TEACHER'S NOTES LESSON 36

Present these key points and questions to the students during the *Activities* section of Lesson 36, as your lecture/discussion. Make sure that as you are speaking, students are taking notes.

DNA sequencing
What do you do after you isolate your favorite gene? One option is to isolate and sequence it. Sequencing is a procedure whereby one can determine the **sequence** or order of the nucleotides in a piece of DNA. How do you do that? *You use almost all the constituents that normally occur in DNA replication such as DNA (from a clone or cDNA), DNA polymerase, primers and normal and modified nucleotides.* Normal nucleotides have a hydroxyl group on the 3' C of the deoxyribose, whereas the modified nucleotides don't. This hydroxyl is what allows binding of one nucleotide to the next. Without the hydroxyl group the covalent bond between the two bases can't form. Therefore, addition of the next nucleotide is blocked and synthesis stops. Remember this modified nucleotide is the radiolabeled or color labeled one. Traditionally, scientists did sequencing reactions in four tubes labeled A, T, C, G. Each tube contained DNA to be sequenced, DNA polymerase, primers and normal nucleotides. Modified nucleotide A was added to tube A, modified nucleotide T was added to tube T and so on. When the reactions were over they were separated by size by gel electrophoresis. Then one read the gel from the bottom to top by reading the band that is the lowest as the first base, the second from the bottom as the second base and so on.

See figure 10-5 in Alberts et. al. 1999 *Essential Cell Biology* p. 319. Nowadays, most labs use automatic DNA sequencing. They use a machine and modified nucleotides that have different colors.

Polymerase chain reaction (PCR)
Show diagram of PCR technique such as fig. 15.6, p. 226 in Starr's *Biology Concepts and Applications*, fig. 10-22 p. 333 in Alberts et. al. Essential Cell Biology, or some other comparable image. PCR is a technique to amplify DNA (genomic or cDNA) very rapidly. What do you need to do PCR? *You need DNA, DNA polymerase (a special one that doesn't degrade at high temperatures), nucleotides and primers (short nucleotide sequences).* You put all of these substances in a tube. You heat the tube to separate the double stranded DNA (one of the easiest ways to open or separate the two chains is by high temperatures or high pH). Then you lower the temperature and the primers (which are designed to match the DNA at the ends of the area you want to amplify). When you lower the temperature the primers can bind and the DNA polymerase copies the sequence by adding nucleotides. You have double the amount of DNA as before. Then you repeat the cycle. You heat the DNA to separate the strands, cool and the primers will bind and the DNA polymerase will copy the DNA. Now you have 8 times as much DNA. Repeat again and you will have 16 times as much DNA. Repeat again and you will have 32 times as much DNA. In a very short time you have a large quantity of the sequence you want.

DNA fingerprints
Show an image of DNA fingerprints such as fig. 15.7, p. 227 in Starr's *Biology Concepts and Applications*, or fig. 10-25 p. 336 in Alberts et. al *Essential Cell Biology*. DNA fingerprints are similar to normal fingerprints. Each person has a unique set of fingerprints and each person has a unique array of DNA fragments. Therefore, we can identify people by their DNA fragments or DNA fingerprints. Even though more than 99% of all DNA is the same in all humans, we look at that less than one percent that is different. The sequences that are different are tandem repeats of DNA.

How do you make DNA fingerprints? You isolate some DNA, use PCR to amplify the fragments, called tandem repeats (the only sequences that are different in humans since 99% of all our DNA is the same), and separate them by gel electrophoresis. Then you look at the bands on the gel and locate differences and similarities.

When can you use DNA fingerprints? *In paternity suits, in identification of criminals and victims, in exonerating innocent suspects.* For example, a girl has a baby and her boyfriend doesn't want to help raise the baby because he says he isn't the father. They can take blood from the baby, the potential father, and the mother and look at the DNA fingerprints. It should prove or disprove if he is the father. Another case is a woman who doesn't know exactly which man is the father of her child. The same technique can be used.

It can also be used in criminal cases. A single hair is found on the floor at a crime scene, or pieces of skin are found under the nails of a murdered person. The DNA from these samples is isolated, amplified by PCR and run on a gel to show the DNA fingerprint. Then blood samples are taken from the suspects, their DNA isolated, amplified and run on the same gel. These bands can be used to identify who is the murderer.

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