DISCUSSION QUESTIONS KEY

Exercise 16: DNA Fingerprinting

1. How long are each of our simulated viruses in base pair (bp) units? Show your calculations. (By the way, real viruses are typically much longer.)

   From the drawing in the Results section students can read from their labeled standard DNA fragments the approximate length of the fragments that make up each of the simulated virus samples. By adding the fragments together, they can determine the total length of each virus. Since drawing their gel and reading their drawing will require a lot of eyeball estimation, their data and answers will vary, but they should show that they understand the concept that the fragments will add up to form the complete DNA molecule. (If you would like students to get more accurate results, you could have them plot the standard bp lengths vs. the corresponding migration distances on semilog graph paper and then determine the lengths of the virus fragments from the graph.)

   CPV

   ~1800 bp + 6557 bp = ~8357 bp

   MFBV

   ~900 bp + ~1800 bp + 4361 bp + 6557 bp = 13,618 bp

   BCV

   ~900 bp + 4361 bp = 5261 bp

2. Restriction sites in DNA are often palindromic sequences. Find the definition of palindrome and write below an example of one for a double stranded sequence of DNA nucleotides.

   In biotechnology a palindromic sequence is a segment of double-stranded DNA where the nucleotide sequence is the same when one strand is read left-to-right and the other strand is read right-to-left. Here is an example:

   GAATTC
   CTTAAG

   Palindromic sequences are significant to biotechnology because the restriction sites in DNA are usually made up of palindromes of nucleotides. Although students could create their own hypothetical palindromic sequence for this question, the example above is for the EcoRI restriction site.

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3. When many restriction enzymes digest DNA they make a staggered cut that leaves single stranded ends called sticky ends. Why are sticky ends called sticky?

The single stranded ends are sticky because they will recombine with complementary single strands of DNA cut by the same restriction enzyme. The base pairs of the two complementary single strands will be attracted to one another (sticky). For example, the recognition site for the restriction enzyme, Eco R1, is:

5'-GAATTC-3'

3'-CTTAAG-5'

Eco R1 makes the cleavage at the site shown and the fragments look like:

AATTC-3'

G-5'

5'G

3'-CTTAA

4. Restriction enzymes are usually named after the species of bacteria from which they were first found. For example, *Bam*HI was first isolated from *Bacillus amyloliquefaciens* strain H, and is the first (I) restriction enzyme isolated from this bacteria. Why are the first three letters of the enzyme name italicized? Explain where the name *Hin*dIII came from.

The first three letters are italicized because these letters come from the scientific name of a microorganism. Proper rules of nomenclature require that scientific names, even abbreviations, always be underlined or italicized. HindIII would be the third enzyme isolated from Hemophilus influenzae strain d.

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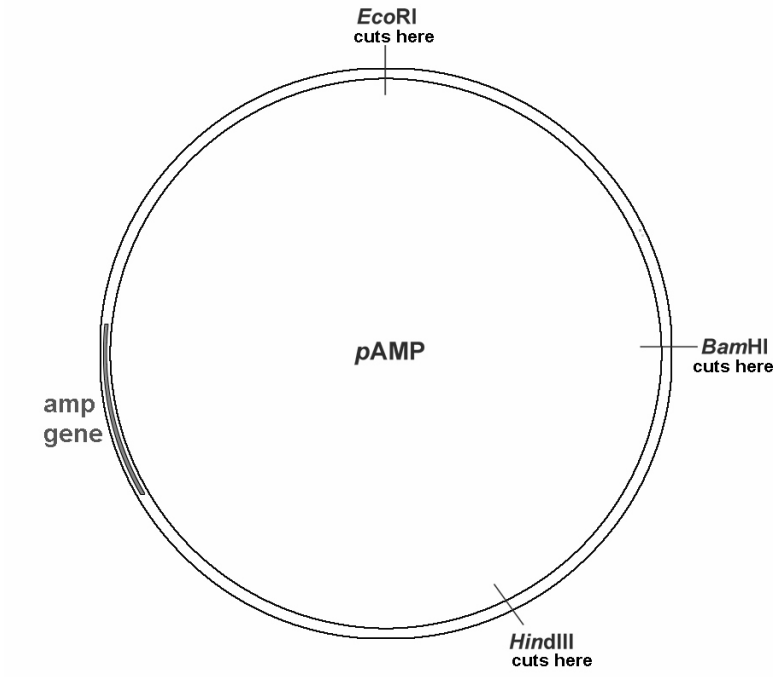
5. Below is a diagram of pAMP, an ampicillin resistance plasmid from *Escherichia coli*, showing restriction sites for the endonucleases, *Eco*RI, *Bam*HI and *Hin*dIII. If this plasmid were exposed to all three enzymes and then run through an electrophoresis gel, how many bands would you expect to see? Counting the bands and starting from the band closest to the wells, which band of DNA would contain the ampicillin resistance gene?

   Total Number of Bands _____3_____

   Number of the Amp Band ____1st_____

   The three enzymes would digest the plasmid into three fragments. The amp gene is in the largest fragment, so it would migrate the shortest distance from the well.



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6. Restriction enzymes come from bacteria. Can you think of a function these enzymes might have in bacteria? (Hint: They help protect bacteria from certain infections.)

   Restriction enzymes help protect bacteria from bacteriophages by digesting the DNA of the infecting phage.

7. Approximately 1 μg of DNA is required for restriction enzyme digests and electrophoresis. This is equivalent to the amount of DNA in 100,000 human cells. Since the amount of DNA in a sample from a crime scene or a diagnostic test would be much smaller than this, what technique would be used to amplify the DNA to create 1 μg of sample?

   PCR or Polymerase Chain Reaction is the technique used for amplifying (artificially replicating) DNA in the lab.

Exercise 22: Antibiotics

1. Were there differences in the sensitivities of the two bacteria in this exercise? If yes, how can you explain these differences? If not, why not?

   Usually there are differences because one species is Gram-positive and the other is Gram-negative. Many antibiotics work differently on these two cell types. For example many antibiotics like penicillin cannot penetrate the outer membrane of Gram-negative cells and therefore are ineffective against these bacteria.

2. Define PPNG.

   Penicillinase producing Neisseria gonorrhoeae is a strain of gonorrhea that has a gene for producing the enzyme penicillinase. This enzyme digests the beta lactam ring of penicillin and allows this strain of bacteria to be resistant.

3. Define MIC.

   Minimum Inhibitory Concentration is the lowest dose of a drug that will stop the growth (but not necessarily kill) a microorganism.

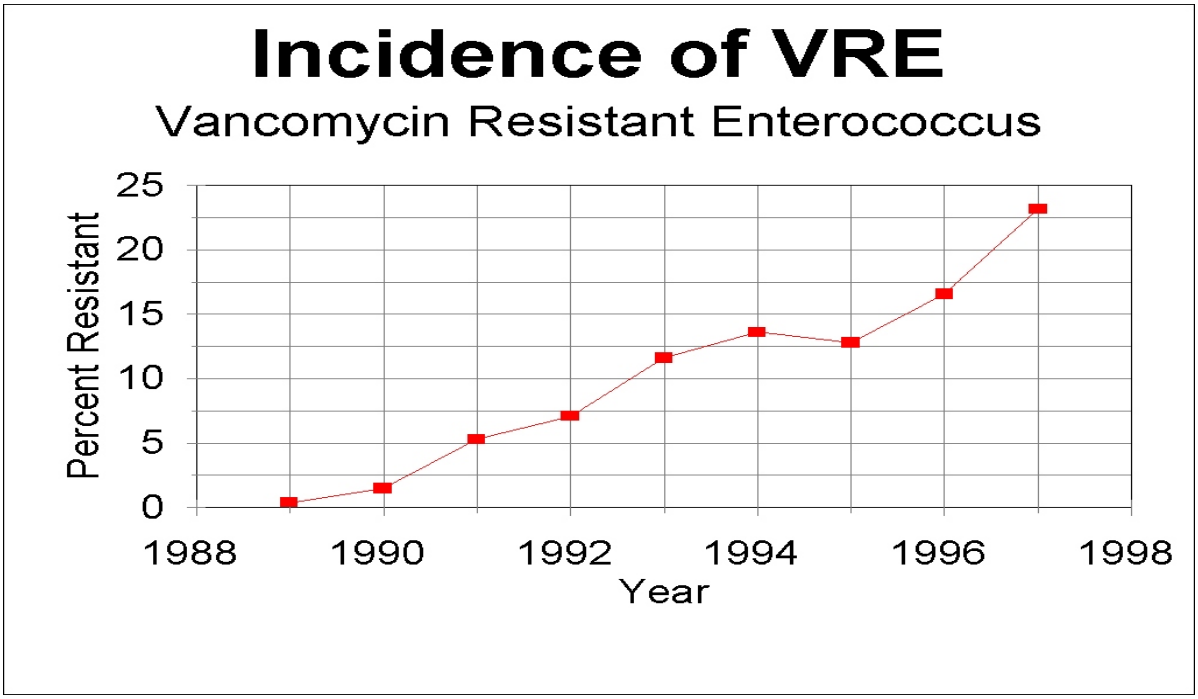
4. Define superinfection

   The normal flora of our bodies contains many opportunists that are held under control by competition with other indigenous species. Sometimes an antimicrobial agent will kill some of the normal flora competition and allow the opportunists to overgrow. The resulting opportunistic infection is called a superinfection.

5. Besides bacterial sensitivity, what are some other factors a physician would need to consider before prescribing an antibiotic for a patient?

   Toxicity, patient age and genetics, interaction with other medications, other diseases the patient might have, etc.

6. MRSA is an abbreviation for Methicillin Resistant *Staphylococcus aureus*. These are strains of *Staph. aureus* that have accumulated resistance to many different antimicrobial agents. The most dangerous MRSA is now resistant to every antimicrobial drug except for vancomycin. In 1989 a strain of *Enterococcus* was discovered that is resistant to vancomycin. In hospital intensive care units the reported incidence of VRE (Vancomycin Resistant *Enterococcus*) has increased from 0.4% in 1989 to 23.2% in 1997. (In between the incidence percentages were 1.1 for 1990, 5.8 for 1991, 7.2 for 1992, 11.6 for 1993, 13.6 for 1994, 12.8 for 1995, and 16.6 for 1996*. Illustrate this climb in VRE with a graph.



7. Describe how the process of natural selection applies to the increase in VRE.

First of all, people do not develop resistance to antibiotics, microorganisms do. Mutations occur that can provide Enterococcus with genes for resistance to vancomycin. These mutations arise randomly and are not caused by use of the antibiotic. However, once the mutation is present and the antibiotic is used, all of the non-mutant, sensitive bacterial cells will be eliminated leaving only the individual bacteria with resistant genes to survive and multiply. These resistant bacteria will replicate and pass on their resistant gene as they multiply, increasing the number of vancomycin resistant microbes. Antibiotics should only be prescribed and used when needed because the more we use these drugs the more common resistant microbes become.

8. What recommendations would you make to prevent or at least postpone the development of vancomycin resistant MRSA?

Avoid using vancomycin except when absolutely necessary. The more we use the drug the more common the VRE will become. High numbers of VRE lead to the possibility of gene transfer (e.g. transduction) to Staphylococcus aureus. This new strain of vancomycin resistant MRSA would then become common due to selection by vancomycin use. Perhaps vancomycin use should be limited to life threatening infections of MRSA that cannot be treated with any other drug. Students might also know about VISA, Vancomycin Intermediate Staphylococcus aureus. This strain of reduced susceptibility to vancomycin has begun to show up in health care settings and even a couple communities recently.