As a result, chain fragments of different length are randomly synthesized.

In the A reaction solution (contains ddA), synthesized fragments all end in A. The G, C and T reaction solutions will generate new strand fragments that end in G, C and T respectively.

The fragments generated from each reaction are indicated in the handout for the example in Fig T20.1.

The length of each synthesized fragment is a measure of the number of nucleotides from the primer to the dideoxynucleotide.

Electrophoresis of the four reaction solutions (one solution in each well), by PAGE allows a separation of the synthesized fragments by length.

Exposure of photographic film to the gel leads to an identification of bands from the β-particle radiation of the ^{32}P labeled fragments and thus the sequence as indicated in Fig T20.1

The dark bands are read from the bottom to the top. The band at the bottom corresponds to the smallest (Primer-ddC) and the band at the top to the largest (Primer-CACCTGAATTACGddT) fragments.

Reading from the bottom to the top then corresponds to the sequence from the 5' to the 3' end of the non-template strand.

The sequence of the template strand would be the complement of the sequence read from the gel.

Note that the 3' end of the non-template strand corresponds to the 5' end of the template strand.

The dideoxy method typically allows one to determine 250 bases in the sequence.

As an exercise, you might try to read the autoradiogram corresponding to an actual dideoxy sequencing gel.

The dideoxy method has been automated as shown in the attached Fig. 8-37.

In one form of the automated method, there is one reaction solution that contains four different fluorescently labeled dideoxynucleoside triphosphates.

The synthesized chain fragments are separated electrophoretically in a single capillary gel tube according to their length.

The terminal dideoxy nucleotide in each fragment is identified by the color of the fluorescent dye that is monitored by a laser beam and detector.

Return