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DNA Sequencing Activity

Sarah O'Leary-Driscoll
Illinois Mathematics and Science Academy, soleary@imsa.edu

DNA Sequencing Activity:

Included at the end of this packet are primer sequences as well as nucleotides. Cut out those pieces and arrange as indicated. Then, work through modeling how sequencing takes place. Once you have your primer in place (don't forget 5' and 3' ends), continue to add nucleotides of appropriate complementarity to your sequence until you pull a dideoxy nucleotide out of the cup. Remember that these nucleotides cannot be added onto, so they are 'chain terminating'. Once that happens, tape your sequence in place and start on the next round. You will do this five times. Then complete the questions below.

Template DNA:

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

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

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

3’	C	A	T	G	A	G	A	C	T	G	A	T	T	G	C	5’

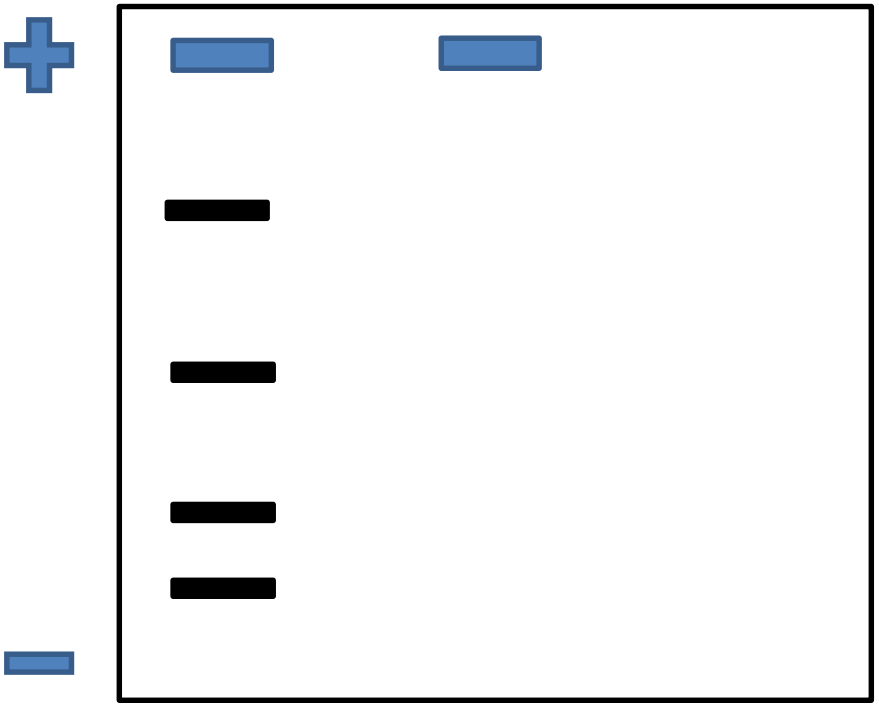
3’	C	A	T	G	A	G	A	C	T	G	A	T	T	G	C	5’

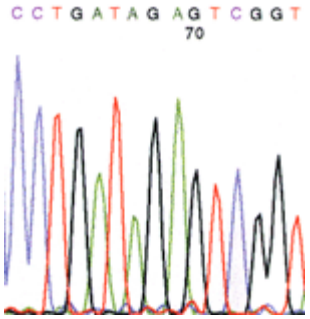
3’	C	A	T	G	A	G	A	C	T	G	A	T	T	G	C	5’

Fragment Size	Ending Nucleotide

Gel Electrophoresis:

If you are unfamiliar with gel electrophoresis, go to the link on moodle for the tutorial, and then draw where you would find the different DNA fragments that you came up with in your sequencing experiment. Label them with the appropriate color for their base. The first lane shows a DNA standard with bands sized 16bp, 8bp, 4pb, and 2bp.





The image above shows the example output of a DNA sequencer. Each of the peaks shows a different base. They are color coded by type of base (i.e. peaks for C are always blue, etc) and the height represents how much fluorescence is found. On the line below, draw what your sequence output would look like. Use appropriate colors, and leave empty any bases you cannot account for.

1. How is this process similar to PCR? What is different about it?

2. Why would it make sense that the step of PCR to replicate DNA fragments often comes before sequencing of those fragments, even though the chain reaction is used in both cases?

3. Why is the primer not accounted for in the final sequencing output?

4. What might be different about this process if the nucleotides were not labeled? What might have to be performed differently for the same output?

5. Why might there be different heights of peaks on the sequencing output? What about multiple colors at a specific location?

6. This example sequencing is of a very short segment of DNA. Sequencing is most efficient with short sequences, with a range of 2 to 150kb (kilobases). How, then, would you be able to use Sanger sequencing to determine the sequence of an entire genome which could be 1000's of kb in size? Why might the need for primers make this more difficult?

7. Once you've completed your part of the sequence, put your data up on the board (band size and nucleotide number). Once everyone has done the same, describe how the data from all of the groups combined will look different in terms of gel and DNA sequence output. How many rounds of PCR do you think were needed in order to get a fully completed sequence for this activity? What about in a more 'real world' scenario?

Activity Prep: Cut out the primers, then cut out the bases and sort them into cups. Mix the dideoxy/tagged nucleotides in with the regular (ddA goes with A, etc)

Primer:

5’	G	T	A	C	T

5’	G	T	A	C	T

5’	G	T	A	C	T

5’	G	T	A	C	T

5’	G	T	A	C	T

5’	G	T	A	C	T

A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA
A	A	A	A	ddA

T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	DdT
T	T	T	T	ddT

G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{Dd}$G
G	G	G	G	$_{dd}$G

C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{Dd}$C
C	C	C	C	$_{dd}$C