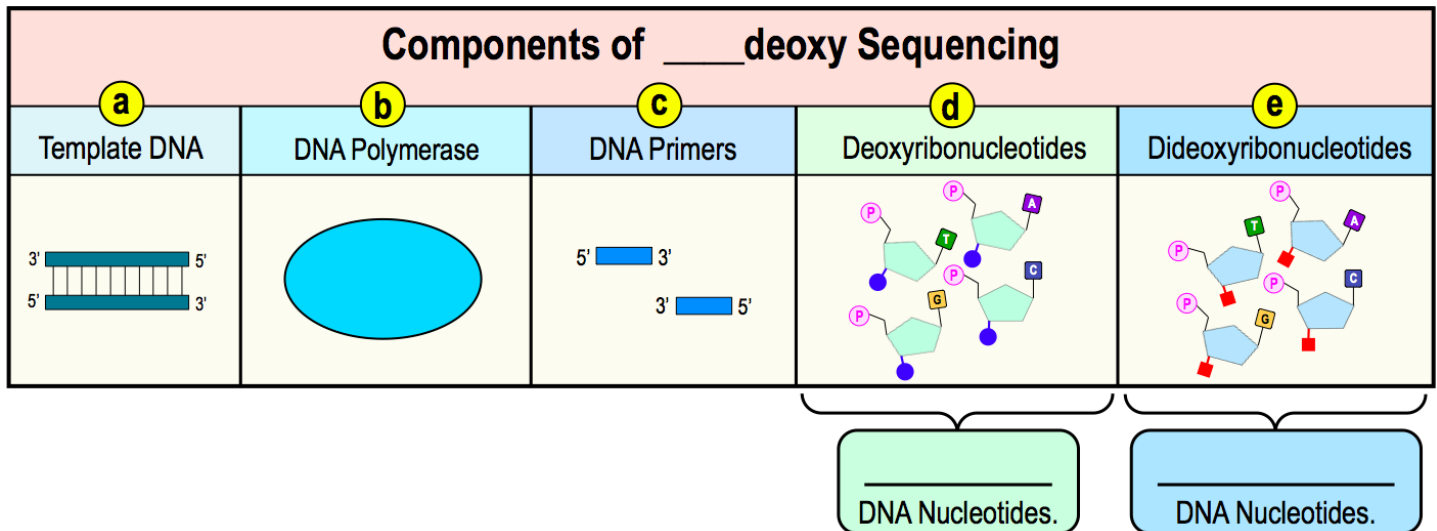


CONCEPT: DIDEOXY SEQUENCING

- _____ **Sequencing:** method using **ddNTPs** as elongation “terminators” to determine the sequence of DNA.
- Discovered in 1977 by Fredrick _____, it is commonly referred to as _____ Sequencing.
 - The first method of DNA sequencing that uses _____ *nucleotides*.

Components of Dideoxy Sequencing

- The components needed dideoxy sequencing reactions include:
 - a** Unknown _____ DNA of interest
 - b** _____ polymerase
 - c** DNA _____ that anneal to the template strand
 - d** All 4 DNA _____ nucleotides (dATP, dTTP, dGTP, dCTP)
 - e** A *small* amount of a *single* _____ nucleotide (either ddATP, ddTTP, ddGTP, ddCTP)
 - Recall: **ddNTPs** _____ DNA synthesis due to the presence of a 3' **H** group.



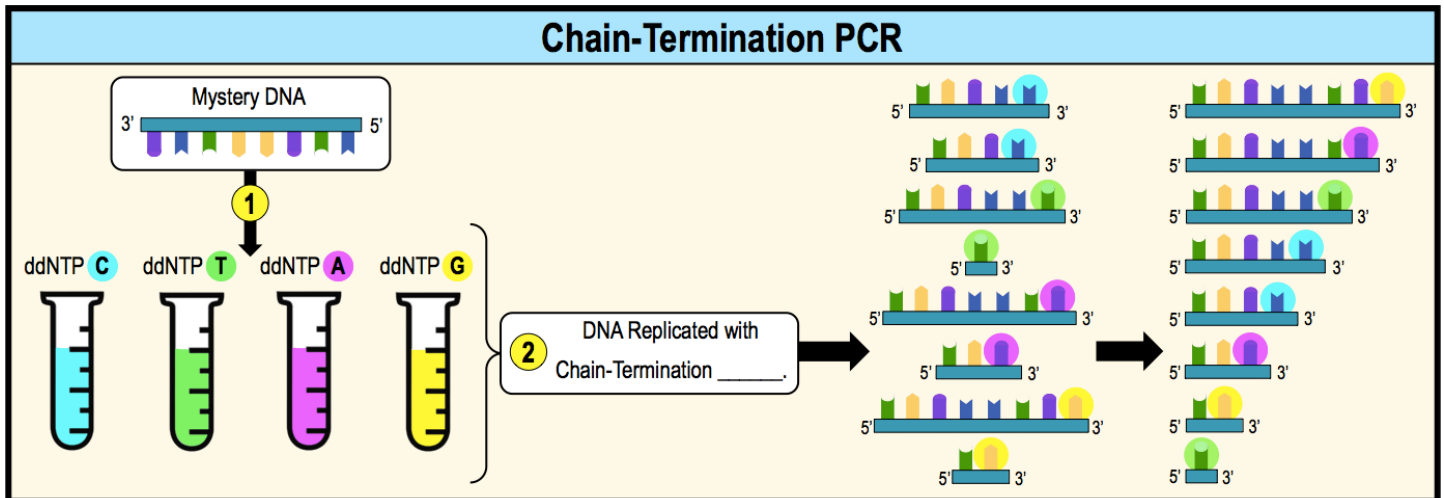
PRACTICE: Which of the following is NOT required for the reactions in dideoxy sequencing?

- a) Deoxyribonucleotides.
- b) RNA polymerase.
- c) DNA primers.
- d) Template DNA.
- e) Dideoxyribonucleotides.

CONCEPT: DIDEOXY SEQUENCING

Chain-Termination PCR

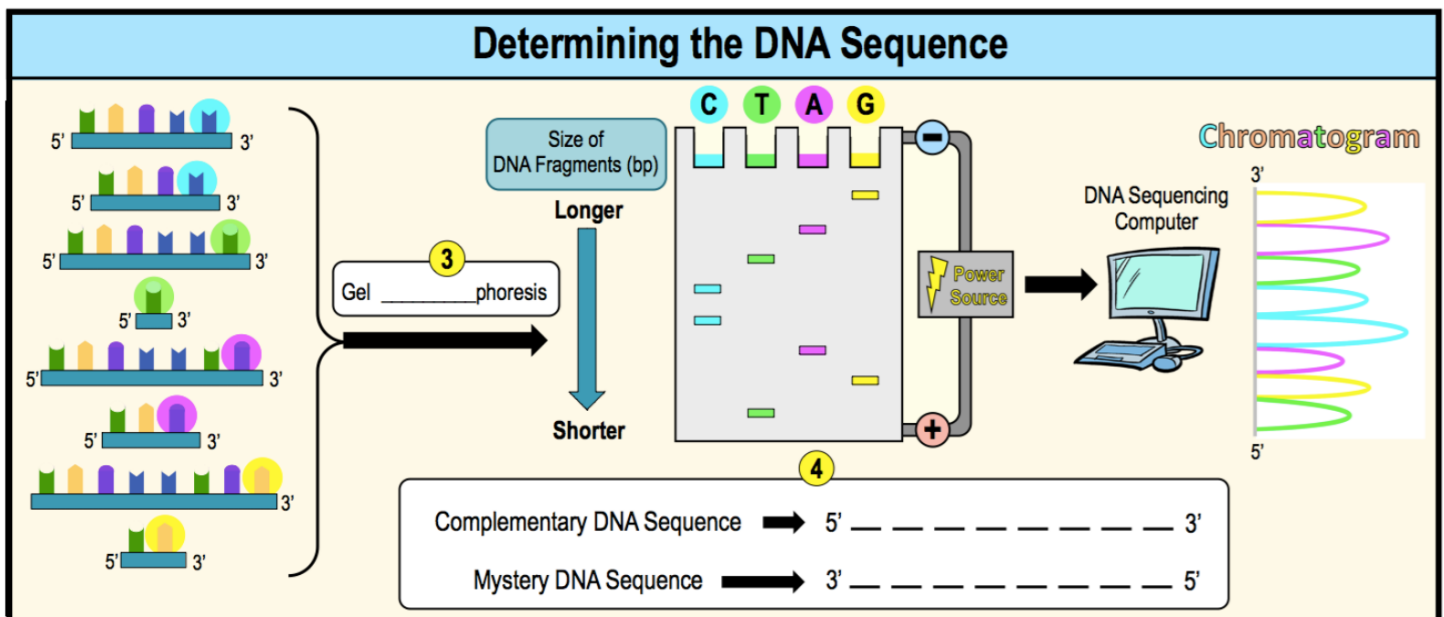
- Recall: DNA synthesis reaction is *terminated* when a **ddNTP** is added to the 3' end of the growing DNA strand.
- The first 2 steps of *Dideoxy sequencing* require setting-up a _____-Termination PCR:
 - 1 _____ separate reactions are set-up each containing a *small amount* of a different **ddNTP**.
 - 2 DNA synthesis produces *fragments* of DNA _____ to the unknown target.



Determining the DNA Sequence from a Gel

- In the *final 2 steps* of *Dideoxy sequencing* the DNA sequence is determined:
 - 3 Fragments from all 4 reactions are separated by _____ using *gel electrophoresis*.
 - 4 _____ determined *manually* (on a gel) OR *autonomously* (on a chromatogram).
 - Gel is “read” *backwards* (from bottom to top) across all lanes to reveal complementary DNA sequence.

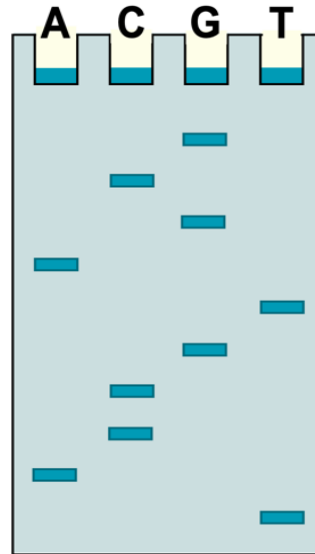
EXAMPLE: Determine the mystery DNA sequence by analyzing the gel electrophoresis results from dideoxy sequencing.



CONCEPT: DIDEOXY SEQUENCING

PRACTICE: According to the gel below, which of the following is the correct sequence on the unknown DNA molecule?

- a) 5' - GCGATGCCAT - 3'
- b) 5' - ATGGCATCGC - 3'
- c) 5' - TACCGTAGCG - 3'
- d) 5' - CGCTACGGTA - 3'
- e) None of the above are the correct sequence.



PRACTICE: Dideoxy sequencing is also known as chain termination sequencing because:

- a) The dideoxy nucleotide prevents further synthesis of DNA due to the lack of a free 5' carbon.
- b) The dideoxy nucleotide prevents further synthesis of DNA due to the lack of a free 3' OH.
- c) The dideoxy nucleotide prevents further synthesis of DNA due to the lack of a nitrogen-containing base.
- d) Chain termination is the same as sequencing by synthesis.
- e) None of the above are correct.

PRACTICE: The final step in a Sanger DNA sequencing reaction is to run the DNA fragments on a gel. What purpose does this serve?

- a) It adds ddNTP to the end of each DNA fragment.
- b) It changes the length of the DNA fragments.
- c) It separates DNA fragments based on their sequence.
- d) It separates DNA fragments generated during the sequencing reaction based on one nucleotide differences in their size.

