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Biology 110

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Sanger Sequencing

**Used to determine sequence of nucleotides in DNA strand**

**Procedure:**

* Denaturing the DNA into single strands through heat
* Annealing: a specific primer binds to complementary spot on DNA strand
* Extension: DNA strand is added to solution with DNA polymerase and all four dNTPs, along with one type of ddNTP
* Electrophoresis: the DNA strands are placed within a lane on a polyacrylamide gel in order to separate the different bands. With a UV light, the size of DNA fragments can be determined by the placement of the bands on the gel when shown under the light. Longer sequences go a shorter distance whereas the shorter fragments go a farther distance.
* When all four reactions have occurred and lined up, the DNA sequence can be determined read in a 5’ to 3’ direction
* Automated sequencing: to sequence more DNA with shorter time. The four ddNTP’s are labeled with a different color dye and ran through electrophoresis. Instead of running the DNA four times, we are able to see the DNA sequence all at once, because of the color dye.

**Explanation**

-a ddNTP binds to DNA strand, but stops extension there because two missing oxygens prevent the next dNTP from binding

Example: ddGTP. The single strand of DNA is placed in a solution with plenty of dNTPs but ddGTP  is only ddNTP.

-when a ddGTP binds to the strand, extension stops.

-therefore there is a guanine anywhere that the strand stops, and a cytosine on the original strand

-by finding the length of many strands (through electrophoresis), the location of all of the guanines can be determined

-this is repeated with ddATP, ddCTP, and ddTTP, until every nucleotide on the strand is determined

Practice Quiz

1. What is not a necessary “ingredient” for Sanger sequencing?
2. ddNTPs
3. heat
4. RNA polymerase
5. DNA primer
6. If a ddATP ends the extension process, then what was the nucleotide on the template strand?
7. adenine
8. thymine
9. cytosine
10. guanine
11. Before DNA can be sequenced, the three steps must be completed in the following order:
12. Extension, Annealing, Denaturing
13. Annealing, Extension, Denaturing
14. Annealing, Denaturing, Extension
15. Denaturation, Annealing, Extension
16. The location of the bands on the gel after electrophoresis is due to
17. the amount of DNA
18. The length of DNA
19. The strength of the sample
20. The weight of the DNA.
21. The following “ingredients” bind to the original DNA template:
22. dNTP
23. ddNTP
24. DNA polymerase
25. all of the above

Essay

1. What is the purpose of electrophoresis in Sanger sequencing?

1. Explain the purpose of the following ingredients used for Sanger sequencing:

-dNTP

-ddNTP

-DNA polymerase

-DNA template

Key

1. C
2. B
3. D
4. B
5. D
6. -put newly extended DNA strands into gel, with the ddGTP sample in one lane, ddATP sample in another, etc.

-run the gel electrophoresis machine to test for length (shortest strands go the farthest)

-using the results of this test, piece together the sequence on the template strand by determining the complement of the nucleotide in the ddNTP that stops extension at that particular strand length

1. -dNTPs bind to the original template to perform extension

     -ddNTPs bind to the original template and stop extension

  -DNA polymerase aids extension by binding to the primer on the original strand and becomes the mechanism for binding dNTPs to the original strand

    -DNA template is the strand of DNA you are trying to sequence