

Sanger DNA Sequencing Simulation

In this laboratory exercise you will be simulating the technique of DNA sequencing. You should review the process of Sanger sequencing before beginning this exercise. The method works by having four separate reaction mixtures and using DNA polymerase to use replicate the template strand in the 5' 3' direction. A Primer is used to begin the process. In each mixture there are normal nucleotides and competing nucleotides that are Dideoxynucleotides. These dideoxynucleotides don't have a 3' OH, so if a normal nucleotide gets put into the new chain another nucleotide could be added to its 3' OH and if a terminating nucleotide gets added to the growing chain that does not have a 3'OH the next nucleotide can not be added and the growing chain is terminated. By having a mixture of normal and terminating nucleotides chains will grow to varying lengths. This is the basis of DNA sequencing.

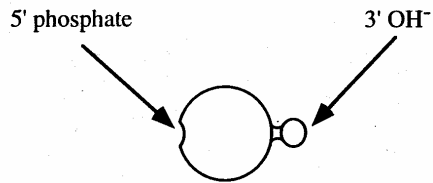
Materials: Reaction Mixture:

Each represents a nucleotide.

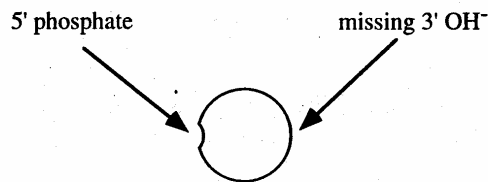
- A Blue Beads*
- T Green Beads
- C Orange Beads
- G Yellow Beads

* Some blue beads have a red dot. That signifies that they are radioactive.

Normal Deoxynucleotide



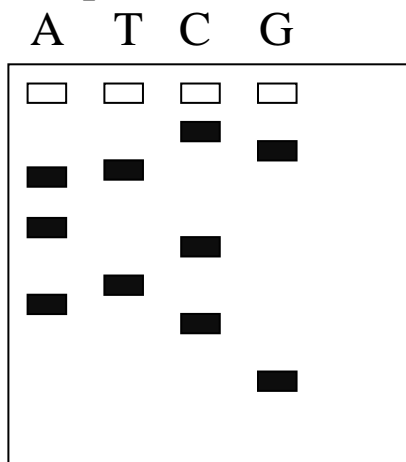
Dideoxynucleotide



Procedures:

1. Cut out the template and primer sequences.
2. Match your primer to the proper sequence on the template strand.
3. Reach into the “reaction mixture and pull out nucleotides at random until you have the next corresponding nucleotide in the growing (replicating) strand. Tape the beads onto your primer facing the 3’ end outward
4. If you come to a nucleotide that can not be added to (Dideoxy A,T,C or G) then get a new primer and start again. Do these as many times as you can. Don’t lose track which DNA strands belong to your group. The pop beads need to go back into your box or jar.
5. When you are done bring your fragments up to the front of room and lay them in the appropriate spot on the sequencing gel drawn on the floor.

Results: Interpretation and reading:



The method used to read this is from bottom toward the wells. Place a ruler across the gel and the first band you come to is the first nucleotide followed by the next and next as you move the ruler toward the wells. Do this for the practice gel above.

Experimental: Do the same thing for your experimental pop-bead sequence. Record your findings.

Real Sequencing gel: Obtain an Autoradiograph and interpret the sequence of one clone in the same manner learning hear. Write it down.

Assignment: Write a summary of how Dideoxy sequencing works by the Sanger method. Link this to the new methods being used that have colored dye linked to each terminating Dideoxy nucleotide.

TEMPLATES

G - T - C - A - C - T - T - G - A - A - G - T - C - C - A - T - A - G - G - C
3' 5'

PRIMERS

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

C - A - G - T
5' 3'

Tape the beads to the 3' end of the primer

REMINDER!

A = BLUE

T = GREEN

C = ORANGE

G = YELLOW