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Biology 110: An outline and a 5 multiple choice, 2 essay sample test on Sanger Sequencing

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**Sanger Sequencing**

**Background**

* DNA is made up of a specific sequence of nucleotides that form sentences that get recorded and translated into the proteins that make an organism.
* Sanger Sequencing also known as Termination Sequencing and Dideoxy Sequencing is the most modern method of DNA sequencing- *finding the order of nucleotide sequences with in certain genes*- by scientists. Prior to sanger sequencing, DNA sequencing was an extremely time consuming process. This method was discovered in 1975 by Frederick Sanger and since then has only been advancing. Twenty five years after it’s discovery, it would be used to sequence the human genome.

**How Does Sanger Sequencing Work?**

Sanger sequencing is modeled after DNA replication. Through a series of managed events, the DNA replication process is controlled. fluorescently dyed, or radioactively labeled Dummy nucleotides, also known as dideoxynucleotide triphosphates (ddATP, ddGTP, ddCTP, and ddTTP), are synthesized without a 3’-OH group, which is required for DNA polymerase to add on additional nucleotides. This prevents DNA polymerase from replicating when the dummy nucleotides are added to the new strand. After multiple episodes of replication with the synthetic nucleotides, the strands will go through electrophoresis. Gel electrophoresis will distinguish the base pairs within the target sequence based on their density and fluorescent dye or radioactive label.

**DNA**

* Is a Double stranded, antiparallel, helical structure composed of nucleotides, each containing a phosphate group, sugar molecule, and nitrogenous base. There are four nitrogenous bases: Adenine, Thymine, Cytosine, and Guanine. The strands in DNA are held together by complementary nitrogenous base pairs that form hydrogen bonds.
* *Adenine binds to Thymine and Guanine binds to Cytosine*

**DNA Replication**

* In DNA replication the strands are separated by the enzyme helicase.
* Single Stranded Binding Proteins keep the strands apart, while primase adds a primer to each strand of DNA.
* Each strand gets replicated at the same time in the same direction.
* DNA polymerase can only synthesize proteins when it is attached to the 3’ sugar group going in the direction of the to 5’ sugar group.
* The leading strand is the strand that goes from 3’ to 5’ in the direction of synthesis, the lagging strand, is the strand that goes from 5’ to 3’ in the direction of synthesis.
* For the leading strand, DNA Polymerase III attaches to the primer and synthesizes nucleotides until it is done.
* The lagging strand DNA polymerase III adds primer and sequences the nucleotides in Okazaki fragments.
* Polymerase I replaces RNA sequence primer with DNA nucleotides.
* DNA ligase attaches to the okazaki fragments to the other primer sequence.
* DNA polymerase III attached to the primer.
* And then all these steps get repeated.

**Sanger Sequencing Process:**

AMplification

* Through the process of DNA cloning or Polymerase Chain Reaction (PCR), genetic material is gathered and primed for in vitro study and analysis.

Heat

* Separates the DNA strands by breaking the hydrogen bonds.

Synthetic Primer added to Mixture

* ddATP, ddGTP, ddCTP, and ddTTP (dideoxynucleotide triphosphates) are all labeled with different fluorescent radioactivity or a dye, which then get added to the solution containing the genetic material.

ddNTPS

* (dNTP) This is deoxynucleotide triphosphate: Adenine, Thymine, Cytosine, or Guanine.
* During DNA replication, the construction of a new strand molecule called a hydroxyl group, which contains an oxygen atom and hydrogen atom, attaches to the sugar of the last dNTP. This bind causes the DNA chain to grow.
* In Sanger Sequencing, “dummy” nucleotides are added to the regular dNTPs that surround the growing DNA strand.

“Dummy” Nucleotides

* Also known as Dideoxynucleotide Triphosphates or ddNTPs, lack the hydroxyl group attached to the sugar of dNTPs. Whenever a ddNTP is added to a growing DNA strand, it is unable to chemically bind with the next nucleotide in the chain. -DNA strands stop growing.

Reading the Sequence: Now and Then

* When Sanger Sequencing was just discovered, four separate reagents were used, one for each type of ddNTP at a time. Gel electrophoresis would then separate the products and order them by size since the shorter segments of DNA move faster and further than the longer segments. Researchers would analyze the length of the truncated stand. End of each strand determined the position of a ddNTP, which halted DNA elongation.
* Now all four ddNTPs are added to a single test tube. Fluorescent color is used to distinguish each ddNTP from each other.  Reaction products are put through a small tube containing a gel matrix. Different sized fragments pass through the tube with are read through a sequencing machine that reads the fluorescent label at each position.
* Sequencing machines have vastly increased the speed and efficiency of DNA sequencing.

How is sequencing used by scientists?

* Advanced many areas of science
  + In Functional Genomics it is used to figure out what certain DNA sequences do and which sequences code for proteins, which have important regulatory functions.
  + In Comparative Genomics, researchers compare genetic material of different organisms in order to learn more about their evolutionary history and degree of relatedness.
  + In Disease Research, this information allows scientists to catalogue certain genetic variations between individuals that may influence their susceptibility to different conditions.

How can People Benefit From DNA Sequencing?

* Genetic testing will reveal more useful information because we will know more about what certain sequences mean.
* Development in more personalized drug therapies.
* Disease resistant plants and animals
* Microbial genome sequencing projects may lead to development of new biofuels and pollutant monitoring systems.
* It can be used in forensic science as evidence for criminal cases.

**Sample Test**

Multiple Choice

1. ddNTPs are

a) nucleotides in DNA

b) synthetic nucleotides that stop DNA elongation

c) synthetic primers that stop DNA elongation

d) nucleotides that initiate DNA replication

1. What is the first step to the process of sanger sequencing?

a) Heat the DNA to separate the strands.

b) Add ddNTPs to regular dNTPs.

c) Collect genetic material through DNA cloning or PCR.

d) Add synthetic primer.

1. Sanger sequencing is used to

a) amplify a set of DNA

b) determine nucleotide sequence of any purified DNA fragment

c) denature a set of DNA

d) determine the amino acid sequence of protein in vitro

1. What causes the chain to stop extending with Sanger sequencing?

a) nucleotides

b) denaturing of helicase

c) DNA polymerase comes to a stop codon

d) dideoxynucleotides

1. What process is sanger sequencing modeled after?

a) DNA replication

b) transcription

c) translation

d) mitosis

MC answers

1. B
2. C
3. B
4. D
5. A

Essay Questions

1. What is Sanger Sequencing?
   1. Introduction:
      1. Sanger sequencing is used to uncover the base sequence in target genes.
      2. The sanger sequencing process leads to results that can be used to determine the genetic makeup of the sequence.
      3. This information is important because it has assisted the advancements in various areas of science.
   2. Body
      1. Amplification
         1. PCR (process)
            1. Denaturation - 90 to 100 degrees celsius
            2. Annealing - 30 to 65 degrees celsius
            3. Extension - 60 to 75 degrees celsius
            4. Repeat process
         2. DNA Cloning (process)
         3. Purpose of process

* makes enough DNA material that can be used for study and analyzation.
  + 1. Heat
       1. Why - separates two strands of DNA
    2. ddNTPs
       1. What - synthetic nucleotides
       2. Why - stop DNA polymerase from continuing elongation
  1. Conclusion
     1. Paragraph describing the purpose and process of sanger sequencing previously described in the intro and body, and how that has impacted our research today in science.

1. Explain the relationship between our knowledge of DNA replication and the influence it has on Sanger Sequence?
   * 1. Introduction:
        1. Why its important to understand DNA replication.
        2. Sanger sequencing was modeled after DNA Replication
        3. They are different and similar in their processes, biomolecular substance, and purpose.
        4. This information has revolutionized science.
     2. Body:
        1. How DNA replication replication works: *see notes on page 1 and 2*
        2. How Sanger sequencing works: *see notes on page 1 and 2*
        3. How was the knowledge of DNA replication used to create Sanger sequencing.
           1. Sanger sequencing follows the same process of DNA replication, except it is controlled in a lab.

ex: heat in place of helicase

* + 1. conclusion:
       1. Why it is important to know information such as DNA replication and Sanger Sequencing.

- identify DNA found at crime scenes

- discover identity of unknown species

- discover identity of unidentified persons

* + - 1. How this information has developed into different categories of science.

- functional genomics

- comparative genomics

- disease research