

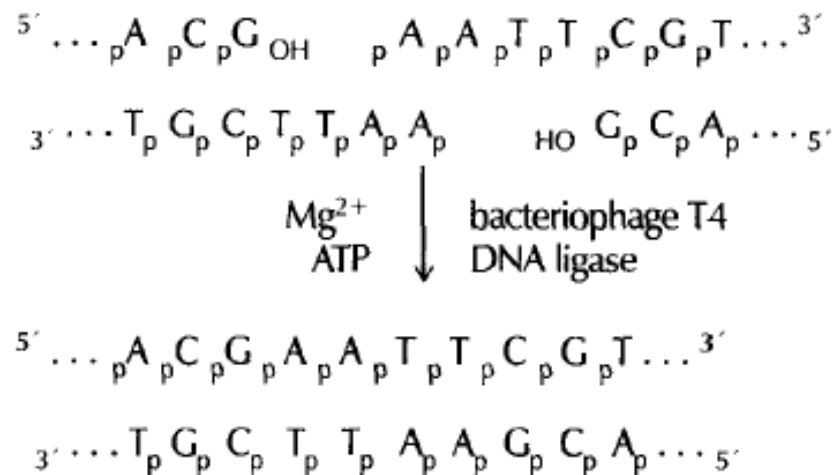
Ligación de DNA

BACTERIOPHAGE T4 DNA LIGASE

Activity: Ligation of cohesive DNA termini or nicks

Substrate: Active on double-stranded DNA with complementary cohesive termini that base pair to bring together 3'-hydroxyl and 5'-phosphate termini. In addition, the enzyme is active on nicked DNA and active, albeit far less efficiently, on RNA substrates. (For a more complete description of substrates, see Engler and Richardson 1982.)

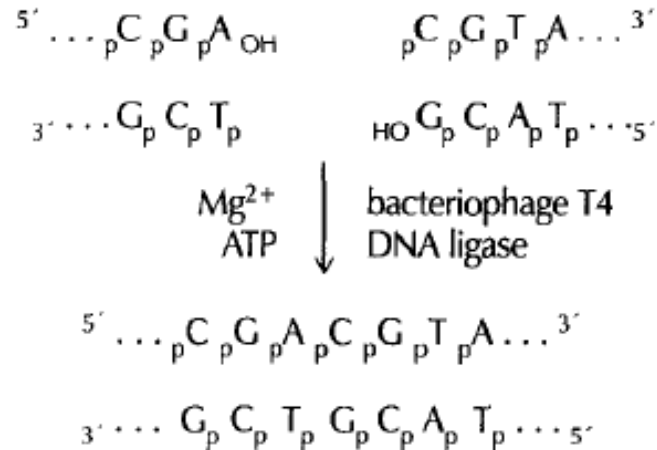
Reaction:



Activity: Ligation of blunt ends

Substrate: High concentrations of blunt-ended, double-stranded DNA containing 5'-phosphate and 3'-hydroxyl termini.

Reaction:



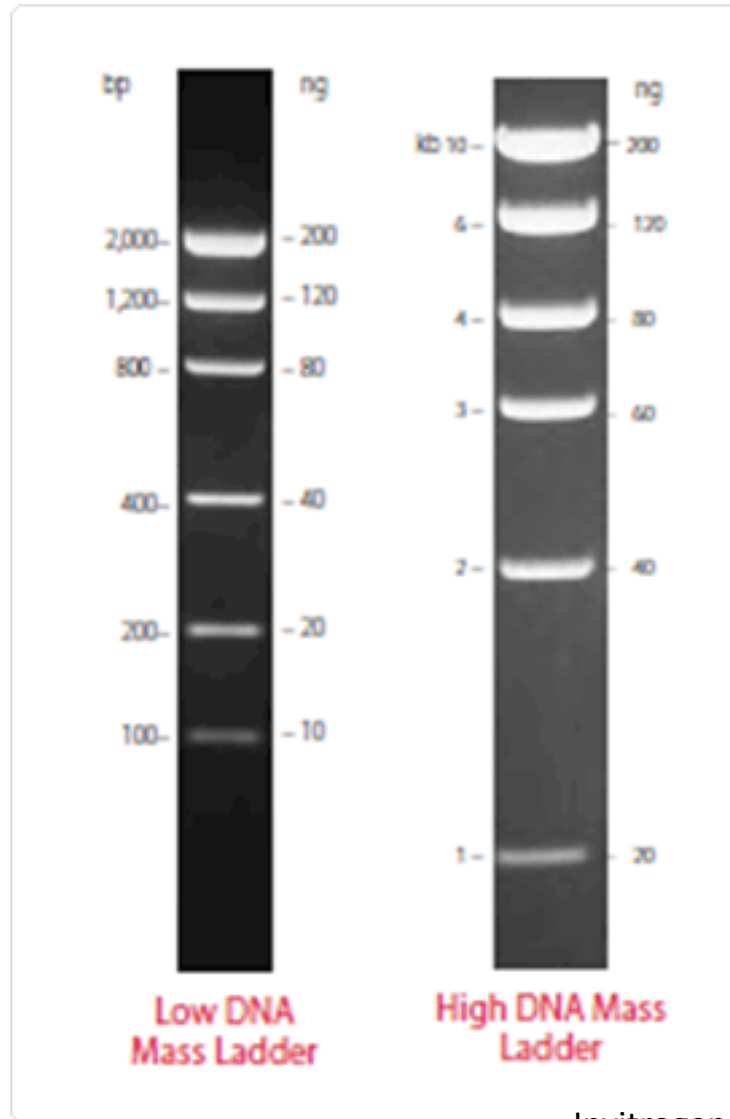
$$\text{ng inserto} = \frac{\text{ng vector} \times \text{kb inserto}}{\text{kb vector}} \times \text{Relación molar inserto:vector}$$

Extremos **romos**= 8:1 , extremos **cohesivos**= 3:1

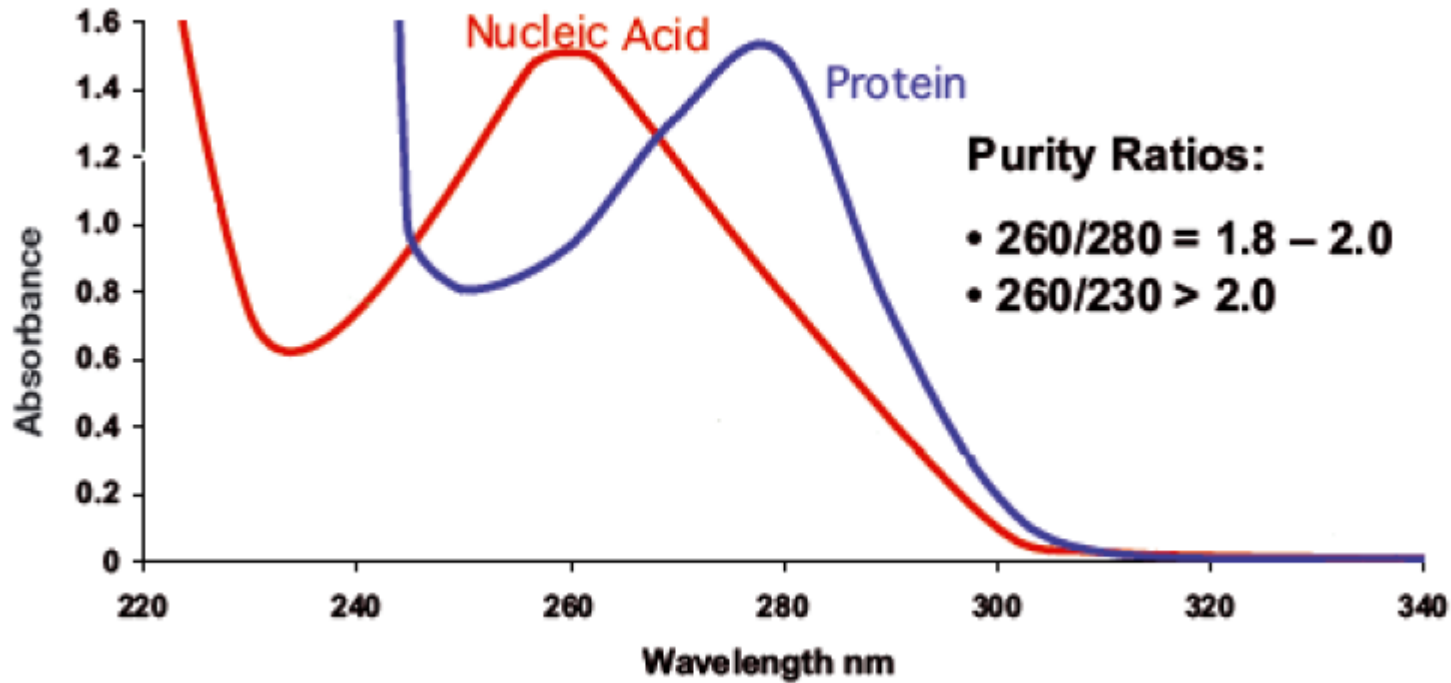
Incubación: **cohesivos** 3h a RT ó ON a 4°C **romos** 4-18h a 15°C

Métodos de cuantificación de DNA

Gel de agarosa con *standard* de masa



Espectrofotometría UV



1 A₂₆₀ unit dsDNA = 50 µg/ml

1 A₂₆₀ unit ssRNA = 40 µg/ml



Fluorescencia

The QuantiFluor™ dsDNA System contains a fluorescent DNA-binding dye that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA) in solution. The quantitation of dsDNA is an important step in many biological applications, particularly in standard molecular biology techniques. This dye shows minimal binding to single stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA.

Qubit Quantitation Platform
Virtual Demo



Quantus™ Fluorometer: Simple, Sensitive DNA and RNA Quantitation



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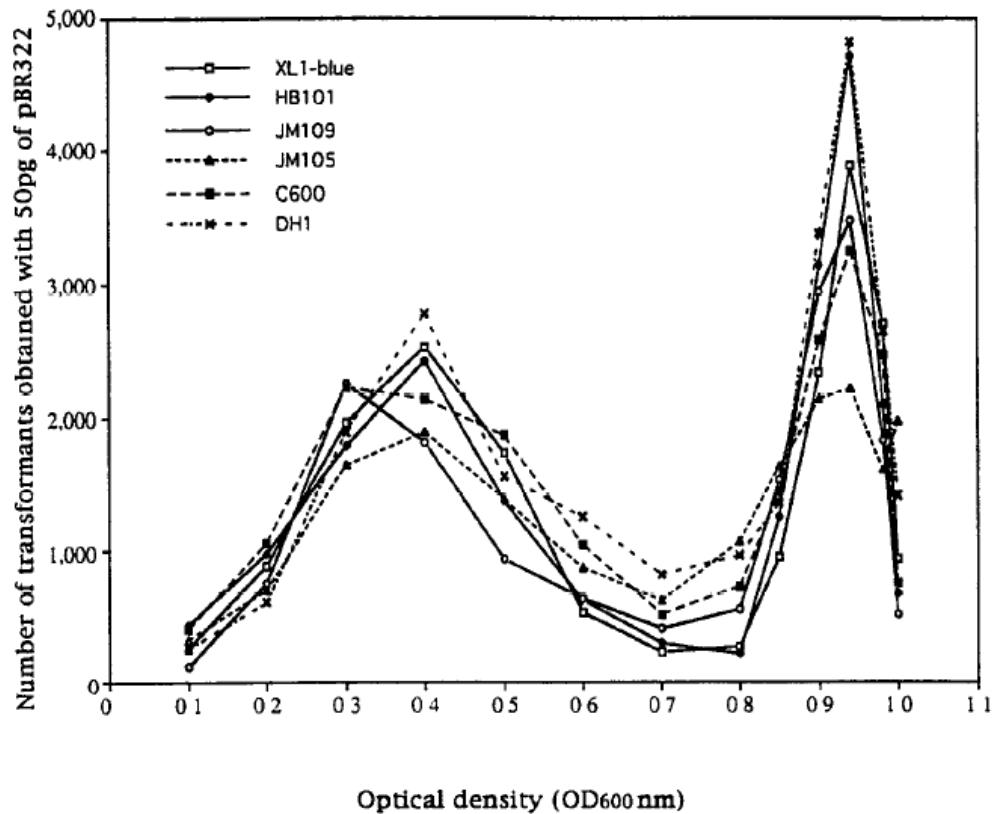
Transformación de *E.coli*

Para poder introducir DNA foráneo con alta eficiencia dentro de bacterias es necesario inducir el “estado de competencia” de las mismas. El tratamiento de cultivos bacterianos frescos, en fase logarítmica de crecimiento, con agentes químicos permite debilitar la estructura de su pared celular haciendo que se puedan introducir en las células DNAs derivados de distintas fuentes. Un requisito fundamental es que la manipulación de las bacterias durante el tratamiento químico, y después del mismo, sea muy cuidadosa, ya que al tener alterada la pared aumenta el grado de fragilidad de las bacterias.

Una alternativa al tratamiento químico es la electroporación, en este caso se somete a las bacterias a un campo eléctrico que desestabiliza a las estructuras de pared y membrana. Durante este proceso se inducen poros temporarios por los que ingresa el DNA en las bacterias. En este caso no es necesario inducir el “estado de competencia” previamente, simplemente las bacterias de un cultivo en fase logarítmica de crecimiento se lavan varias veces para eliminar todas las sales que puedan estar presentes.

Método Inoue

1. Harvesting of bacterial cells at the logarithmic phase of growth;
2. Incubation of cells on ice throughout the procedure; and
3. Prolonged exposure of CaCl_2 .



Electrocompetentes



La eficiencia de transformación se expresa como número de colonias obtenidas/ ug de DNA plasmídico superenrollado.

Screening de bacterias transformadas

- α –complementación
- *Colony* PCR
- Miniprep
- Mapa restricción
- Orientación inserto PCR o ER

Secuenciación de DNA: Maxam-Gilbert

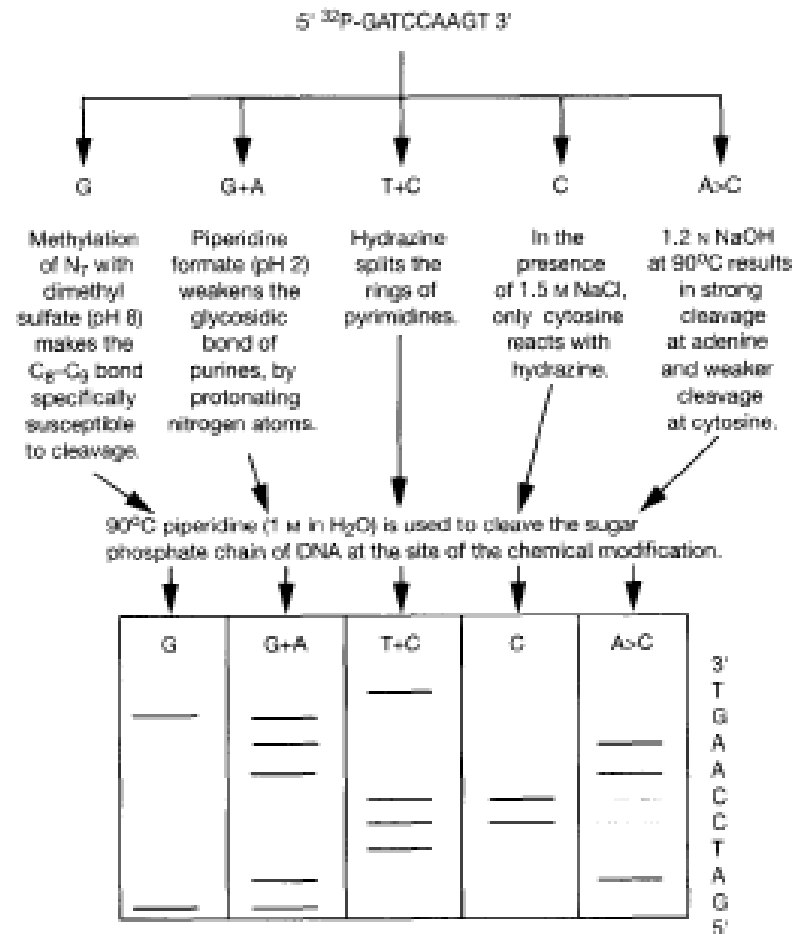
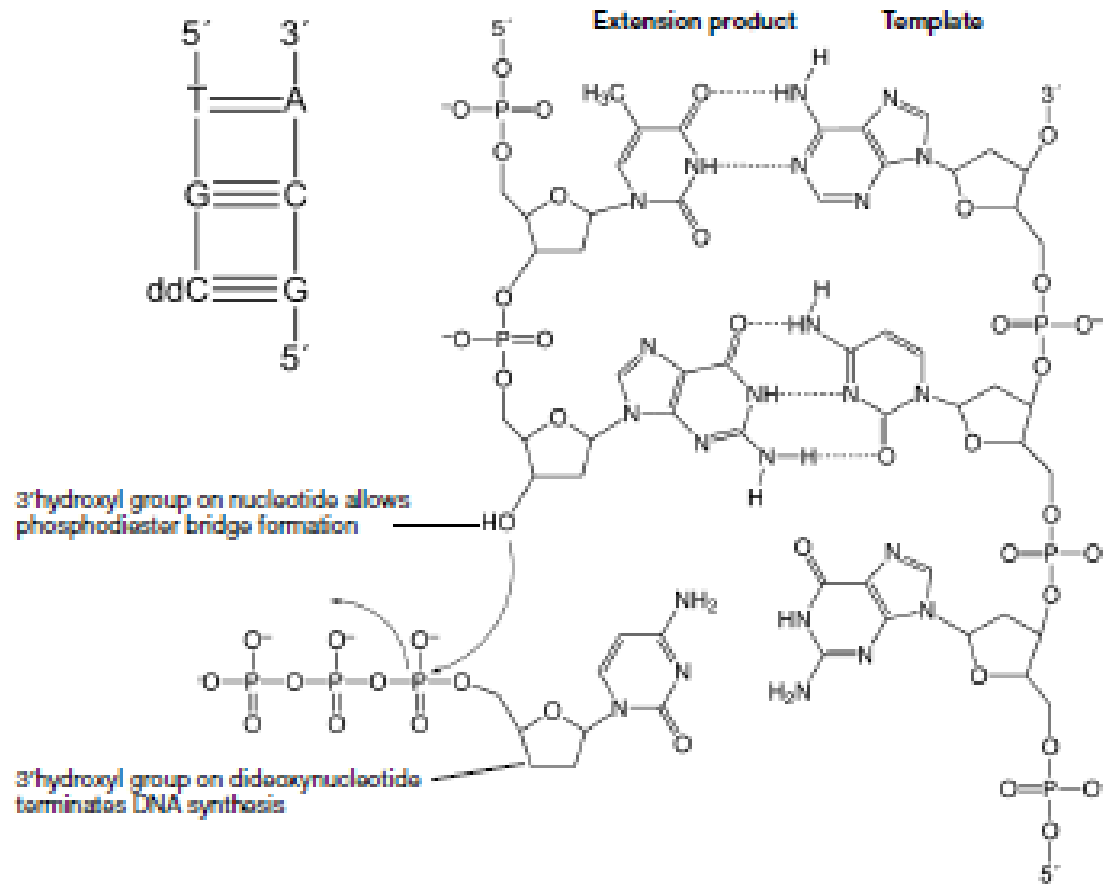
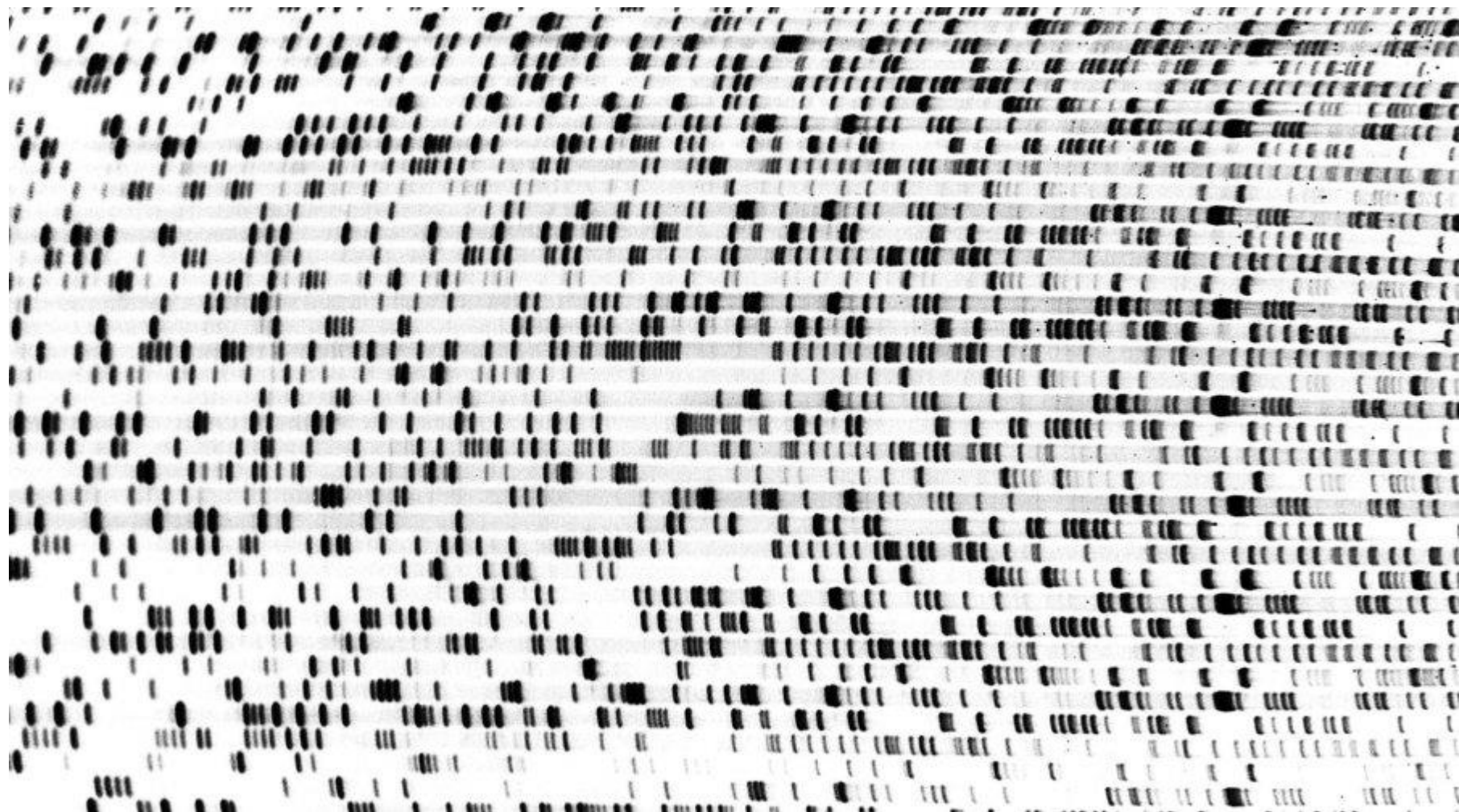


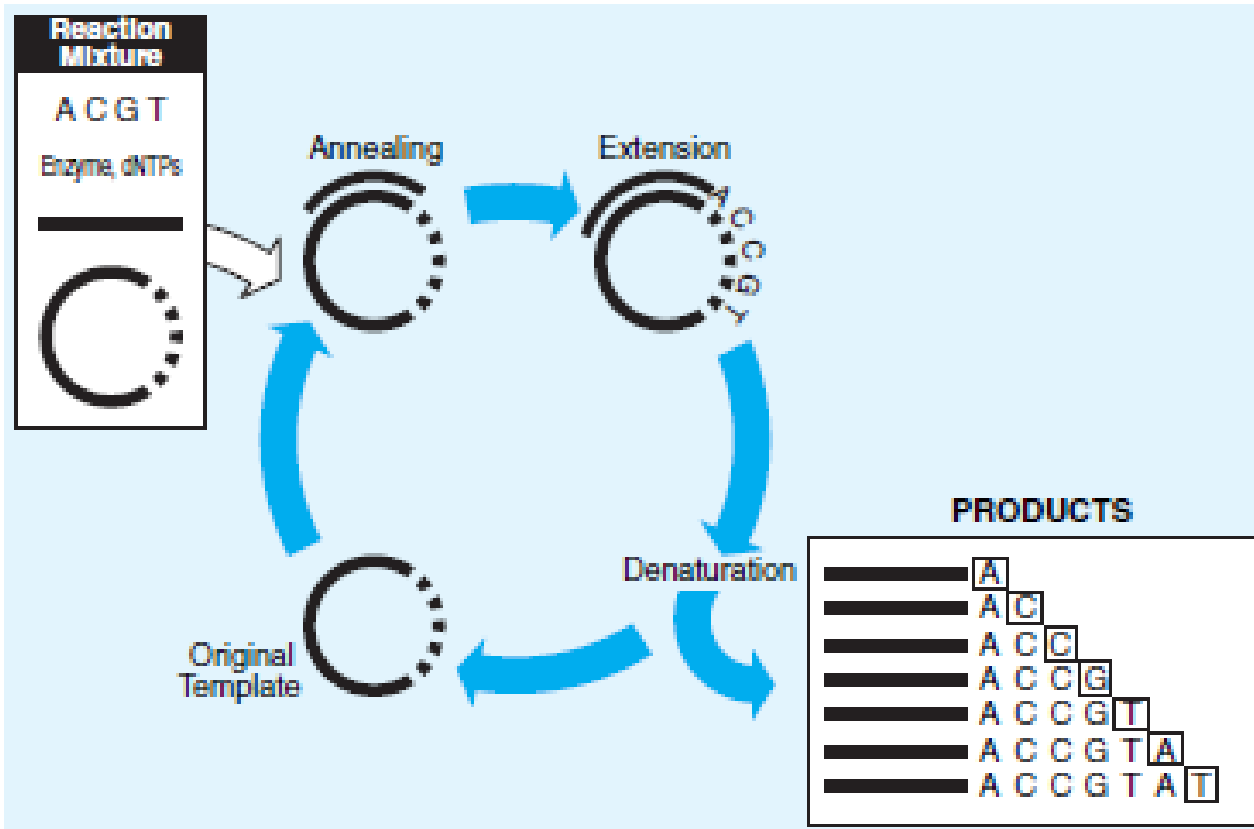
FIGURE 12-7 Sequencing by the Maxam-Gilbert Method of Chemical Degradation

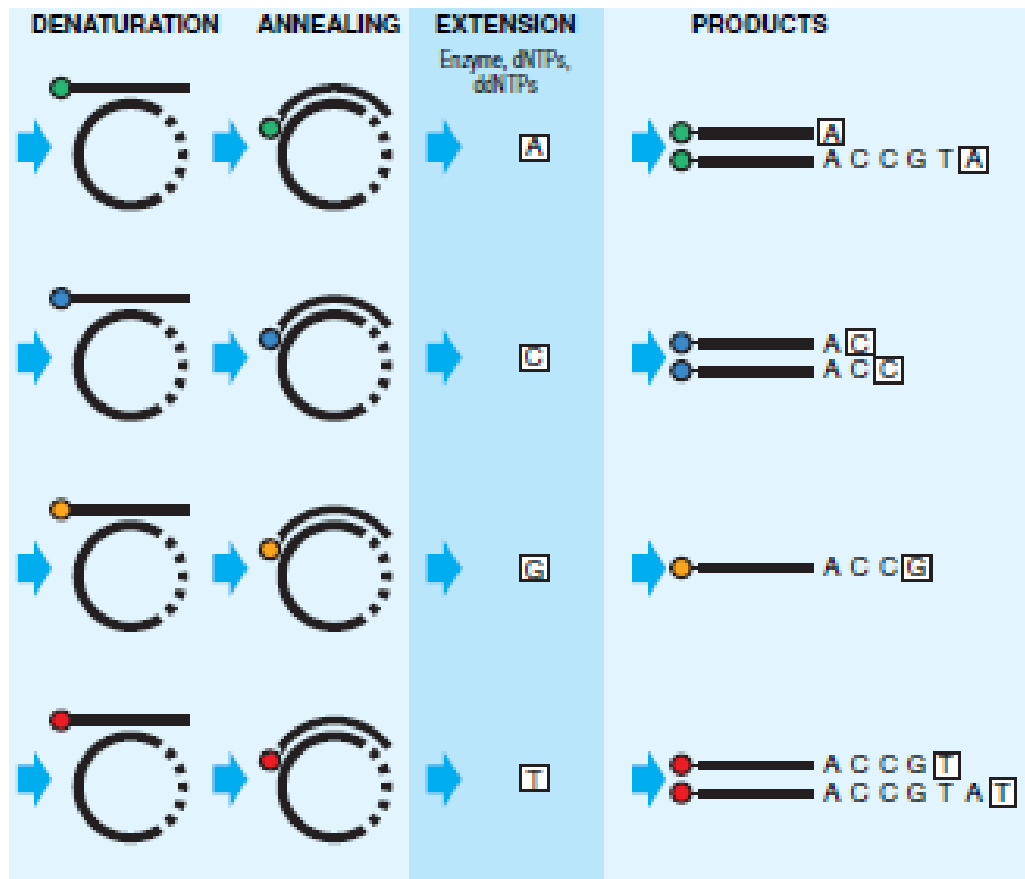
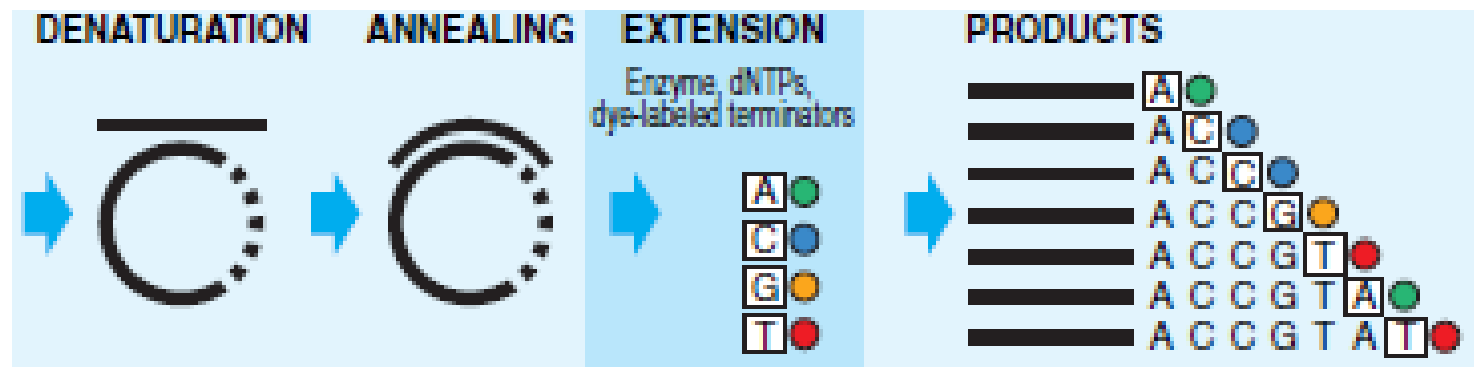
The oligonucleotide in this example (5' GATCCAAGT 3') is subjected to five chemical cleavage reactions (G, G+A, T+C, C, A>C). The resulting products of each of the reactions are separated by electrophoresis through a denaturing acrylamide gel and visualized by autoradiography, and the original sequence is determined from the "ladders" of oligonucleotide products. For details of the chemistry of these reactions, please see the accompanying text and Table 12-15.

Secuenciación de DNA: Sanger









Electroforesis capilar

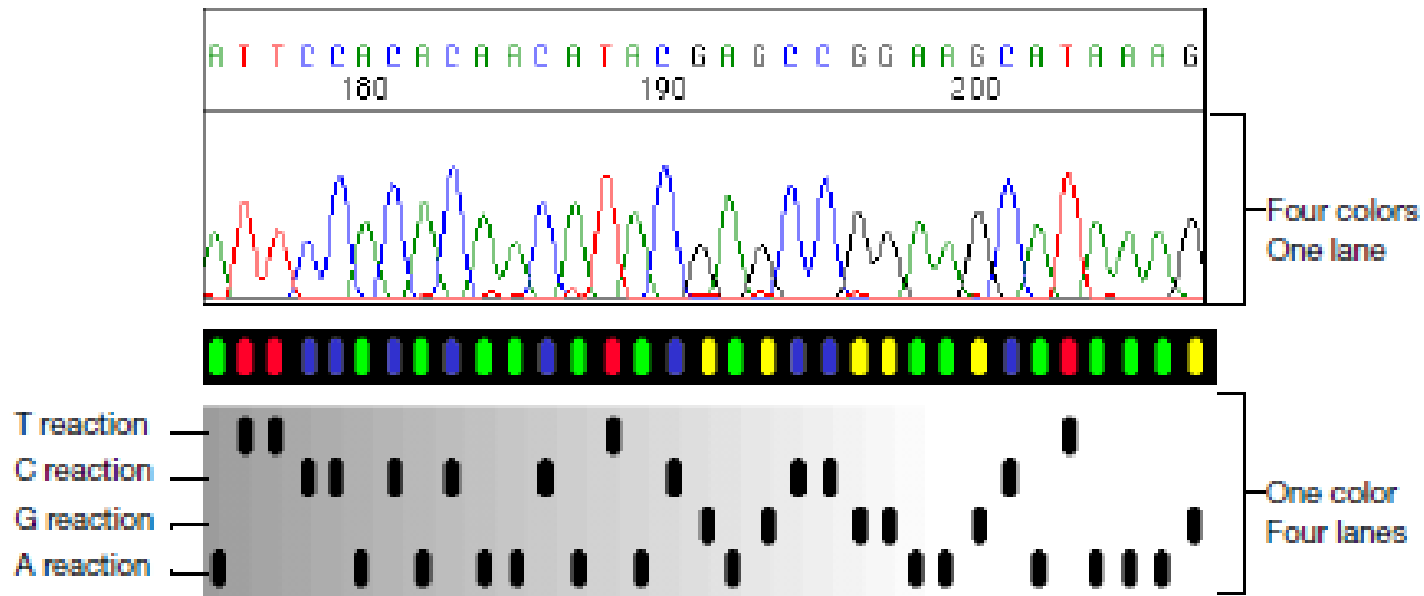


Figure 8 Fluorescent sequencing compared with radioactive sequencing