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**Sanger Sequencing**

* Also referred to as chain-termination sequencing or dideoxy sequencing
* Used to sequence the human genome

**How it works**

* It is modeled after the natural process of DNA replication
  + It uses dummy nucleotides to end the replication process whenever a specific nucleotide is encountered
    - Occurs over and over again.
* Because of the repetitive process nucleic acids of different lengths are accumulated.
  + They can be used to determine the position of the nucleotides in the sequence.

**The Sequencing Experiment**

* Before sequencing can be done there must be multiple copies of the DNA segment made
  + This can be done by either cloning the DNA or triggering the PCR
* The strands are then heated to become separated
  + The synthetic primer is then added
* The primer’s sequence is complementary to the first piece of target DNA strands.
  + This means that the DNA target and primer bind with each other.
* The next step in Sanger sequencing is to expose the target sequence to the DNA polymerase and significant amounts of all four nucleotides.
* These nucleotides in their unbound form have three phosphate groups called deoxynucleotide triphosphates (dNTPs)
* In the making of the DNA strand a molecule called a hydroxyl group attaches to the sugar of the last dNTP
  + Causing the strand to grow
* In this sequencing there is a special type of nucleotide that is included with regular dNTP that surround the growing strand.
* These special nucleotides are called dideoxynucleotide triphosphates (ddNTPs).
  + They lack the hydroxyl group that is attached to the sugar of the dNTPs.
* When a ddNTP is added to the strand it will stop the growth (replication)
  + It is unable to chemically bind with the next nucleotide in the chain.
* The Sanger sequence manipulates many copies of the template strand at once.
* An over abundance of dNTPs is needed for the DNA synthesis proceed until a ddNTP is added.
* After the supply dNTPs has been exhausted a group of new DNA strands of varying lengths.
* These strands all have a terminal ddNTP that indicated if an A, T, C, or G occurs in that position on the template strand.

**Reading Sequence**

* When DNA sequencing was first introduced, four separate reagents were used, one for each type of ddNTP
* These four reactions were separated by gel electrophoresis organizes DNA fragments by size.
* The more recent sequencing technique combines all four ddNTPs in a single test tube
  + Each of the ddNTPs is labeled by a fluorescent color
* They are not read manually instead passed through small tube containing a gel-like matrix
* A laser detects the dyes when the ddNTPs are put into the sequencing machine (sequencer)

**Multiple Choice**

### 1.) What is the function of dideoxynucleotides in Sanger DNA sequencing? a. They act as primers for DNA polymerase. b. They act as primers for reverse transcriptase. c. They cut the sequenced DNA at specific sites. d. They allow only the specific sequencing of the RNAs of a genome. e. They stop synthesis at a specific site, so the base at that site can be determined.

**Answer: E**

**2.) In the Sanger DNA Sequencing method, what causes the chain to stop elongating?**

1. The incorporation of a regular DNA nucleotide
2. The incorporation of a dideoxynucleotide
3. Denaturation of the double-stranded test fragments
4. When the DNA polymerase encounters a stop codon
5. When denaturation causes the primers to disassociate from the strands

**Answer: B**

**3.)Which is *not* a part of the Sanger Sequencing method?**

1. Dideoxynucleotides
2. DNA polymerase
3. Electrophoresis
4. Reverse transcriptase

**Answer: E**

**4.) What causes the double stranded DNA to separate?**

1. Heat
2. DNA polymerase
3. Electrophoresis
4. ddNTPs

**Answer: A**

**5.) Electrophoresis organizes the DNA fragments by which characteristic?**

a. shape

b. nucleotide

c. size

d. name

**Answer: C**

**Essay Question**

**How can DNA Sequencing help to benefit all people? Give multiple examples, for present and the future.**

**Key**

* DNA sequencing can help to identify disease-causing genetics – mutations – which will lead to the improvement and expansion of genetic testing
  + Also it will lead to more targeted drug therapies in the future
* The microbial genome sequencing projects may lead to the development of new biofuels and pollutant – monitoring systems.
* Today
  + It can be seen agriculture and disease-resistant plants and animals.
  + DNA sequencing also used in forensic science providing crucial information in criminal cases.
    - The FBI uses a national database the genetic profiles of known offenders.
* DNA sequencing along with PCR can help improve the forensic science
  + Refer to the video featuring the process of expanding New York State’s DNA databank and give an opinion on why this is a good or bad idea.