DNA Sequencing with paper nucleotides

Activity Type
Group work

Time Needed
20-25 minutes

Purpose
To illustrate the process of DNA sequencing using paper nucleotides, dideoxy nucleotides, templates, and primers

Abstract
<table>
<thead>
<tr>
<th>Activity/Exercise</th>
<th>Duration</th>
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<tbody>
<tr>
<td>Distribute supplies to groups</td>
<td>5 min</td>
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<tr>
<td>Group exercise</td>
<td>20 min</td>
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<tr>
<td>Problem-solving worksheet</td>
<td>20 min</td>
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<td>Review worksheet answers</td>
<td>5 min</td>
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Supplies (per group)
- 4 plastic cups containing nucleotides and dideoxy nucleotides (one cup has both A and ddA, etc.)
- 1 double stranded DNA template (attached)
- 5 single stranded DNA primers (attached)
- 1 roll of tape
- colored pencils

Pre-class prep
- Copy and cut out attached paper DNA templates and nucleotides
- Assemble supplies for each group (note: all supplies can be borrowed from the HHMI teaching laboratory, NS1 1202)

In Class
Have the students form groups of 3-5 and give them the following or similar instructions:
1. Anneal one of your primers to your DNA template. Which is the 5’ end of your primer and which is the 3’ end? Which is the template strand and which the coding strand?
2. Decide which nucleotide you will need to extend your primer – i.e. the nucleotide that will be complementary to the next nucleotide in your template. Take a nucleotide out of the appropriate cup (without looking) and tape it to the 5’ end of your primer.
3. Continue extending your new DNA strand in this way until one of two things happens:
   a. You run out of template sequence.
   or
   b. You place a dideoxy nucleotide at the end of your DNA strand.
4. Remove your newly synthesized DNA strand and set it aside. Begin the cycle again with a new primer. Continue until you have used up all 5 primers.

5. Using a blank piece of paper, place the DNA strands as they would appear on an electrophoresis gel. Indicate which end of the paper is the cathode and which is the anode (i.e. the positive and negative poles).

6. Draw the electropherogram that would result from your 5 DNA strands. Choose a color for each dideoxy nucleotide and draw a key for your diagram.

**Things to Ask or Emphasize**

Questions to pose during the exercise:

1. DNA sequencing uses a modified PCR reaction. What molecules must go into that reaction? (Hint: you should be able to list at least 4 of them.) In the exercise above, you have been playing the role of which molecule?
2. What are the 3 steps of a PCR cycle and what happens during each step?
3. Why do dideoxy nucleotides cause the DNA strand to stop elongating?

Additional problems to focus on in class (see PCR and Sequencing Worksheet):

1. Designing primers and calculating Tm: Give students a series of DNA sequences and ask them to design primers of different lengths for each sequence. Have students calculate the Tm for each primer, and determine whether that primer can actually be used for PCR (a primer usually has to be >15 basepairs to have a Tm high enough for use in PCR).
2. Reading a sequence from a gel: Now that students have produced a gel and/or electropherogram, have them work backwards from such data to deduce the DNA sequence from which the data were produced.

**Comments**
Cut along the lines to separate each of the nucleotides (A, C, T, G) and dideoxynucleotides (ddA, ddC, ddT, ddG).

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Cut on the thick black line around the 10-nucleotide template.

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

Cut around each of the five-nucleotide primers on the thick black lines.

C A T G A
C A T G A
C A T G A
C A T G A
C A T G A