

Lecture 3 - DNA Sequencing

Chain termination sequencing - Be able to describe how it works (Sanger sequencing) and what's needed (Template, ONE primer, DNA polymerase, dNTPs and ddNTPs).

Chain termination Sequencing - Method of sequencing DNA that uses dideoxynucleosides to terminate synthesis of DNA chain.

- Provides the research w/ the exact order of nucleotides for on segment of DNA.
- Provides confirmation of mutations, insertions or deletions of a gene, and can be still used sequencing a whole genome.

1) Isolating the **Template DNA Sample** (the sample of DNA that contains the segment of DNA or target DNA in that is to be sequenced)

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Understand the structural difference between dNTPs and ddNTPs. Which one can a nucleotide be added to? How is chain termination sequencing different from PCR?

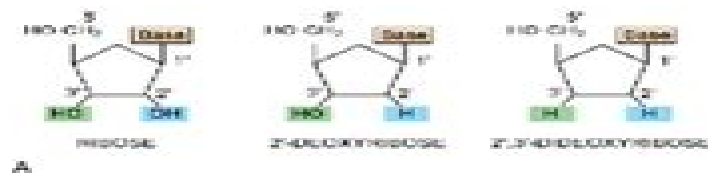
Deoxynucleoside triphosphate (dNTPs) - the building blocks of DNA that are used by DNA polymerase to synthesize a complementary DNA to a template or target DNA.

- dNTPs have a deoxyribose sugar connected to one of the four bases: Adenine(A), Guanine(G), Cytosine(C), Thymine(T) on the 1' carbon, and three phosphate attached to the 5' carbon of the deoxyribose.

Dideoxynucleoside triphosphate (ddNTPs) - An analog of deoxynucleosides triphosphate that is missing the 3' -OH, and therefore, are not competent to have another nucleotide added onto it.

- ddNTPs have a dideoxyribose sugar connected to one of the four bases, Adenine(A), Guanine(G), Cytosine(C), Thymine(T) on the 1' carbon, and three phosphate attached to the 5' carbon of the dideoxyribose.

STRUCTURES OF RIBOSE, DEOXYRIBOSE, AND DIDEOXYRIBOSE



DIDEOXYRIBOSE BLOCKS ELONGATION



When ddNTPs were radiolabeled, how were sequences read from the gel? How did sequencing reactions change when ddNTPs were labeled with fluorophores?

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