Chapter 9: Genomics and DNA Sequencing

- DNA sequencing
- Human genome project
- Genomics

DNA Sequencing

- **Purpose**

  The way to get the base sequence of DNA

- **Principles**

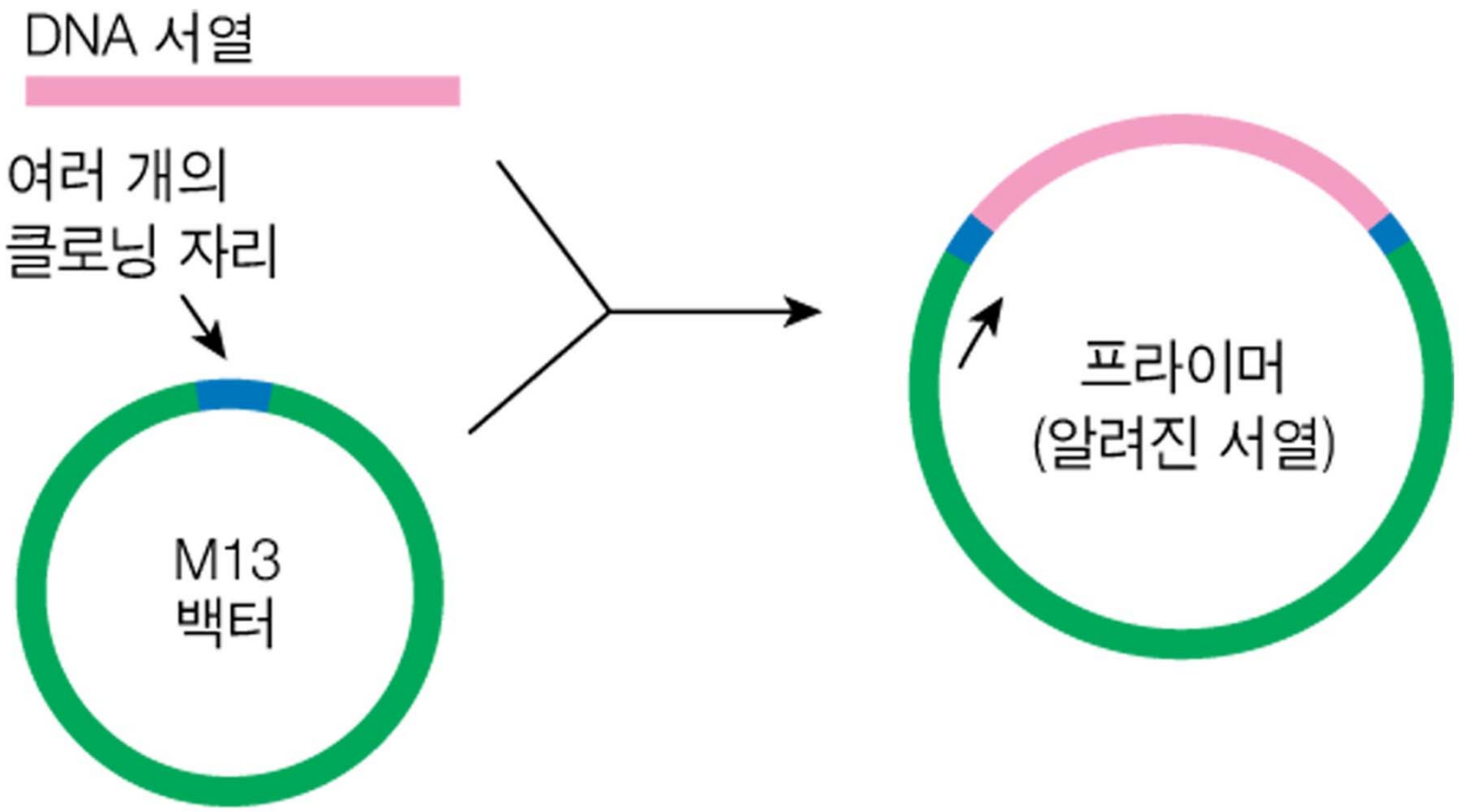
  1. Generate sub-fragments of all possible lengths from the DNA to be sequenced
  2. Group them according to which base they end in
  3. Separating them by electrophoresis

- **Main Methods**

  1. Chemical degradation method (Maxam and Gilbert, 1977)
  2. Chain termination method (Sanger and Coulson, 1977)
  3. Automated chain termination method
  4. Next Generation Sequencing (NGS)

DNA Sequencing

First, the DNA molecule to be sequenced must be cloned in a sequencing vector (usually 13), and a primer matched to a vector sequence is needed



DNA Sequencing

- **Process of DNA sequencing based on chain termination method**

  Target DNA fragment to be sequenced: ACGATTAG

1. Generate sub-fragments of all possible lengths from the DNA to be sequenced by using PCR

   ACGATTAG
   ACGATTA
   ACGATT
   ACGAT
   ACGA
   ACG
   AC
   A

   So, now we generated eight fragments different in size

Basic Tools and Techniques

2. Group them according to which base they end in

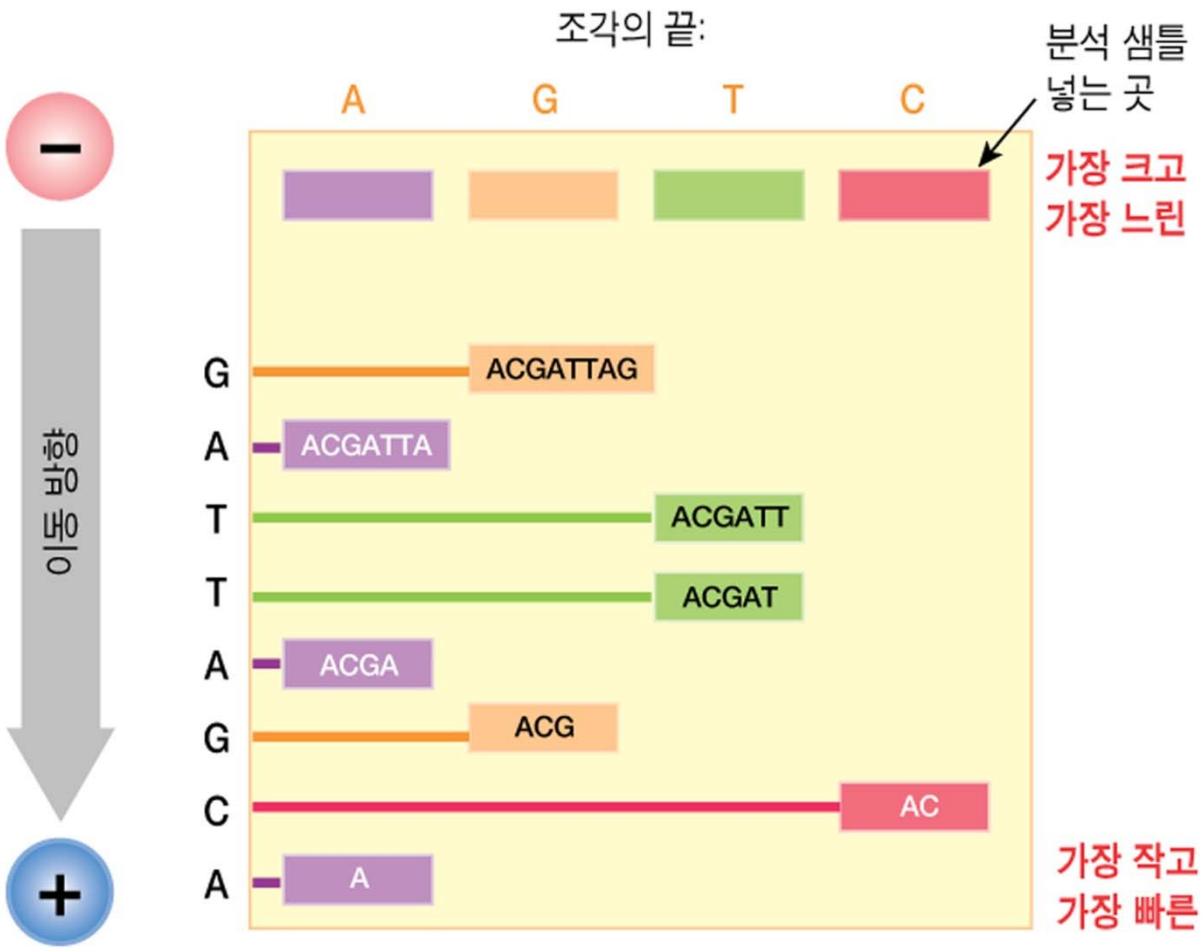
Ending in A	Ending in G	Ending in T	Ending in C
ACGATTA	ACGATTAG	ACGATT	AC
ACGA	ACG	ACGAT	
A			

How can we do this grouping?

Let's see the next slide

Basic Tools and Techniques

3. Separating them by electrophoresis using Polyacrylamide gel (sequencing gel)

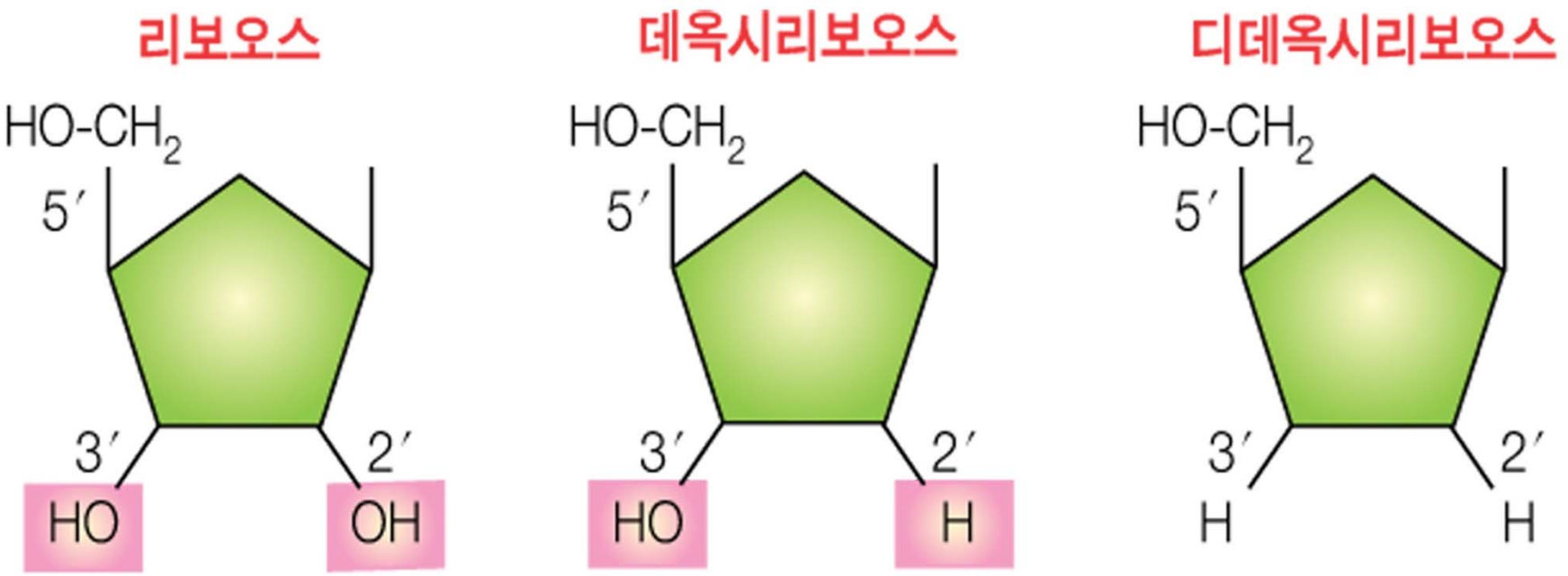


DNA Sequencing

Q. How to generate sub-fragments of all possible lengths from the DNA to be sequenced by using PCR?

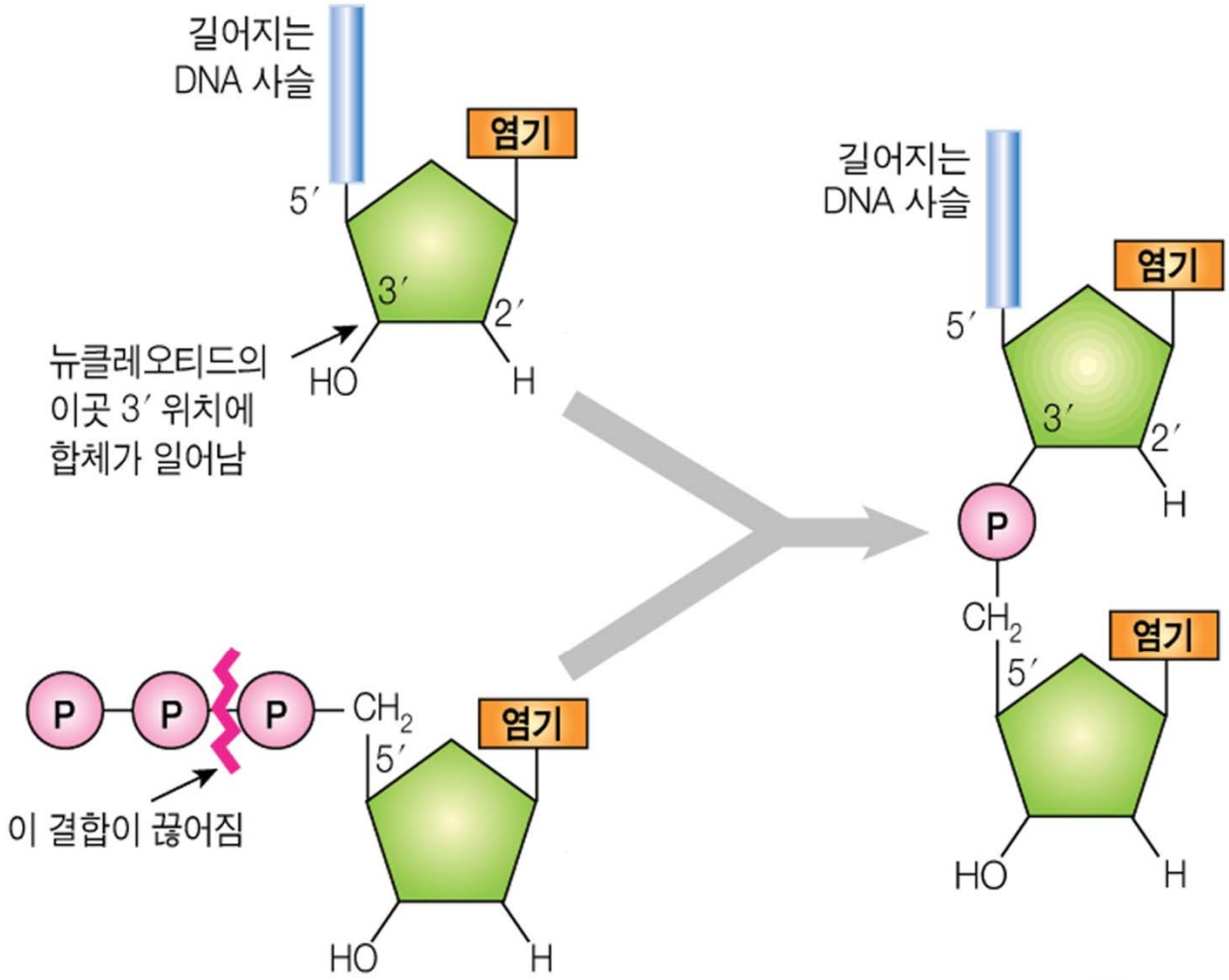
- Chain termination method (dideoxy method)

Ribose **Deoxyribose** **Dideoxyribose(ddNTP)**



Chain termination method

- **Dideoxy analogs of normal DNA precursors cause premature termination of a growing chain of nucleotides**



Chain termination method

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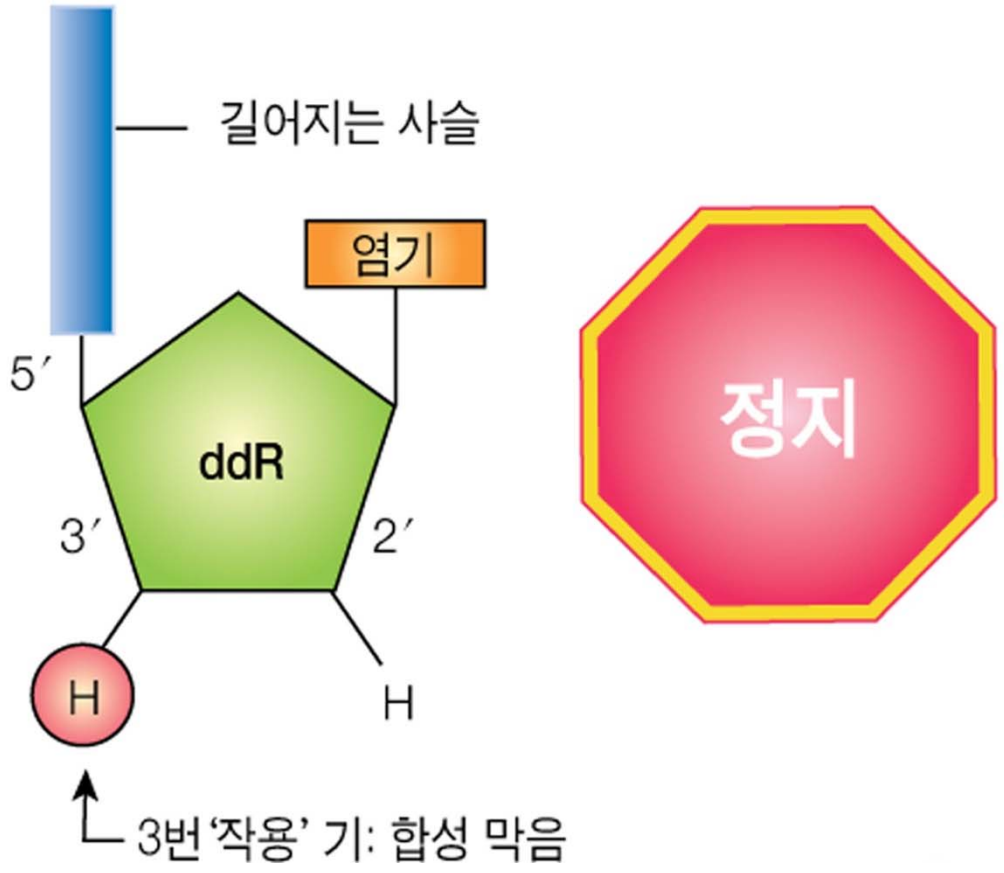
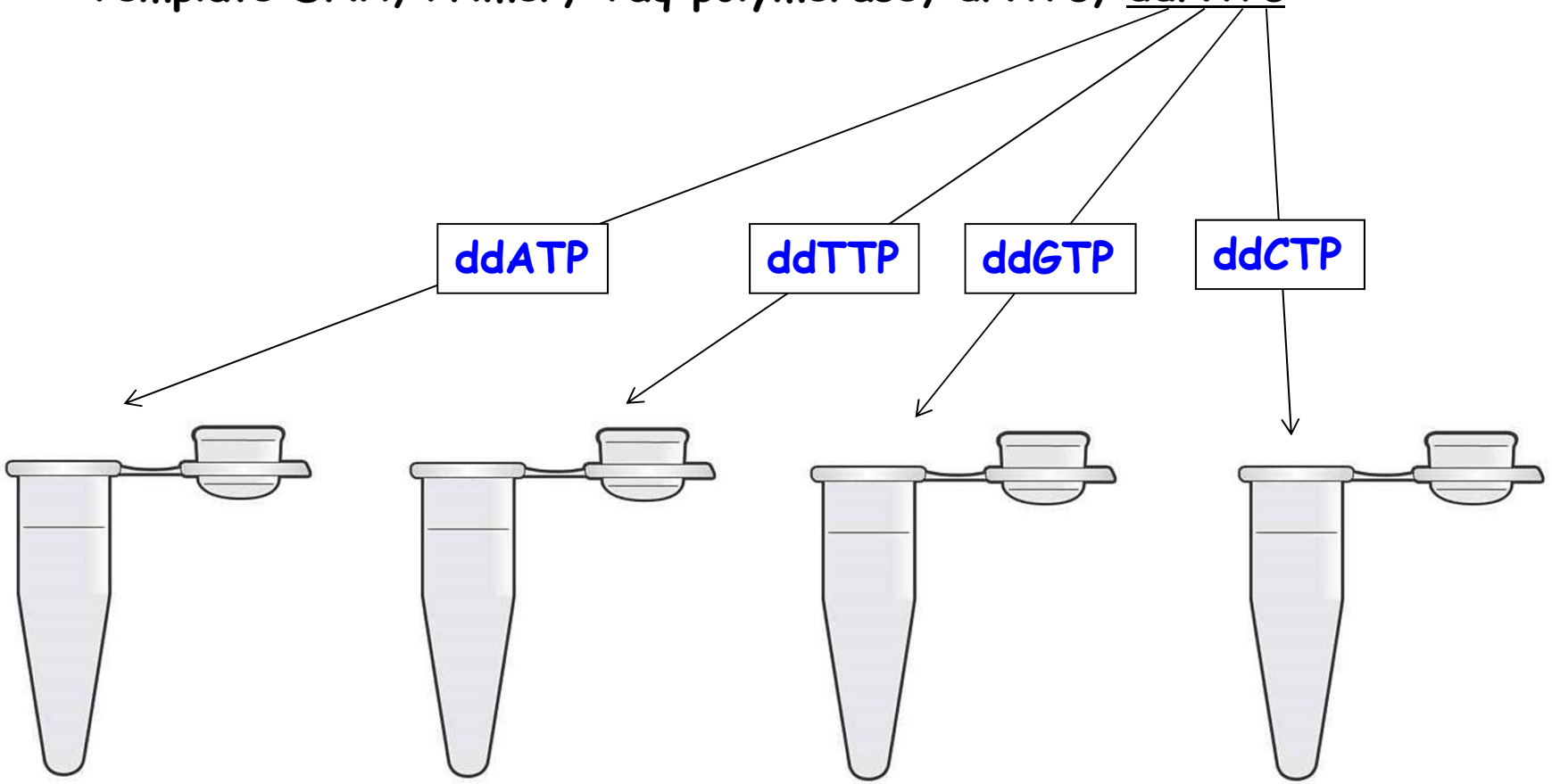


Chain termination method

- Components of PCR mixture needed for chain termination method

- Template DNA, Primer, Taq polymerase, dNTPs, ddNTPs



Chain termination method

- **When nucleotides containing dideoxyribose is incorporated into a growing nucleic acid chain in PCR,the PCR is terminated.**

본래의 서열:

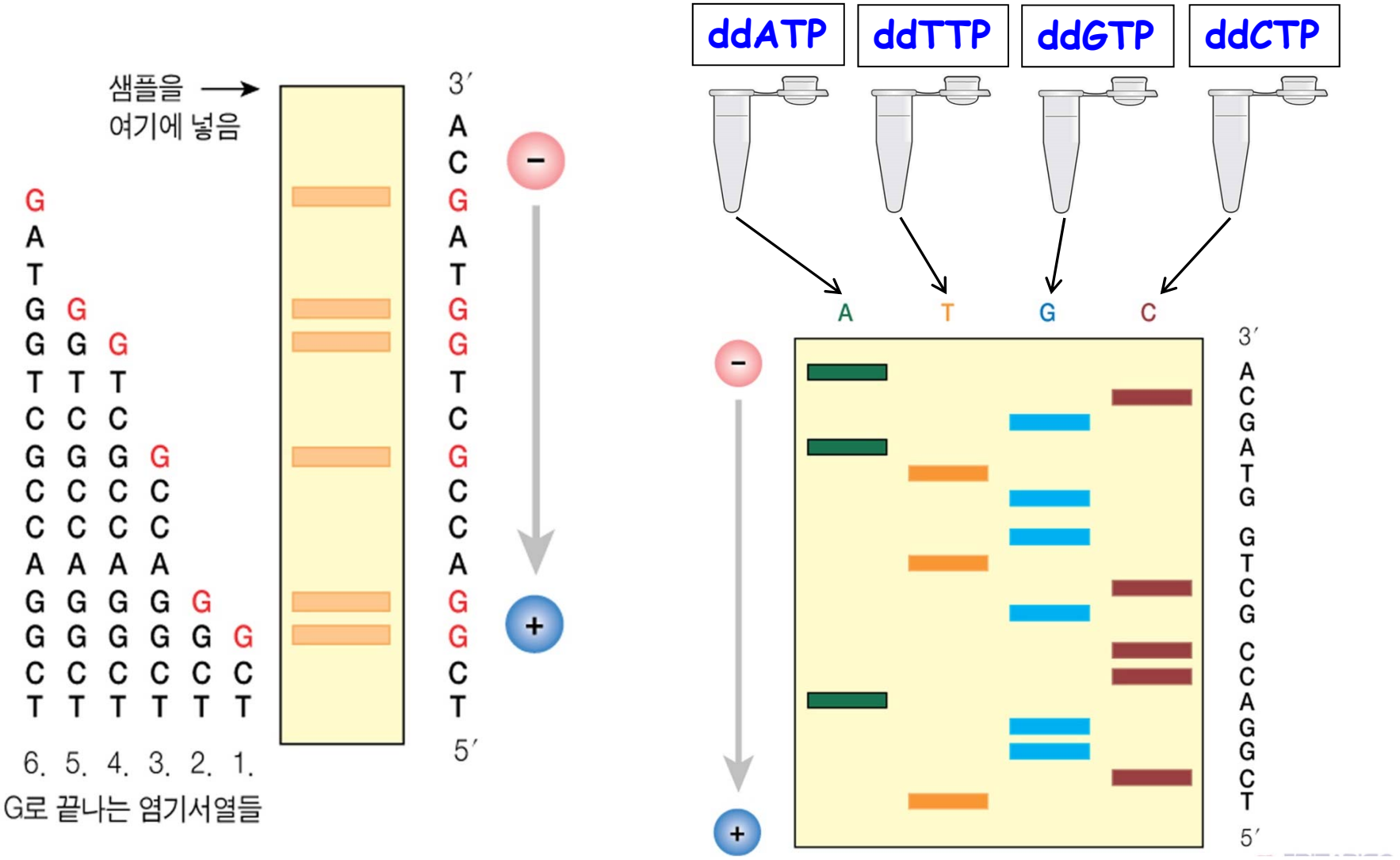
T C G G A C C G C T G G T A G C A

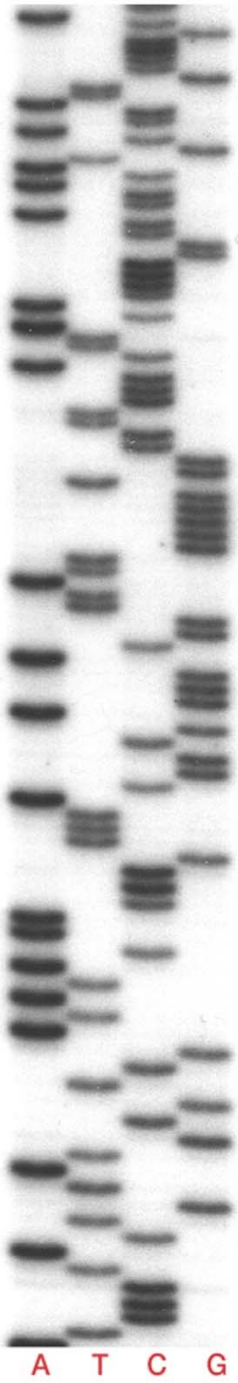
dGTP와 ddGTP를 섞어 사용하여
생긴 여러 G 종결사슬들

1. T C G
2. T C G G
3. T C G G A C C G
4. T C G G A C C G C T G
5. T C G G A C C G C T G G
6. T C G G A C C G C T G G T A G

Chain termination method

- Separation of PCR-amplified DNA fragments by electrophoresis





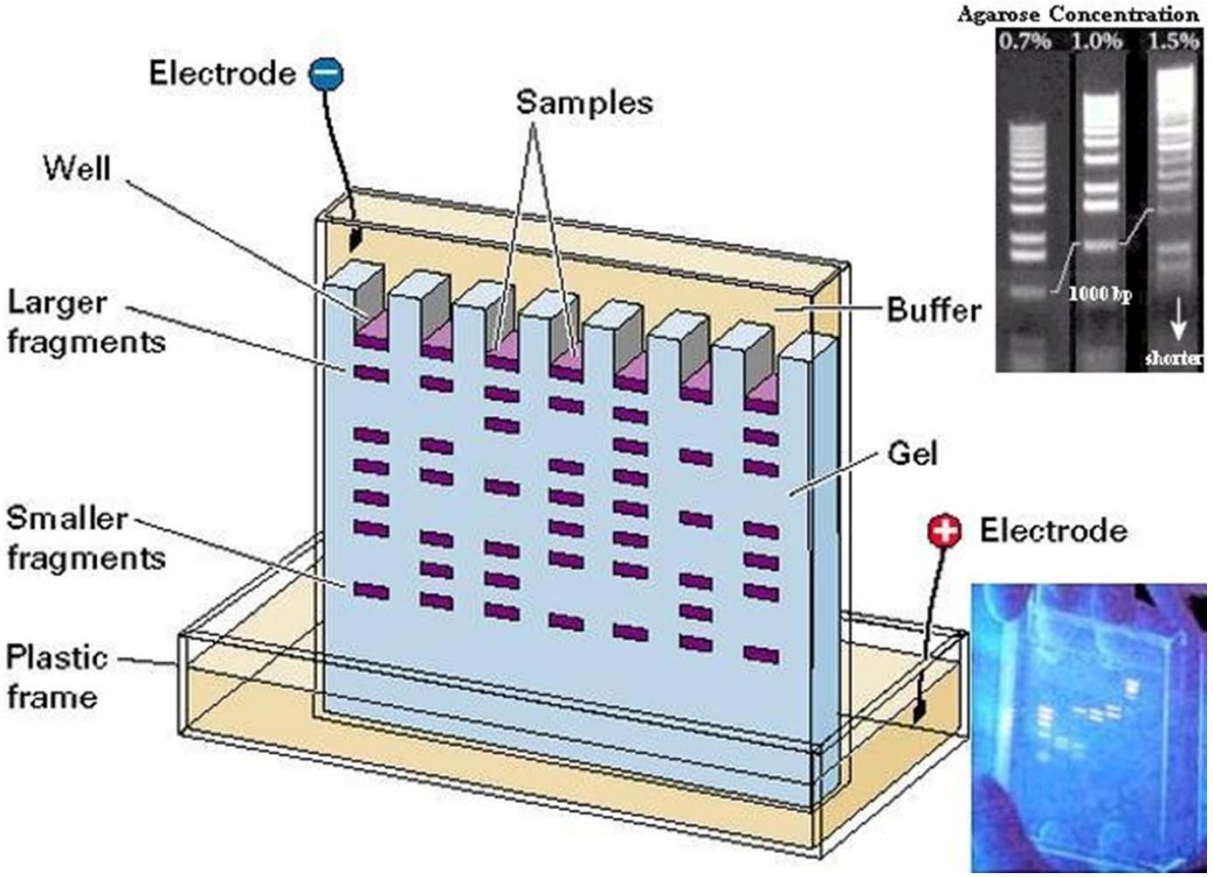
Let's give it a try!

Automated sequencing

- Everything is the same as the previous method, except for the followings

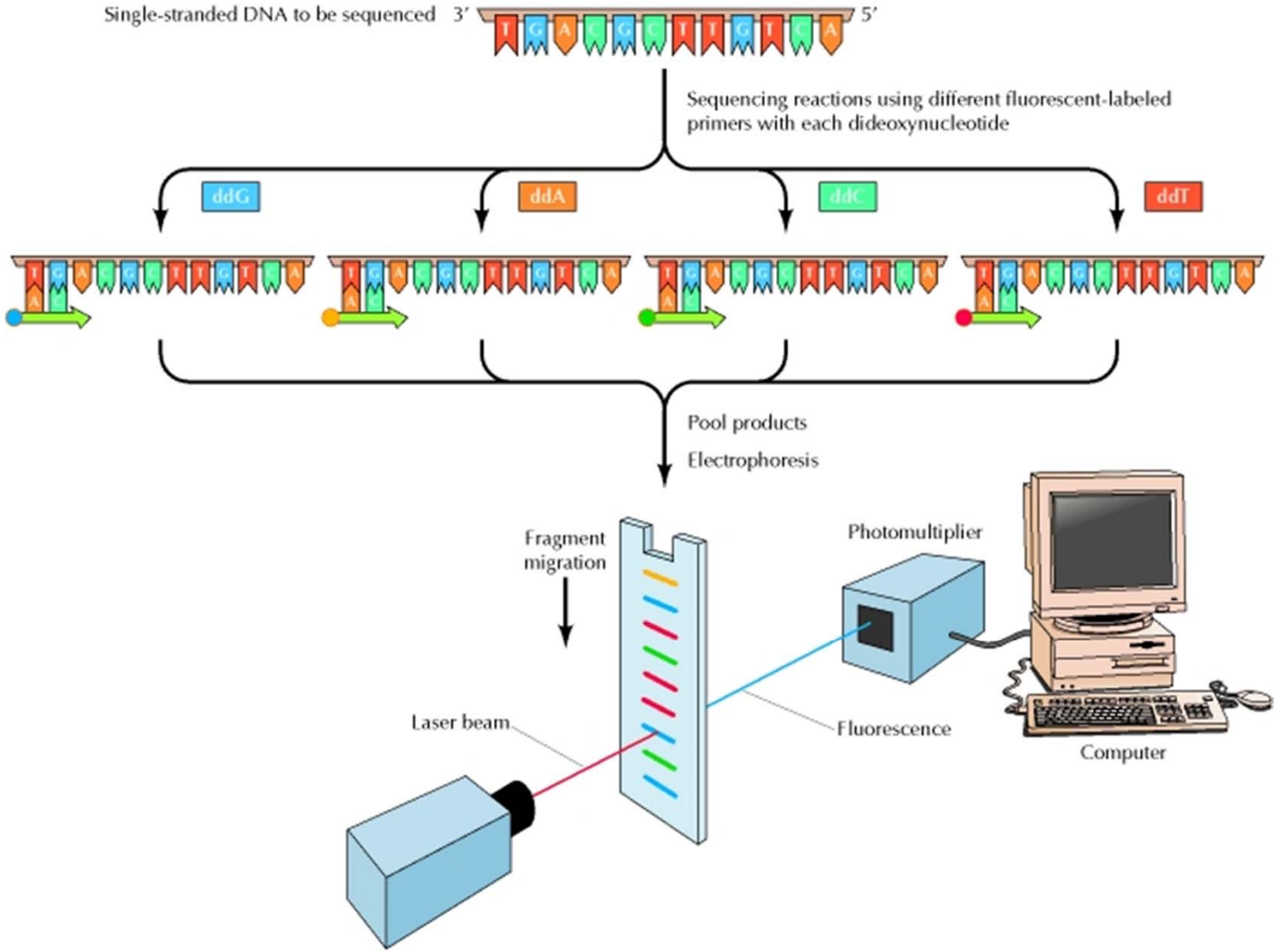
1. ddNTPs are labeled by attaching a fluorescent dye with four different colors [fluorescent chemicals with four different wave length(signal)], so each chain terminated DNA fragment carries a single label at its 3' end.
2. PCR is performed in a single sequencing reaction tube with all four ddNTPs, because molecules terminated with different ddNTPs can be identified by their distinctive fluorescent signals
3. When run the PCR products on the gel, DNA fragments are detected by a special type of imaging system
   - computer to read the DNA sequence
   - reaction products are loaded into a single well of polyacrylamide gel (capillary electrophoresis system)
   - run past the fluorescence detector

Manual DNA sequencing



Manual DNA sequencing

Process of automated sequencing



Automated DNA sequencer