# BIOTECHNOLOGY

DNA Sequencing

Fig 20.12

# Human Genome Project

- **Genome**: an organisms' complete set of DNA
- Human genome has about 3 billion base pairs
- **Human Genome Project** is an international collaborative research effort (1998-2003) to sequence and map all genes in human beings (homo sapiens)

Reference: http://www.genome.gov/11006943

# The Race!

- Most of the sequencing was performed in university and research centers in the USA, UK, France, Germany, Japan, China
  - **Small-scale shotgun** sequencing
- Parallel project conducted by private company Celera Genomics (Craig Venter)
  - **Shotgun** sequencing

# Video Clips

- Private Project sequencing:

http://content.dnalc.org/content/c15/15537/private_project_sequencing.mp4
(http://www.dnalc.org/resources/3d/30-private-project-sequencing.html)

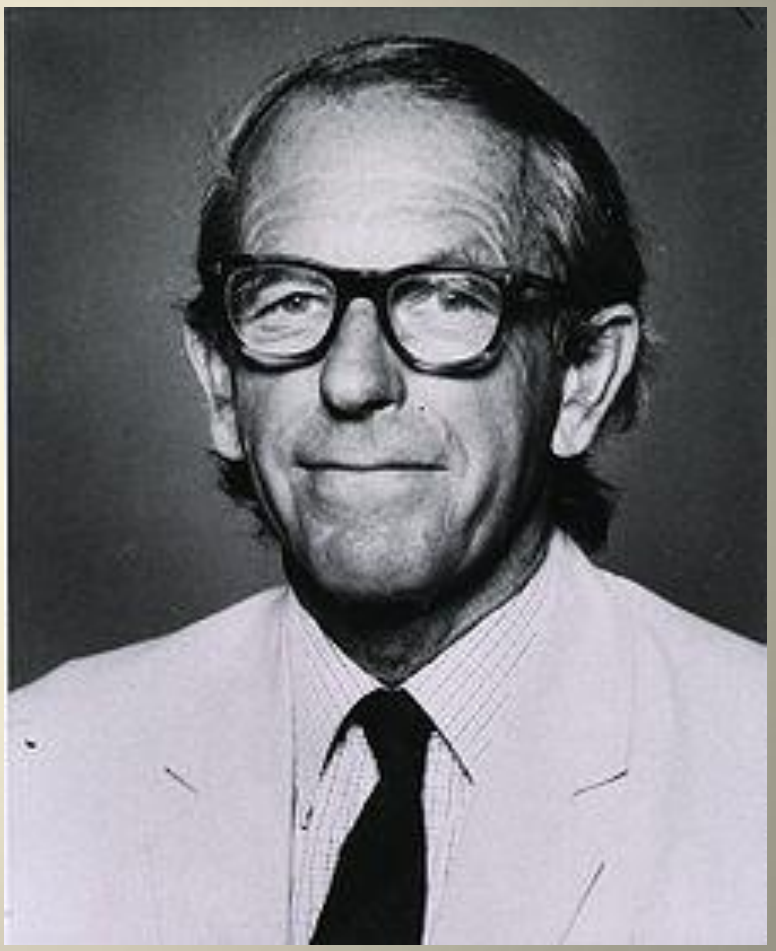
- Comparing Public & Private Project sequencing:

http://content.dnalc.org/content/c15/15477/public_project_sequencing.mp4
(http://www.dnalc.org/resources/3d/28-public-project-sequencing.html)

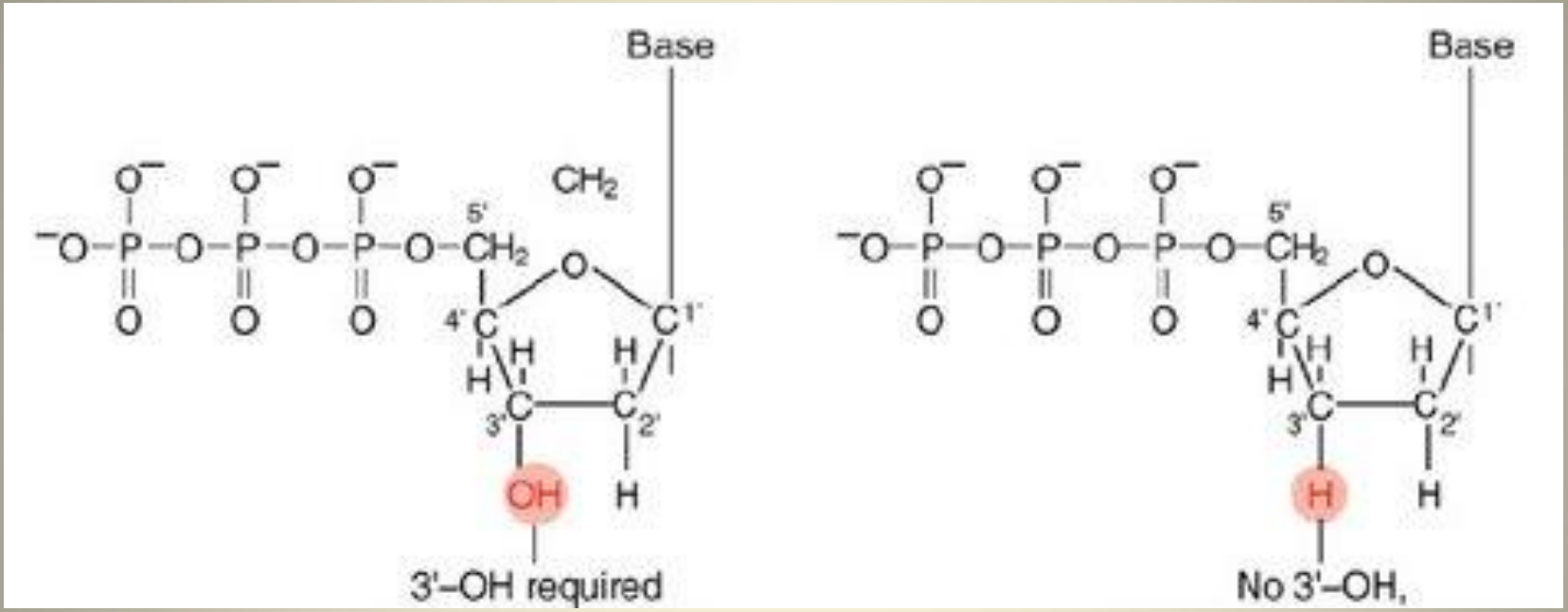
(brackets) indicate links to same animation but with addition resources available on the webpage including the narrative.

# Fredrick Sanger (1975)

- Developed **dideoxy termination sequencing** method
- 1980 received Nobel Prize in Chemistry for sequencing method
- 1958 received Nobel Prize in Chemistry for his work on the structure of insulin



# Dideoxyribonucleotides (ddNTP)

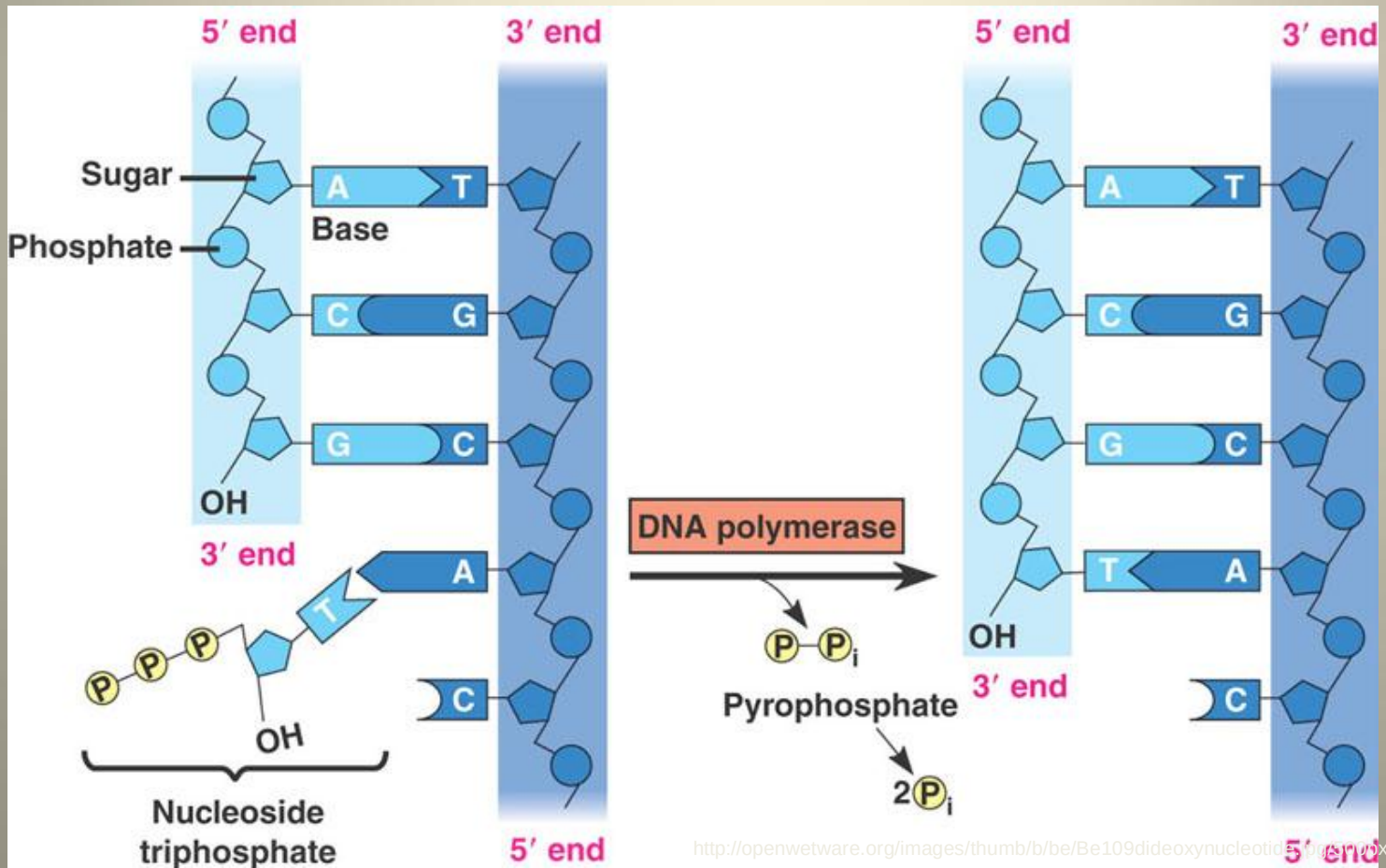



- Di = two, Deoxy = removed oxygen
- Dideoxy NTPs have oxygens missing at both the 2’ and 3’ position

http://openwetware.org/images/thumb/b/be/Be109dideoxynucleotide.jpg/500px-Be109dideoxynucleotide.jpg

# Recall: DNA Elongation

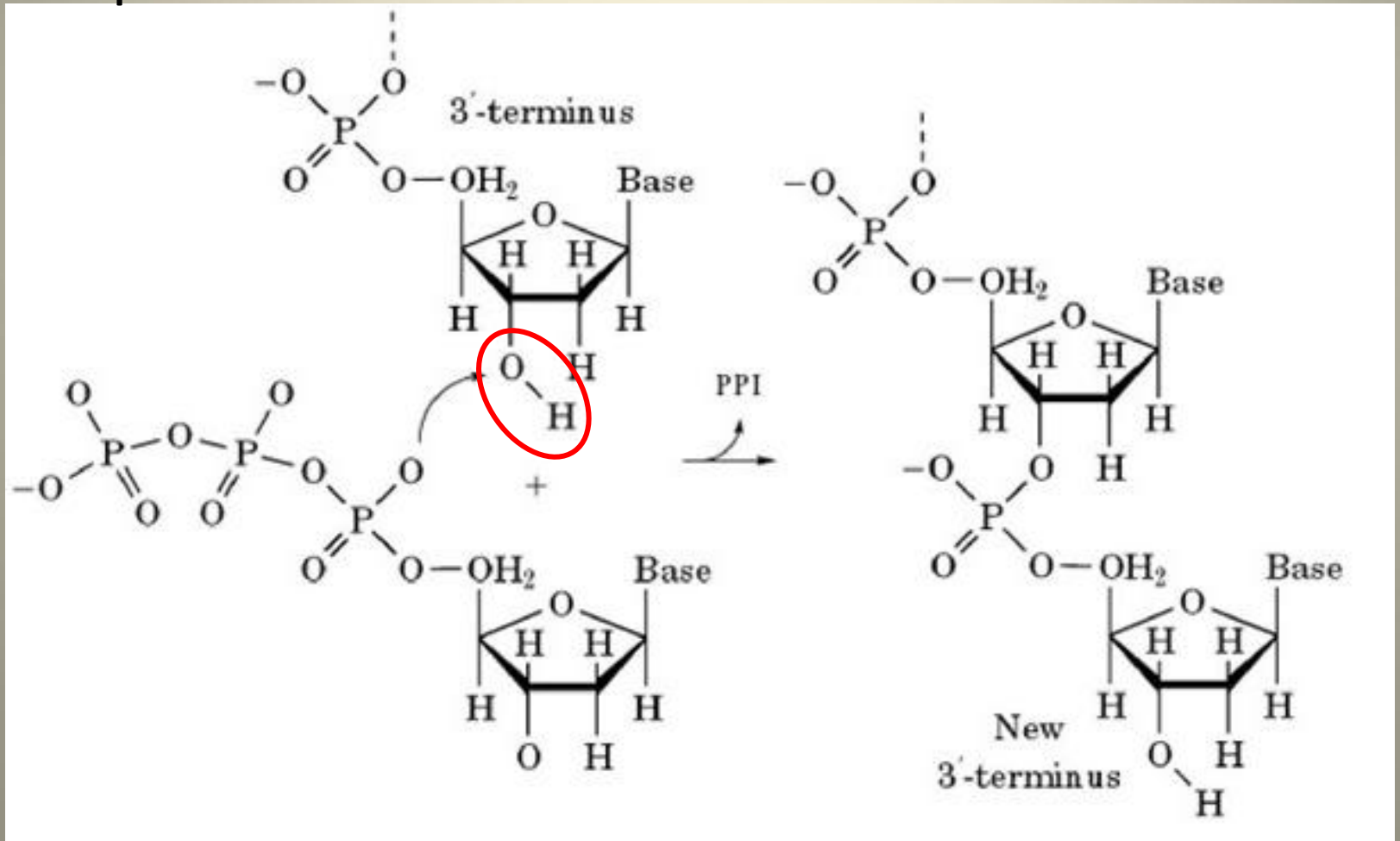
- What problem do you forsee if ddNTPs are used in replication?




http://openwetware.org/images/thumb/b/be/Be109dideoxynucleotide.jpg/300px-Be109dideoxynucleotide.jpg

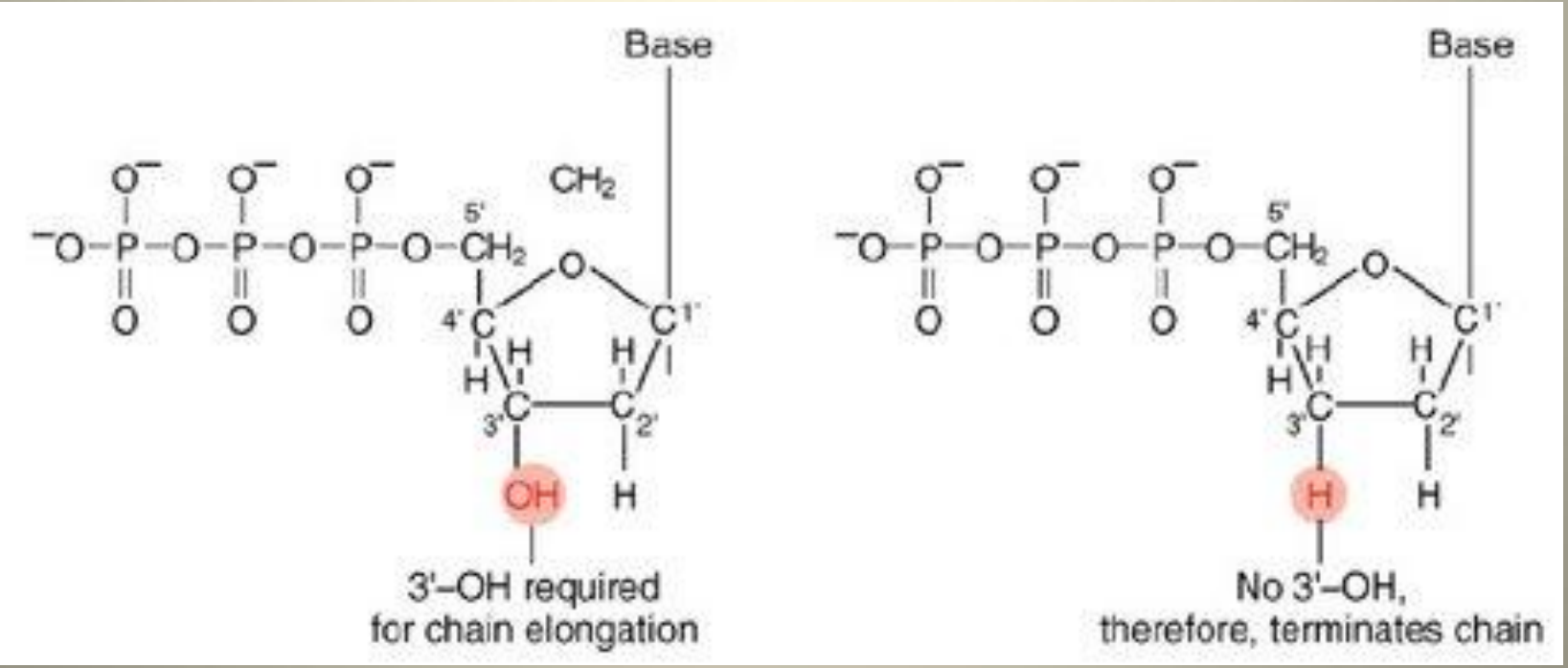
# Recall: Polymerization Reaction

- 3’OH is needed to react with the phosphate group on the 5’ end of the next nucleotide to form a phosphodiester bond



# Dideoxyribonucleotides (ddNTP)

- When a ddNTP is RANDOMLY incorporated into the newly synthesized strand of DNA, synthesis stops




http://openwetware.org/images/thumb/b/be/Be109dideoxynucleotide.jpg/500px-Be109dideoxynucleotide.jpg

# Sanger Sequencing

- Method is based on DNA replication by DNAP
- Materials:
  - DNAP
  - Primer (radioactive)
  - Template DNA
  - dNTPs (deoxyNTP)
  - ddNTPs (dideoxyNTP)

# Sanger Sequencing Setup

- Each test tube has deoxyA, G, C, T
- In addition each also has ONE of the 4 ddNTP

| Tube A | Tube G | Tube T | Tube C |
|---|---|---|---|
| 15% dATP | | | |
| 25% dGTP | | | |
| 25% dTTP | | | |
| 25% dCTP | | | |
| 10% ddATP | | | |

# Sanger Sequencing Setup

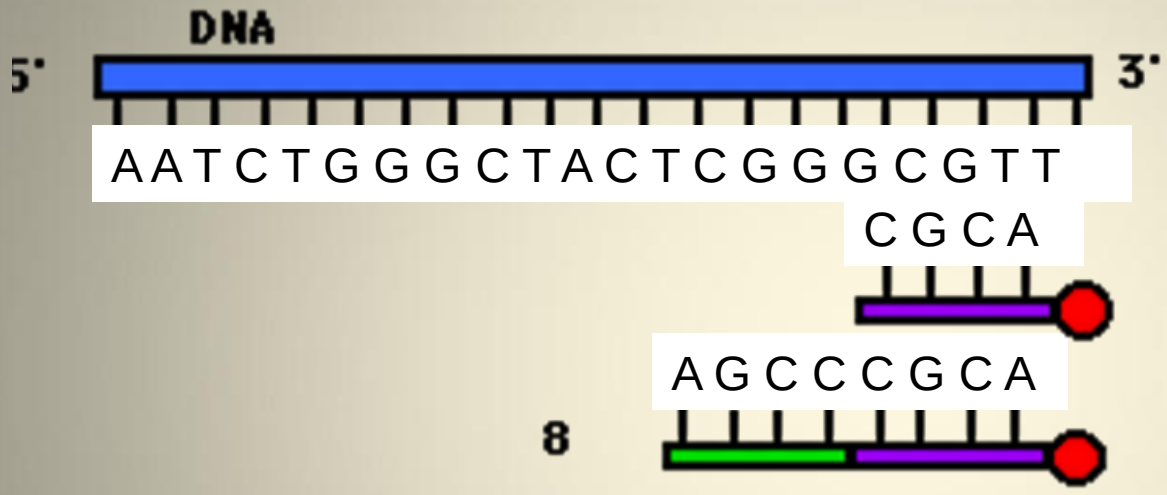
- What do you think will happen if you allow DNA replication to occur in the presence of both deoxyATP and dideoxyATP?


| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Setup

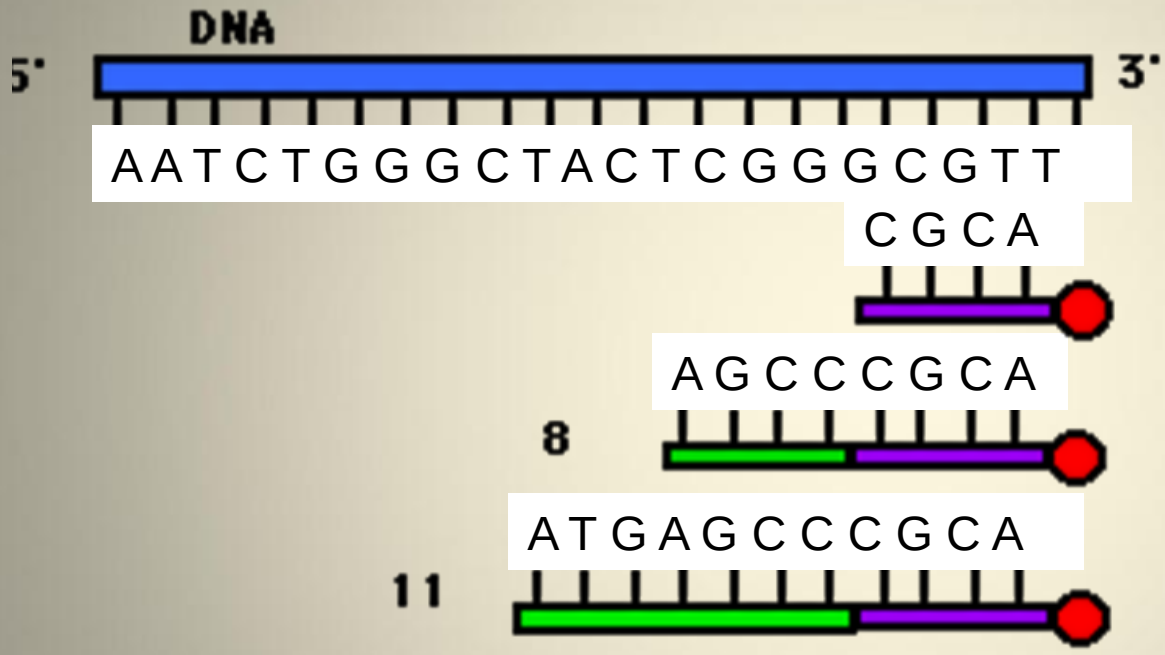

| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Setup



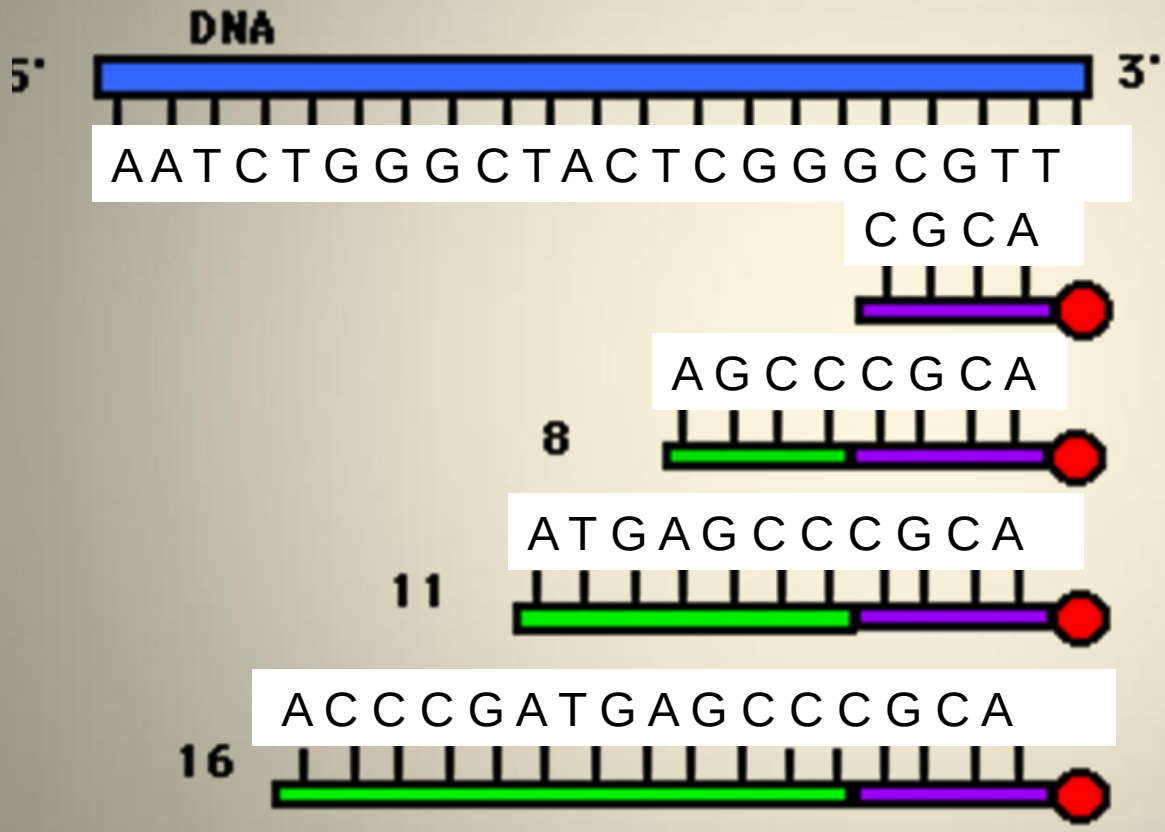

| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Setup



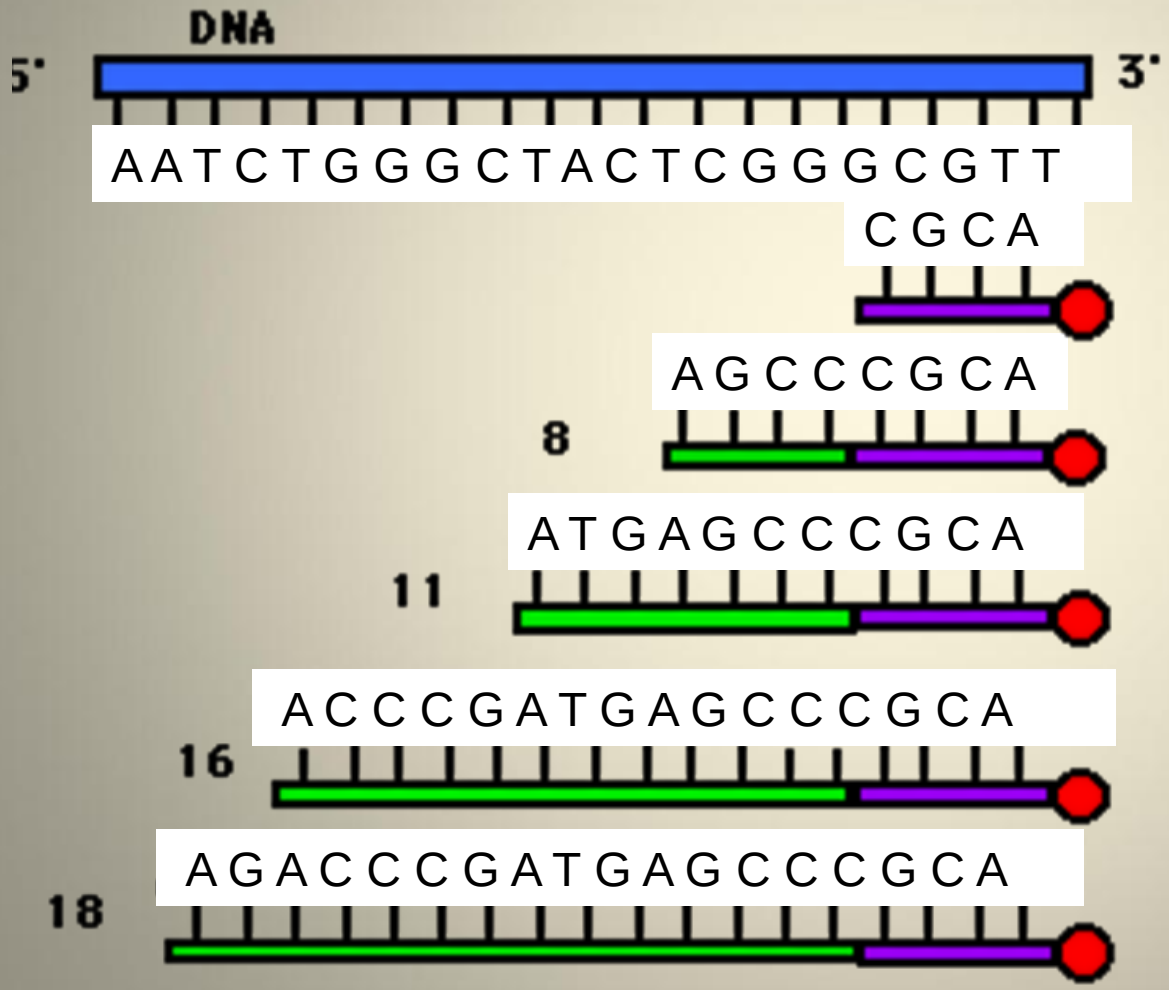

| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Setup




| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Setup




| Tube A |
| --- |
| 15% ATP |
| 25% GTP |
| 25% TTP |
| 25% CTP |
| 10% ddATP |

# Sanger Sequencing Process

- The newly synthesized strands of DNA have different lengths depending on when a ddNTP was incorporated into the strand
- The process is repeated 4 times, with different ddNTPs

# Sanger Sequencing Setup

- Complete the chart for the other tubes

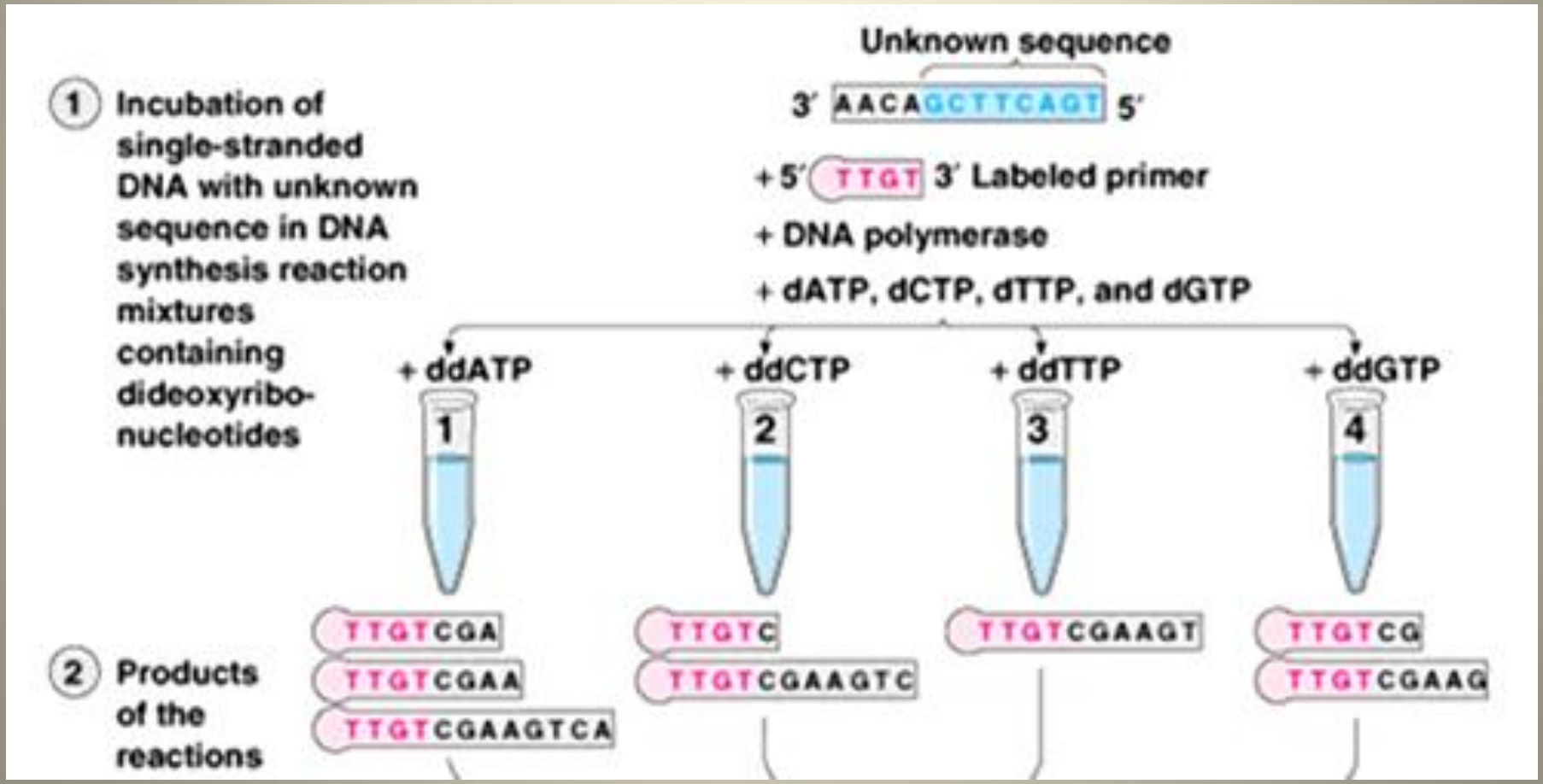
| Tube A | Tube G | Tube T | Tube C |
|---|---|---|---|
| 15% dATP | 25% dATP | 25% dATP | 25% dATP |
| 25% dGTP | dGTP | 25% dGTP | 25% dGTP |
| 25% dTTP | 25% dTTP | dTTP | 25% dTTP |
| 25% dCTP | 25% dCTP | 25% dCTP | dCTP |
| 10% ddATP | | | |

# Sanger Sequencing Setup

| Tube A | Tube G | Tube T | Tube C |
|---|---|---|---|
| 15% dATP | 25% dATP | 25% dATP | 25% dATP |
| 25% dGTP | 15% dGTP | 25% dGTP | 25% dGTP |
| 25% dTTP | 25% dTTP | 15% dTTP | 25% dTTP |
| 25% dCTP | 25% dCTP | 25% dCTP | 15% dCTP |
| 10% ddATP | 10% ddGTP | 10% ddTTP | 10% ddCTP |

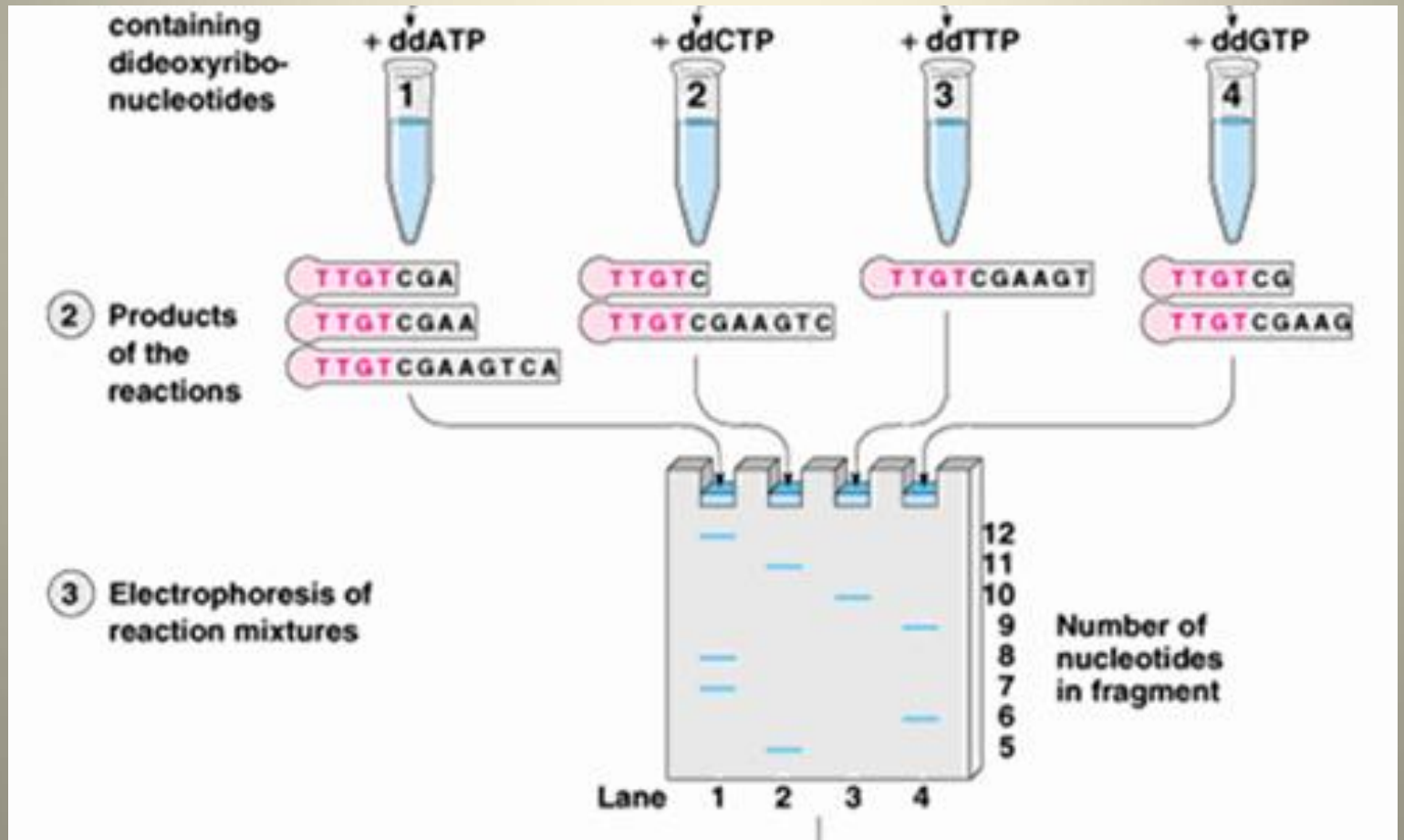
# Sanger Sequencing Setup

- Each reaction vessel only has ONE of the 4 possible ddNTPs

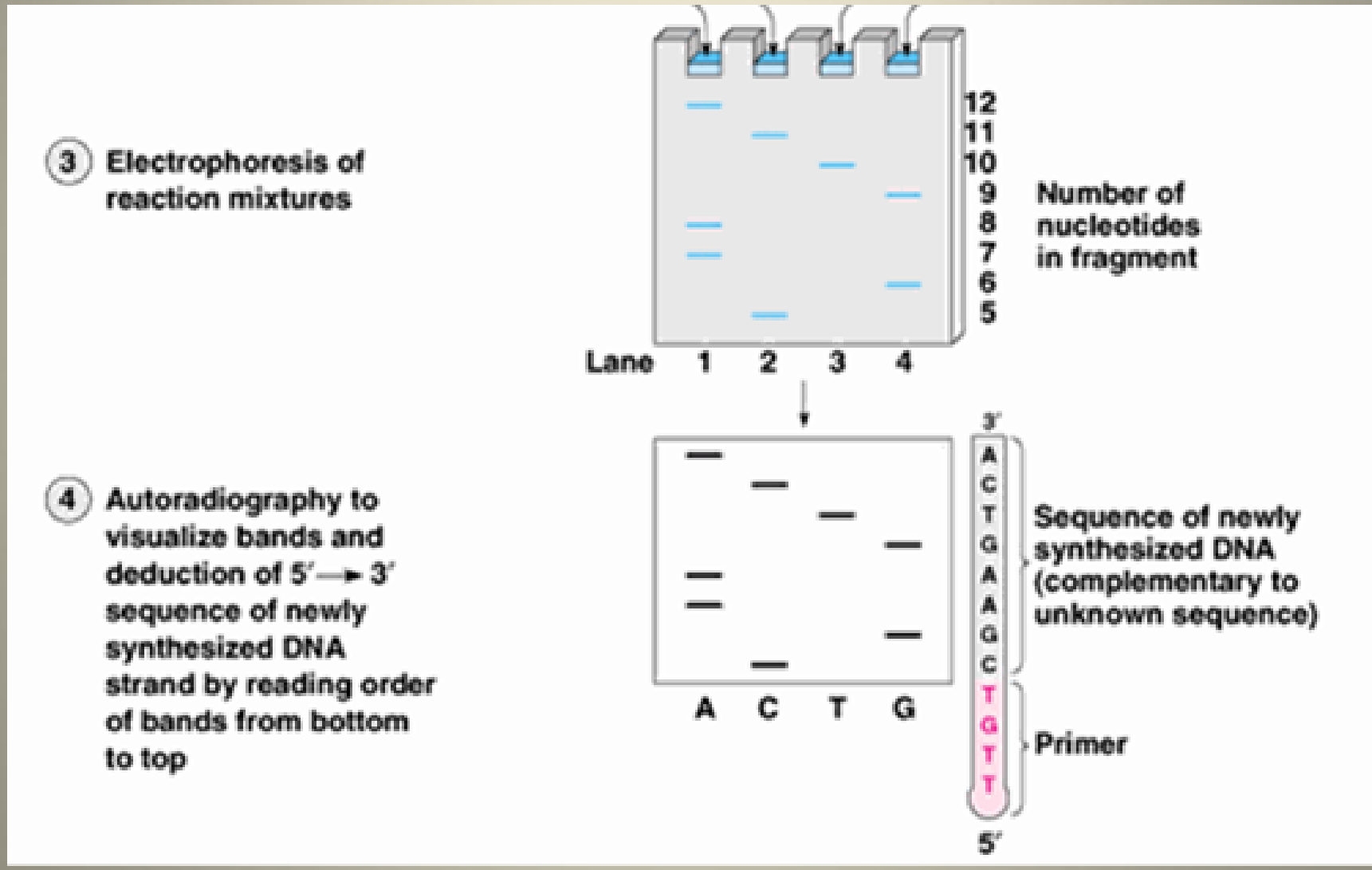


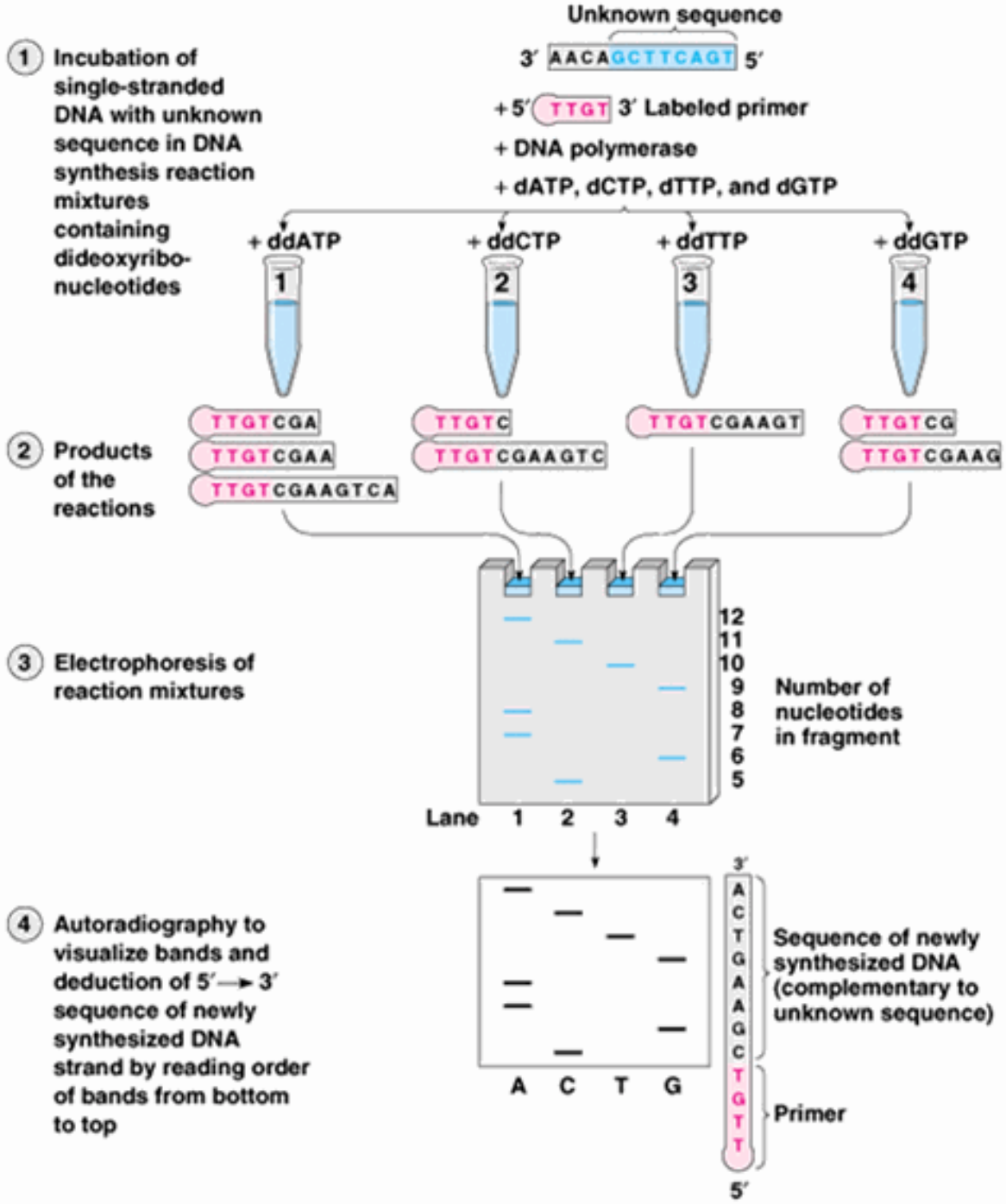
# Sanger Sequencing Visualization

- The strands of different lengths will be separated using gel electrophoresis onto 4 lanes, one for each ddNTP reaction



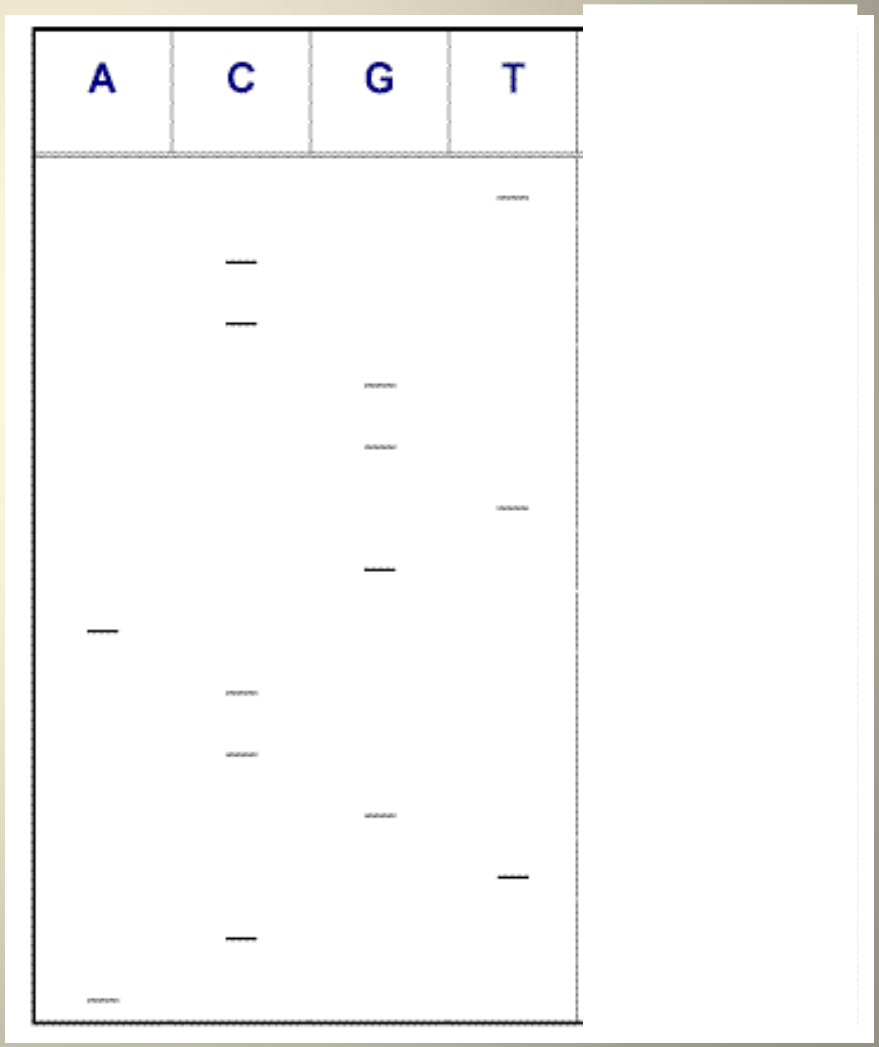
# Sanger Sequencing Visualization




Unknown sequence
3′ AACAGCTTCAGT 5′
+ 5′ TTGT 3′ Labeled primer
+ DNA polymerase
+ dATP, dCTP, dTTP, and dGTP
1 Incubation of single-stranded DNA with unknown sequence in DNA synthesis reaction mixtures containing dideoxyribo-nucleotides
+ ddATP
+ ddCTP
+ ddTTP
+ ddGTP
1
2
3
4
2 Products of the reactions
TTGTCGA
TTGTCGAA
TTGTCGAAGTCA
TTGTC
TTGTCGAAGTC
TTGTCGAAGT
TTGTCG
TTGTCGAAG
3 Electrophoresis of reaction mixtures
12
11
10
9
8
7
6
5
Number of nucleotides in fragment
Lane 1 2 3 4
4 Autoradiography to visualize bands and deduction of 5′ → 3′ sequence of newly synthesized DNA strand by reading order of bands from bottom to top
A C T G
3′
A C T G A A G C T G T T
5′
Sequence of newly synthesized DNA (complementary to unknown sequence)
Primer

# Sequence Interpretation

- What information would you need to be given about this gel electrophoresis to be able to determine the 5’ and 3’ end of the sequence?
- Label the 5’ and 3’ ends of this DNA sequence



# Sequence Interpretation

- What information would you need to be given about this gel electrophoresis to be able to determine the 5’ and 3’ end of the sequence?
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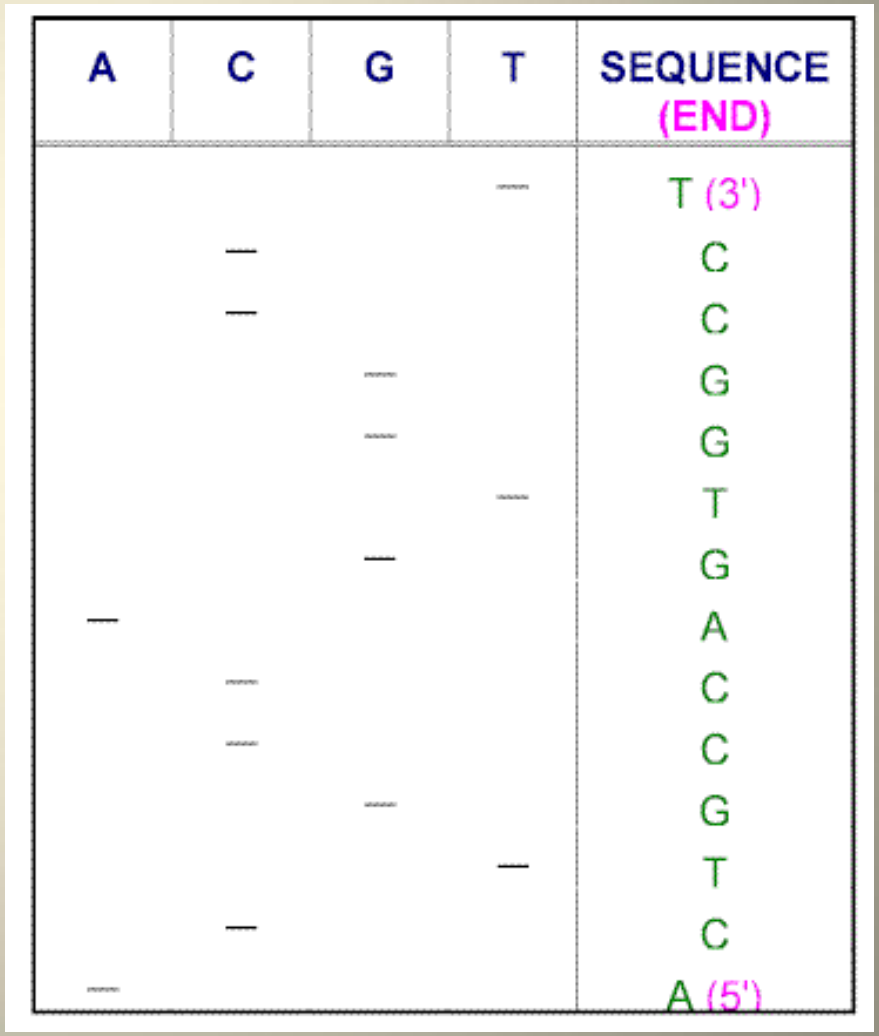
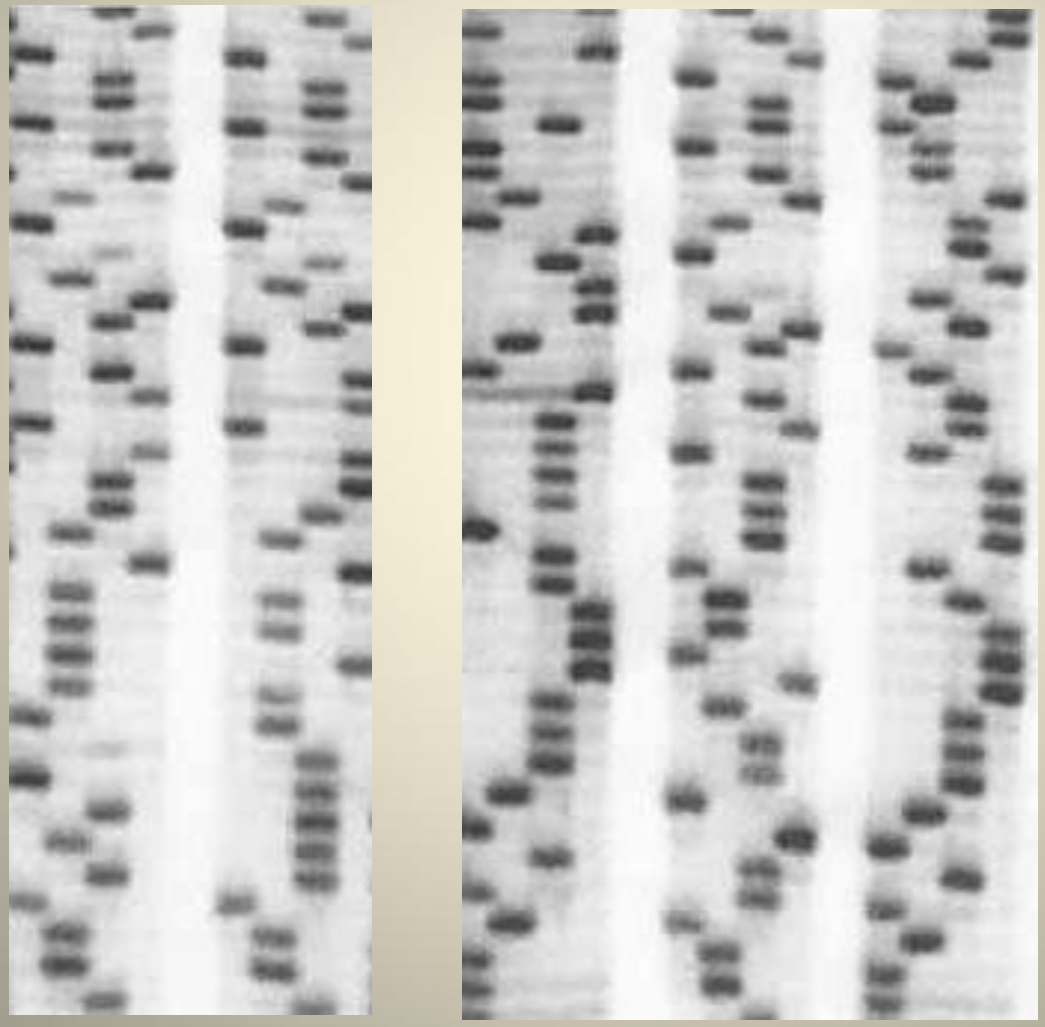


# Example of a real sequencing gel



# Sanger Sequencing Animation

- http://content.dnalc.org/content/c15/15479/29_sanger_sequencing.mp4

(http://www.dnalc.org/resources/3d/29-sanger-sequencing.html)

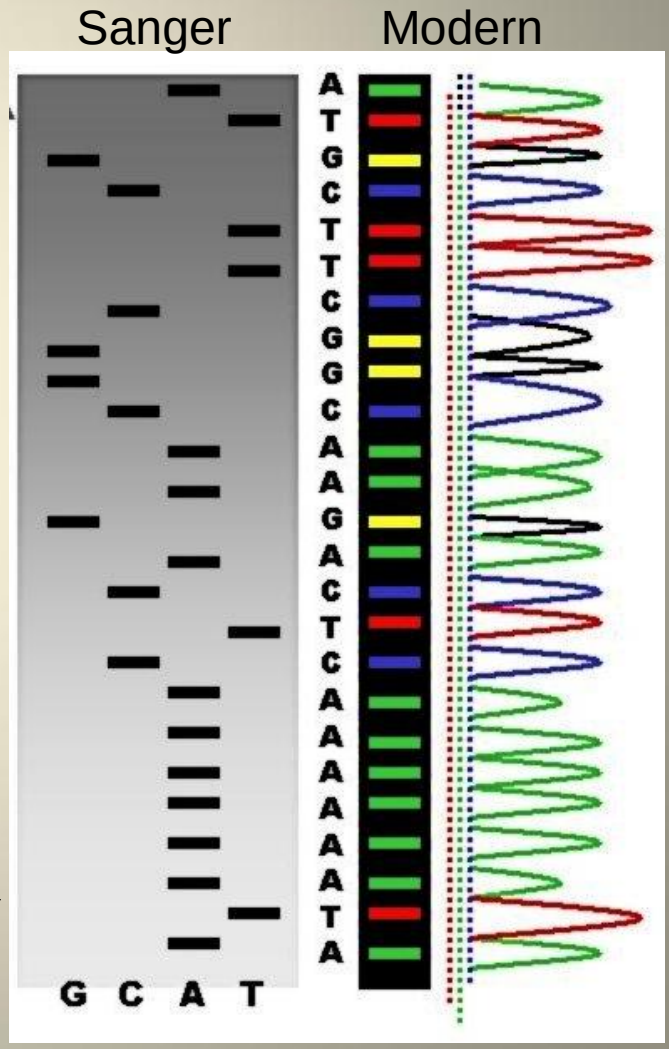
(http://www.dnalc.org/view/15479-Sanger-method-of-DNA-sequencing-3D-animation-with-narration.html)

- http://smcg.ccg.unam.mx/enp-unam/03-EstructuraDelGenoma/animaciones/secuencia.swf

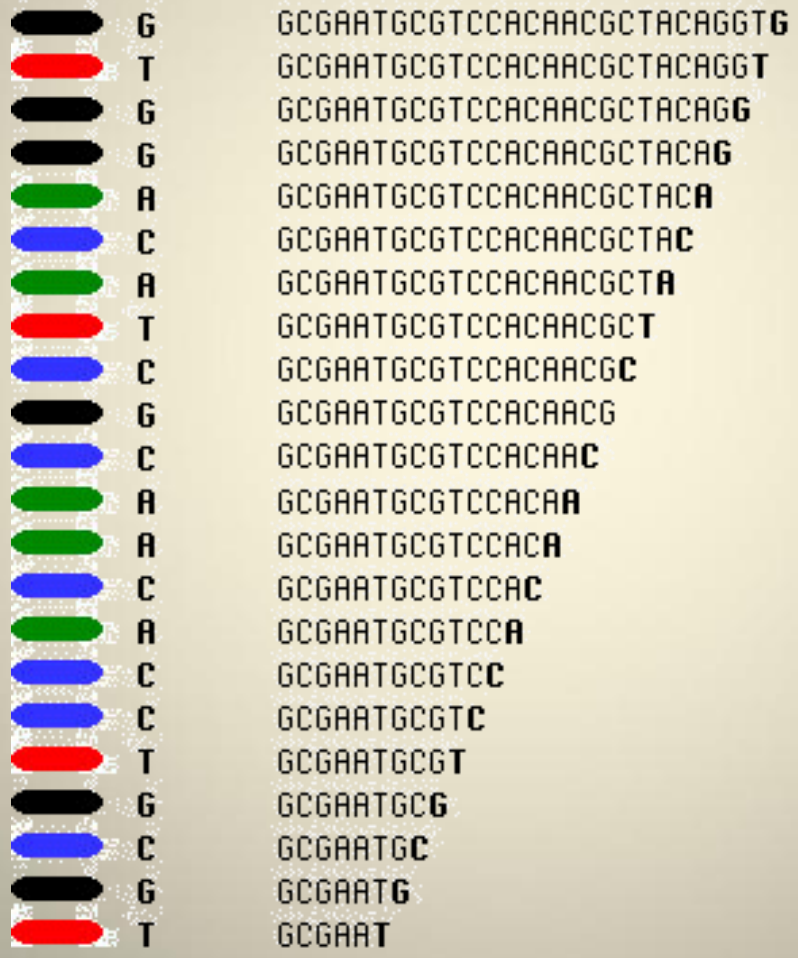
(Brackets) indicate links to same animation but with addition resources available on the webpage including the narrative

# Modern sequencing

- Theory is exactly the same as Sanger sequencing but the method is simplified
- **4 differently coloured fluorescent dyes** used to **label the 4 different ddNTPs in a single test tube**
- All 4 fluorescent ddNTPs can be **run on one lane** on a gel electrophoresis



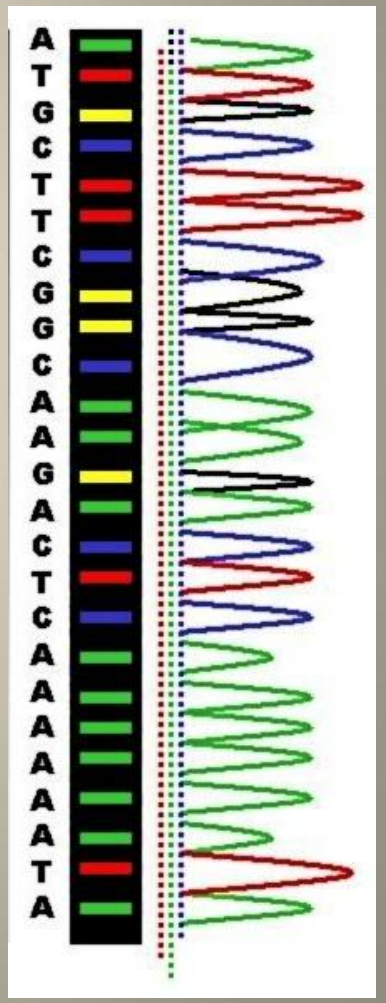
# Modern sequencing



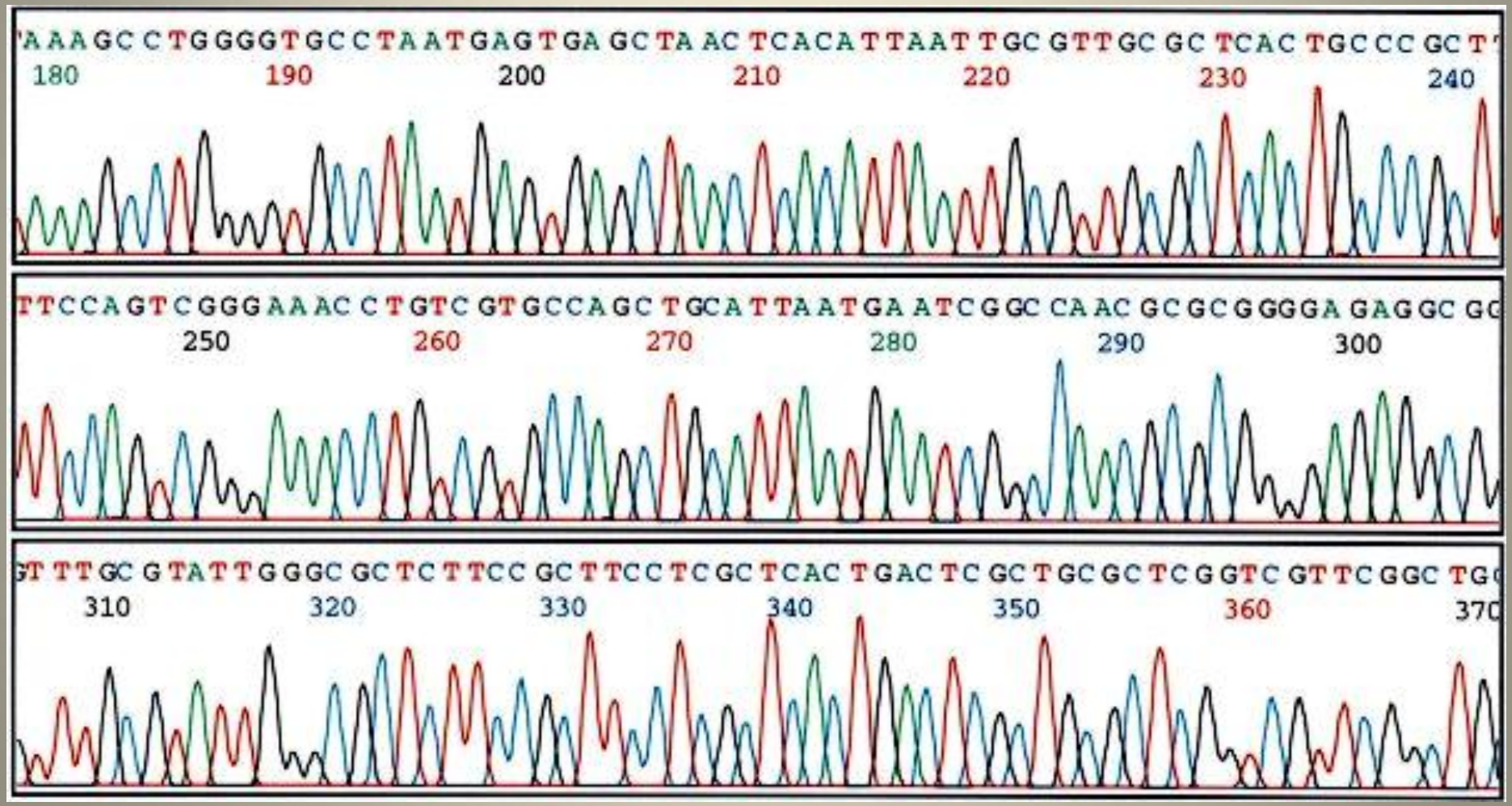

GCGAATGCGTCCACAACGCTACAGGTG
GCGAATGCGTCCACAACGCTACAGGT
GCGAATGCGTCCACAACGCTACAGG
GCGAATGCGTCCACAACGCTACAG
GCGAATGCGTCCACAACGCTAC
GCGAATGCGTCCACAACGCTA
GCGAATGCGTCCACAACGCT
GCGAATGCGTCCACAACGC
GCGAATGCGTCCACAACG
GCGAATGCGTCCACAAC
GCGAATGCGTCCACA
GCGAATGCGTCCAC
GCGAATGCGTCCA
GCGAATGCGTCC
GCGAATGCGTC
GCGAATGCGT
GCGAATGCG
GCGAATGC
GCGAATG
GCGAAT
GCGAA

# Modern sequencing

- Fragments are still separate by size but show up as coloured bands
- Colours have different wavelengths which can be read by a computer
- Computer translates the colours into the order of the nucleotides

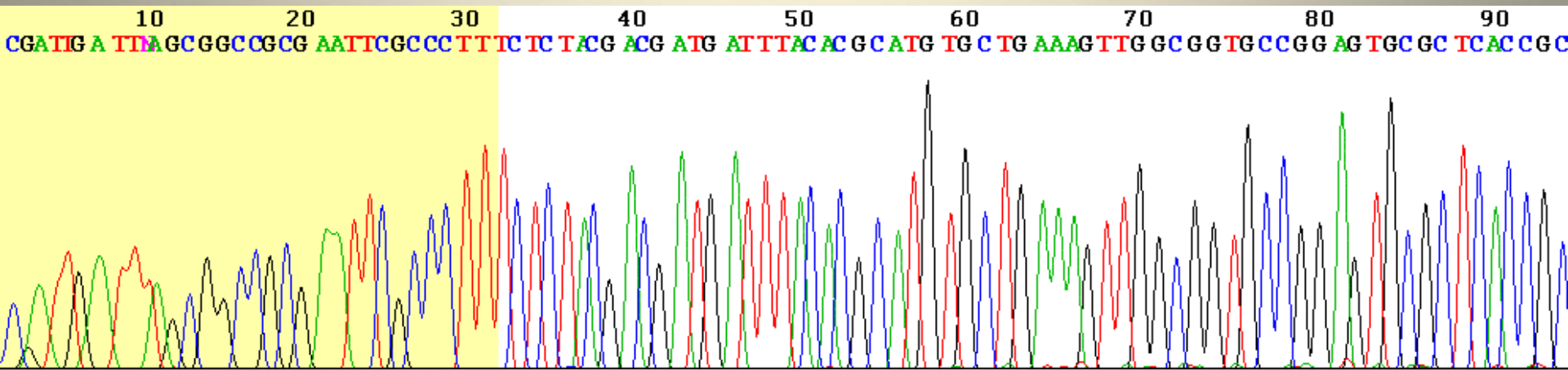


# Modern Sequencing



# Modern Sequencing

- Given that the sequence in the diagram below is read 5’ to 3’ from left to right, which end of the diagram represents the positive end of the gel?



# DNA Sequencing Activity

- Form groups of 4
- Read the instructions carefully!
- Each group should have 1 coloured paper (gel) and 4 white papers (sequences)
- Each person in the group should complete one sheet of white paper
- Once your group has finished, let your instructor know so that you can switch gels to practice reading each other's sequences