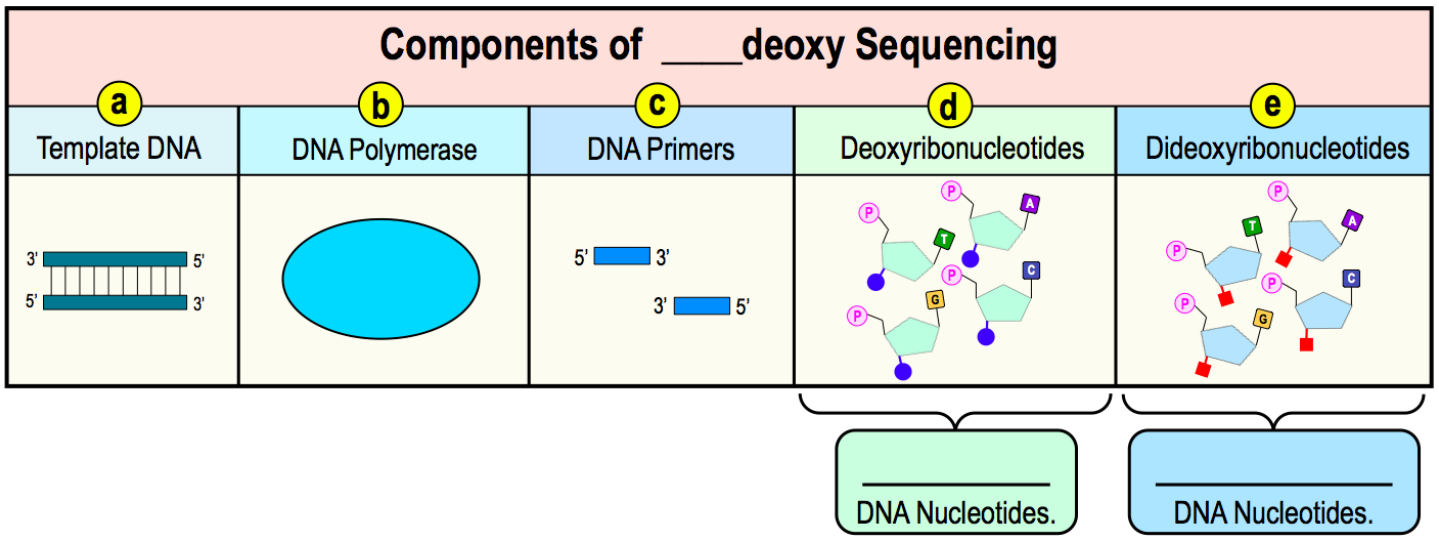
CONCEPT: DIDEOXY SEQUENCING

- _____ **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.

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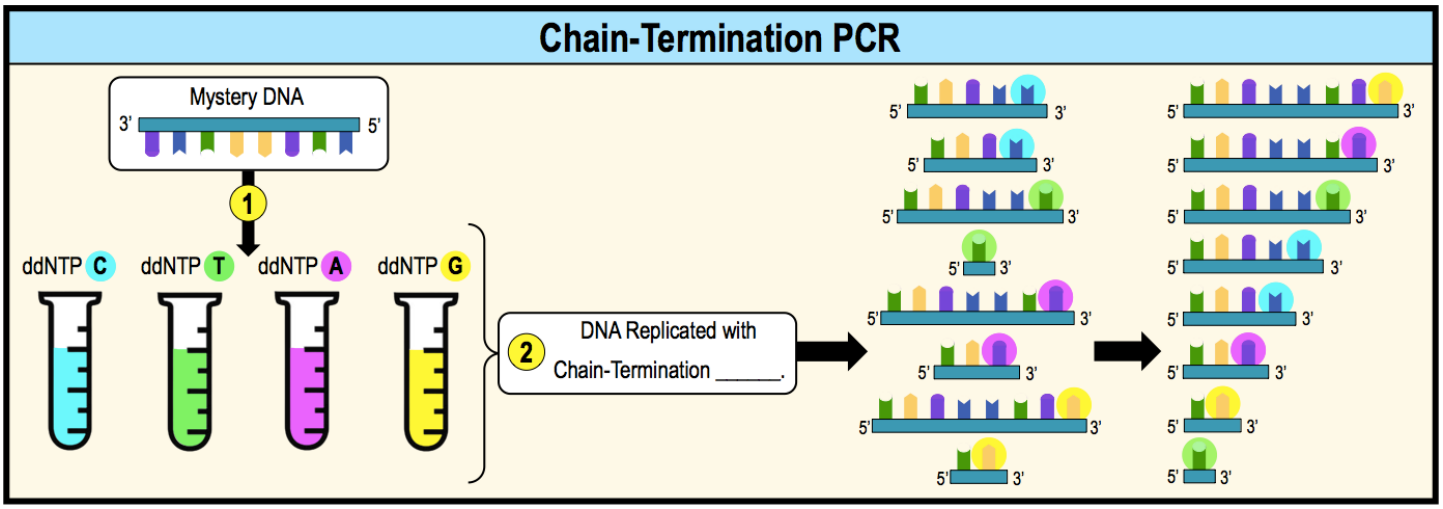
Chain-Termination PCR

●*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.

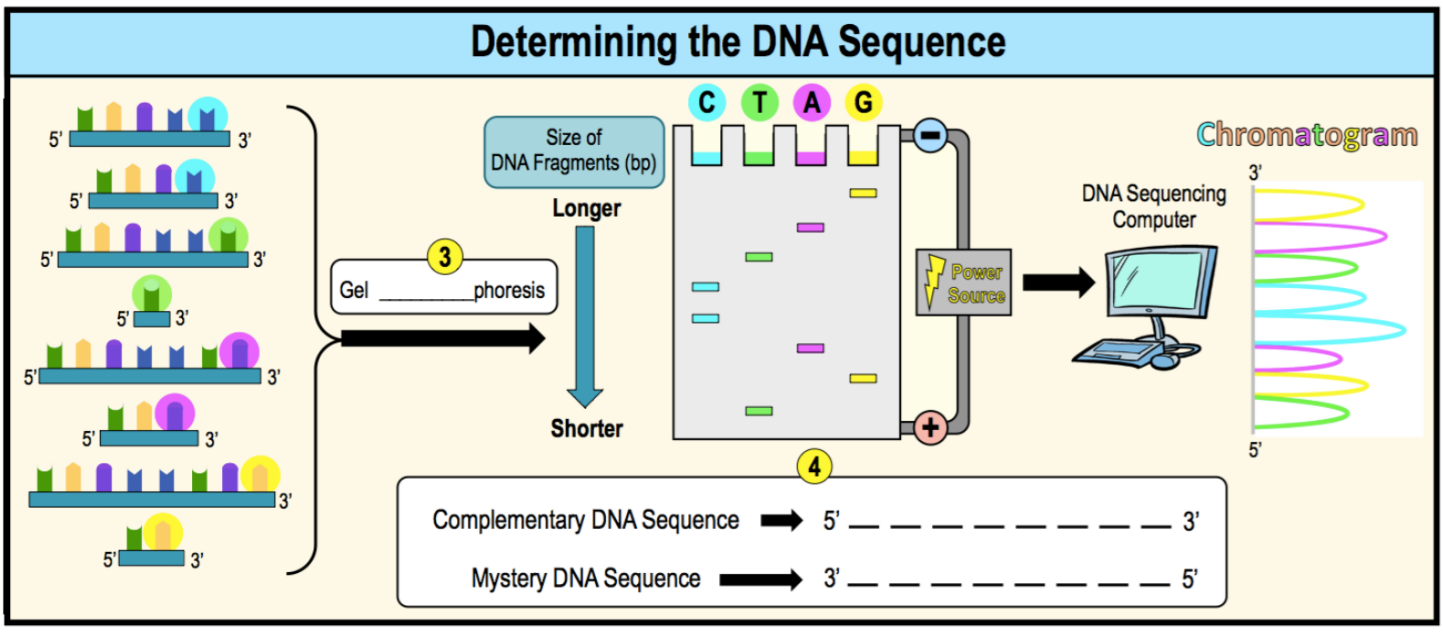


Determining the DNA Sequence from a Gel

●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.

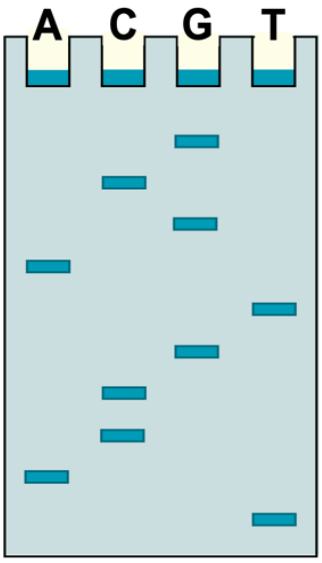
EXAMPLE: Determine the mystery DNA sequence by analyzing the gel electrophoresis results from dideoxy sequencing.



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.