in each reaction is adjusted such that chain termination is equally
likely to occur after each base in the newly synthesized DNA chain which corresponds to that in the dideoxynucleoside
triphosphate. Most often, a normal nucleotide is incorporated and the chain grows. However, occasionally a
dideoxynucleotide is incorporated and chain extension is terminated. This results in each reaction containing a

collection of DNA chains of all possible lengths that end in the base carried by the dideoxy nucleoside triphosphate in the reaction.

Scene 4

The DNA strands in the four reactions are then separated in parallel lanes using gel electrophoresis. Gel electrophoresis separates DNA chains according to their sizes with the shortest chains running fastest and therefore ending up nearer the bottom of the gel. The DNA-containing bands are made visible by radioactively or fluorescently labeling one of the nucleoside triphosphates or the primer. The sequence of the newly synthesized strand can then be determined by simply reading up the gel to see which nucleotide ends the chain at each successive position. Note that the sequence of the template strand is the complement of the newly synthesized strand, keeping in mind that the two strands are antiparallel.