Sequencing methods

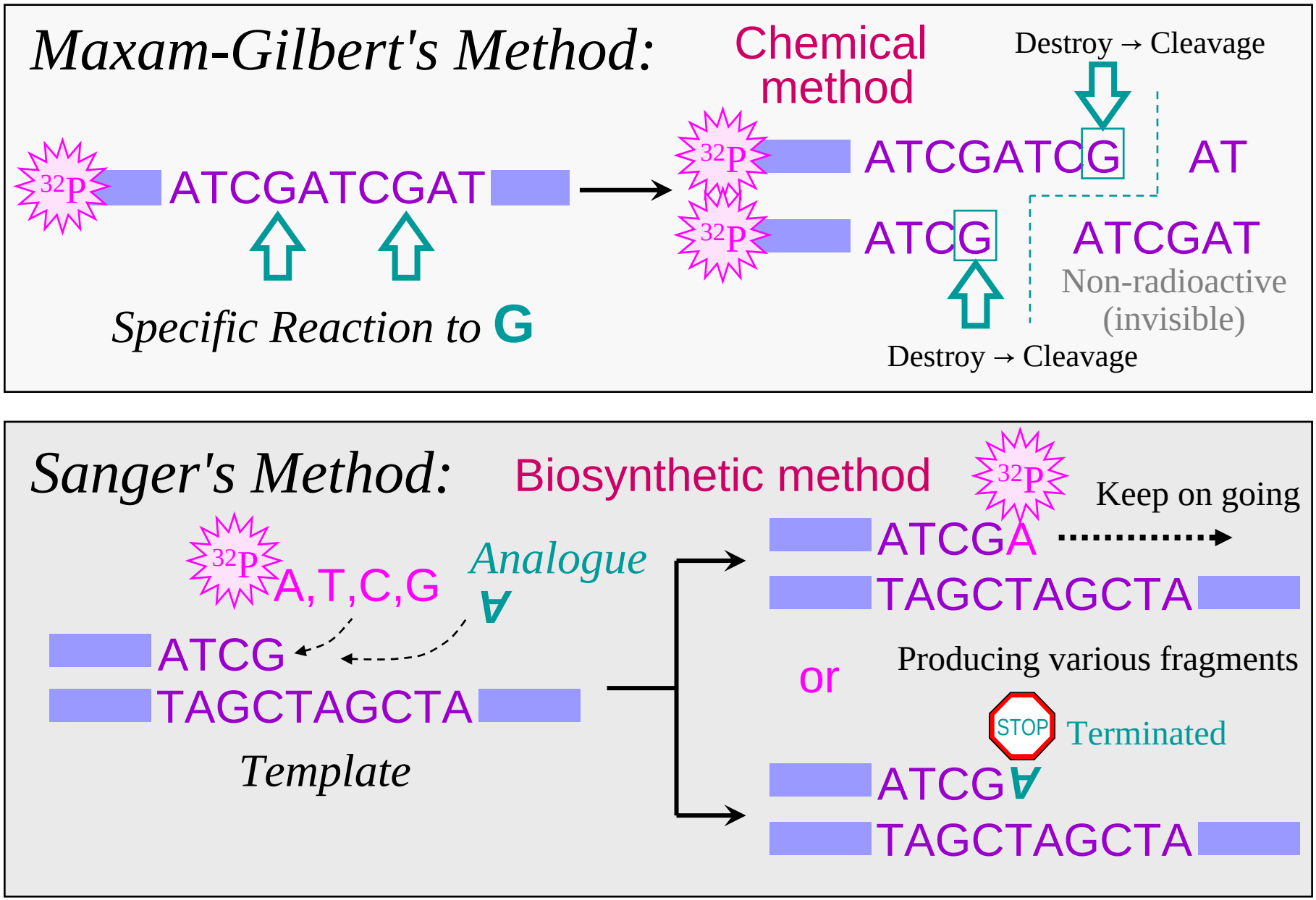
- The process of determining the order of the nucleotide bases along a DNA strand is called **DNA sequencing**

- In 1977 two separate methods for sequencing DNA were developed: the **chain termination method** or **cycle sequencing** (Sanger et al.) and the **chemical degradation** method or **Maxam-Gilbert sequencing**
(Maxam and Gilbert)

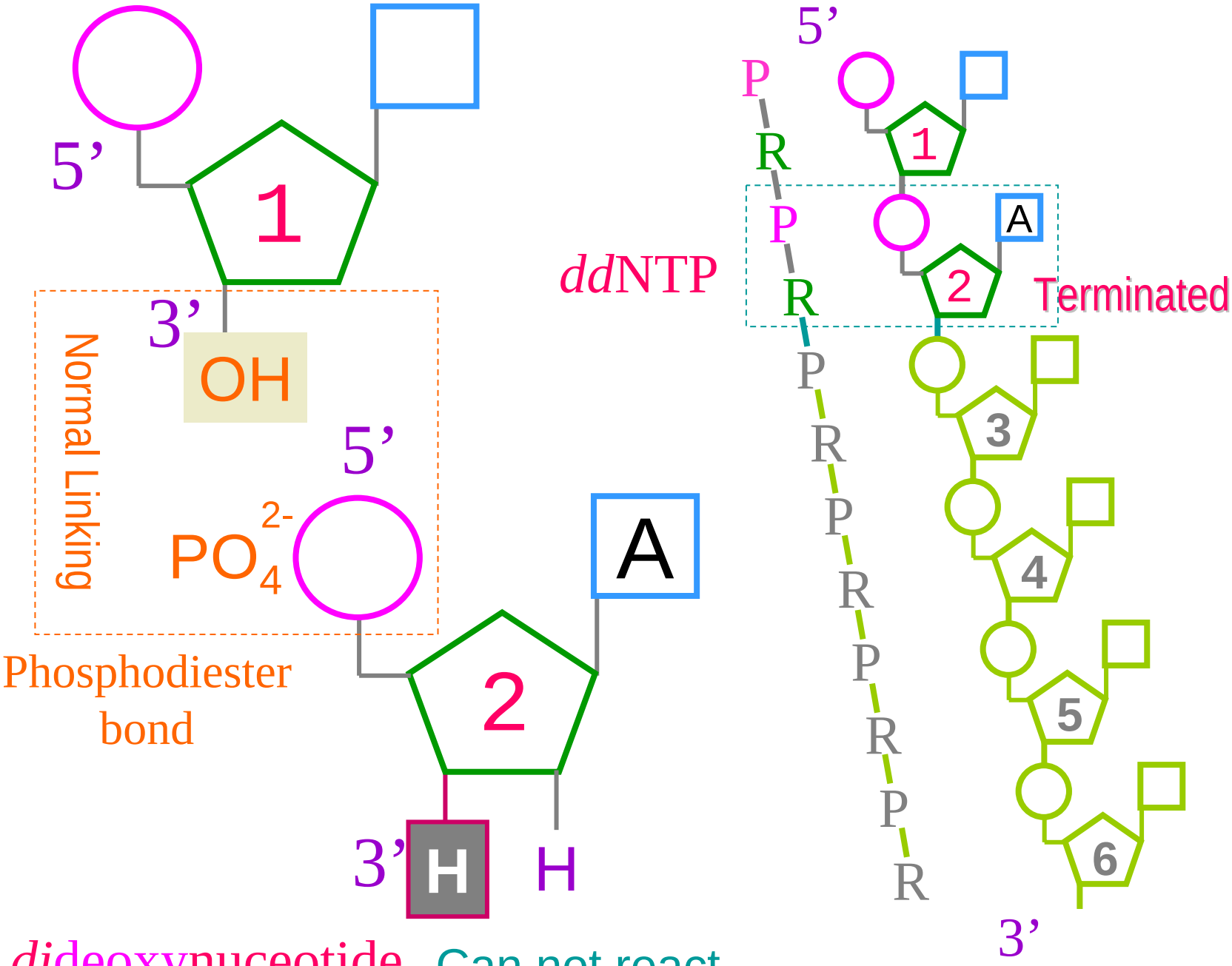
- Both methods were equally popular to begin with, but, for many reasons, the cycle sequencing method is the method more commonly used today

- This method is based on the principle that single-stranded DNA molecules that differ in length by just a single nucleotide can be separated from one another using polyacrylamide gel electrophoresis

How to Obtain DNA Fragments

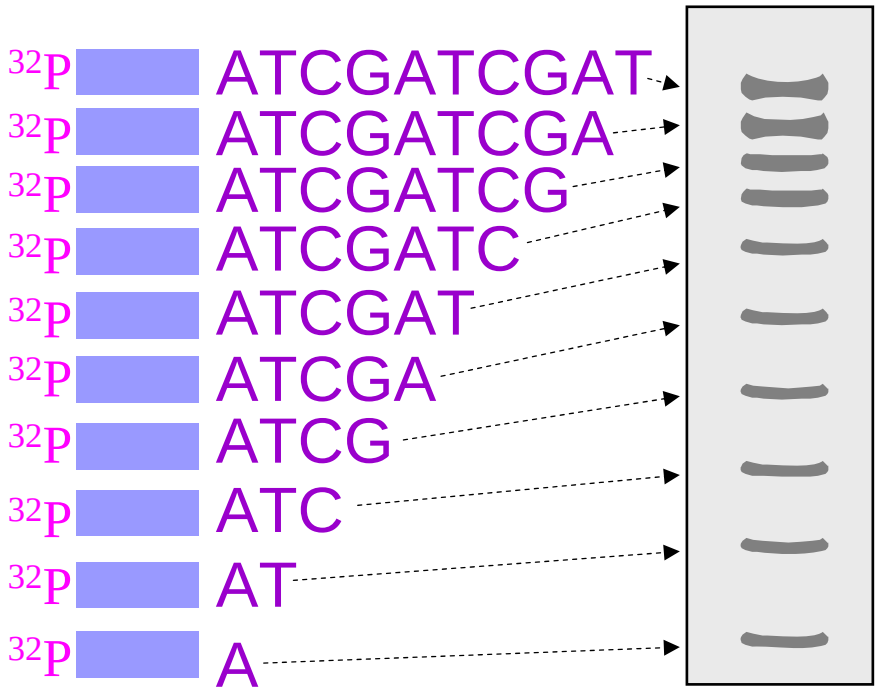


Sanger's Method: How Terminated



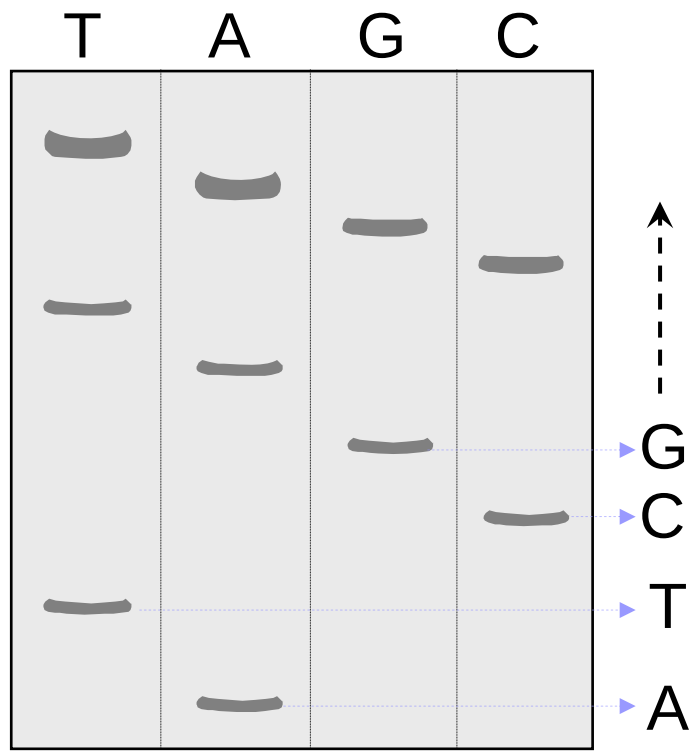
How DNA Sequence Is Determined?

DNA fragments having a difference of one nucleotide can be separated on gel electrophoresis



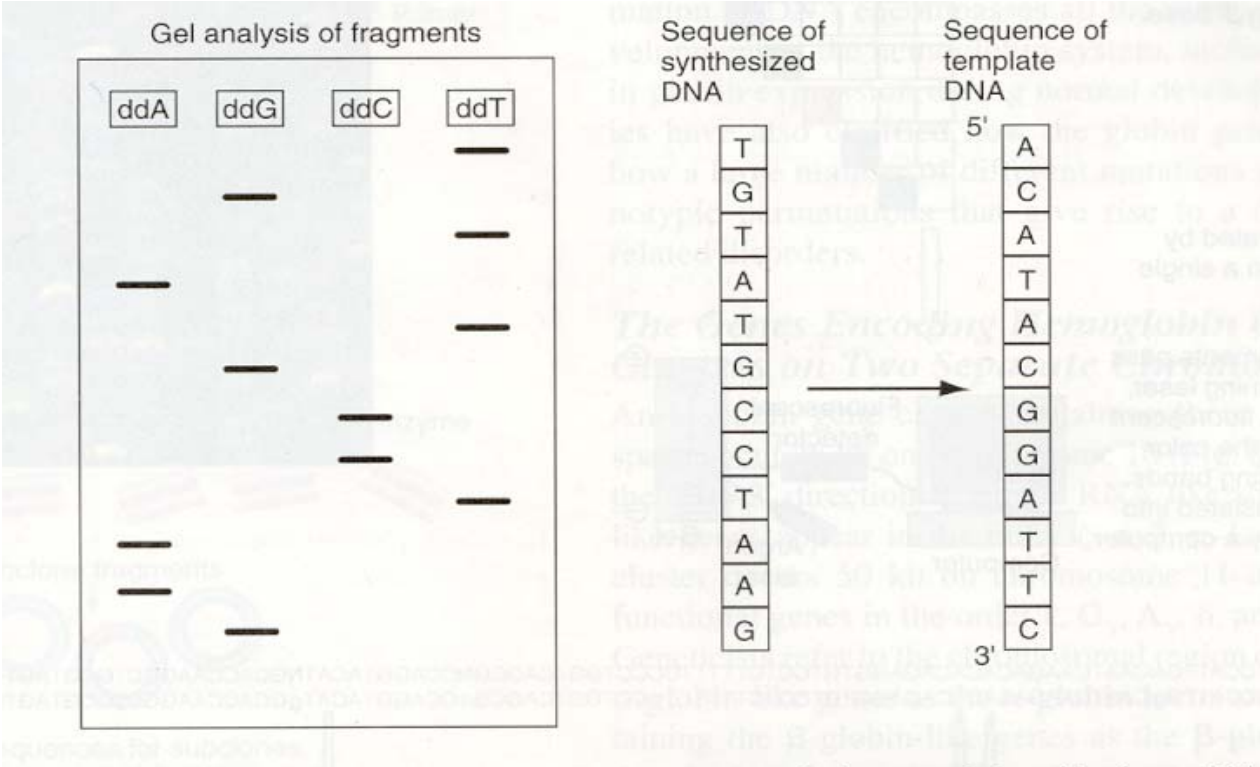
But these bands can't tell us the identity of the terminal nucleotides

Polyacrylamide Gel Electrophoresis

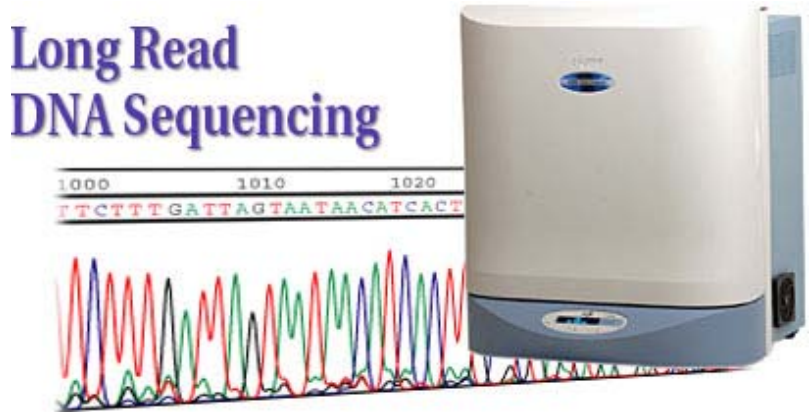


If those band with the same terminal nucleotide can be grouped, then it is possible to read the whole sequence

Juang RH (2004) BCbasics


Gel analysis of fragments
ddA
ddG
ddC
ddT
Sequence of synthesized DNA
Sequence of template DNA
5'
3'
T
G
T
A
T
G
C
C
T
A
A
G
A
C
A
T
A
C
G
G
A
T
T
C

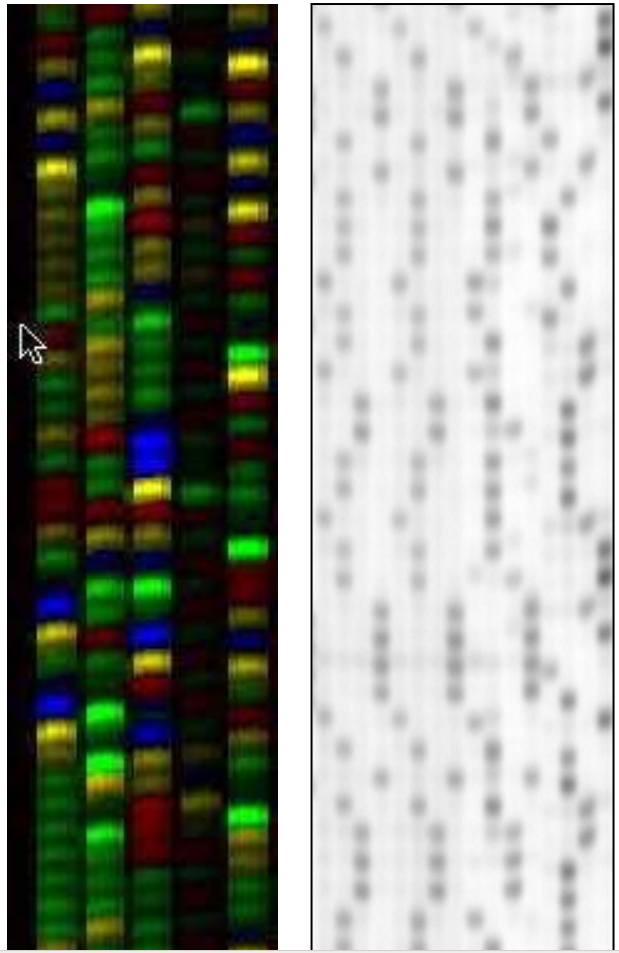
Sequencing systems



LICOR DNA 4300

ABI 3100

DNA Sequencing



Chromatogram file