Biology Behind the Crime Scene
Week 4: Lab #4 Genetics Exercise (Meiosis) and RFLP Analysis of DNA

Genetics Exercise: Understanding how meiosis affects genetic inheritance and DNA patterns

Cells that are involved in reproduction undergo meiosis. During meiosis, the parental diploid (2n) cell gives rise to four haploid (n) daughter cells. Meiosis is divided into two parts: meiosis I and II. Just before meiosis I, the cell duplicates its chromosomes. In meiosis I, the cell separates homologous chromosomes into 2 cells. Then in meiosis II (which is virtually identical to mitosis) these two cells each go through a division that separates sister chromatids. The overall effect of meiosis is to make four cells that contain one-half the number of chromosomes that are in somatic (non-reproductive) cells.

During Interphase I, the cell will duplicate its chromosomes. Then in Prophase I, you will see **homologous** chromosomes (homologous – chromosomes similar in size and appearance, one from each parent) lining up beside each other. This is where **crossing over** occurs. Crossing over leads to recombination or swapping of genetic material between the two homologous chromosomes. During Metaphase I, these homologous chromosomes are pulled apart by spindle fibers. During Anaphase and Telophase I, the homologous chromosomes are further pulled apart and, in most organisms, cytokinesis (the splitting of the cytoplasm) occurs.

Meiosis II begins with Prophase II, when the chromosomes once again start to line up at the equator of the cell. Once these chromosomes have lined up end to end in Metaphase II, in Anaphase II the spindle fibers start to move each of the **sister chromatids** to the opposite poles of the cell. This has the effect of separating the two sister chromatids of each chromosome. Telophase II and cytokinesis occur at last; at this point the chromatids are fully separated and a new nuclear envelope is formed.

The end result of meiosis is the production of four genetically different daughter cells from one parent cell, each with half (haploid) the chromosome number of the parent cell (diploid). Note that the daughter cells are different from each other as well as different from the parent that gave rise to them. In most animal species, these daughter cells can become gametes (sperm or eggs) that can combine with another gamete. The fusion of 1 haploid (n) gamete cell with another haploid (n) gamete cell produces a new diploid (2n) cell. Meiosis occurs in both males and females. You will be using pop beads today to simulate the process.

It is estimated that approximately 30,000 – 50,000 genes are contained in the 23 pairs of homologous chromosomes (or 46 chromosomes) of human DNA (remember most people have 44 autosomes and 2 sex chromosomes). Each chromosome contains a series of specific genes. Each of the homologous chromosome pairs contains similar genes. Each gene holds the code for a particular protein. For all the DNA contained in chromosomes, genes only comprise about 5% of the total chromosomal DNA. The other 95% is non-coding DNA. This non-coding DNA is interspersed in blocks between functional segments of genes and within genes, splitting them into segments. The exact function of the non-coding DNA is not known, although non-coding DNA may allow for the accumulation of mutations and variations in genomes. Non-coding DNA can be useful for DNA fingerprinting in forensics. The human genome contains repetitive DNA elements called tandem repeats that are located in various spots within our genome. These repeats can be analyzed to determine "uniqueness" of an individual based upon the number of tandem repeats at specific locations (loci) on the chromosomes.

Virtual DNA-gel Blot Creation

Last week you cut different DNA samples – each group members' DNA and a "crime scene" DNA sample. Over the last week instructors created virtual blots based on what may have resulted from these samples. Had you done the blots yourselves in lab, you would have followed the instructions in the **"Instructors' Procedures Appendix" – be sure to read this over because there may be quiz questions on it** but you do not need to print it and bring it to lab. This week we will analyze the resulting "DNA fingerprints," the fragments of different sizes created when the restriction enzymes cut these different sequences.

We can take advantage of **r**estriction **f**ragment **l**ength **p**olymorphisms (**RFLP**) as well as tandem repeat sequences. RFLP is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites. In RFLP analysis, the DNA sample is broken into pieces (digested) by restriction enzymes. The restriction enzyme can recognize and cut DNA wherever a specific short sequence occurs. The resulting restriction fragments are separated according to their lengths (size) by the process called gel electrophoresis. DNA fragments are mixed with a dye and loaded into an agarose gel. The gel is then placed in a chamber filled with a conductive buffer solution. A direct current gets passed between wire electrodes at each end of the chamber. DNA fragments are negatively charged, and when placed in an electric field will be drawn toward the positive pole and repelled by the negative pole. The matrix of the agarose gel acts as a molecular sieve through which smaller DNA fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in what appears as a single "band" of DNA in the gel. This is a very simple method for separating DNA molecules by size as well as for visualizing and purifying them. The fragments are then transferred to a membrane via the Southern blot procedure. Remember that differences in DNA sequences between alleles, between surrounding sequences for the same gene, and between pieces of non-coding DNA cause the DNA to have a different number of the "cut sites" for any given restriction enzyme for any given individual person. For example, if a cut site sequence is found 5 times in Person A's segment of DNA, it will be cut into 6 pieces of various lengths. If that same cut site sequence is found only 3 times in Person B's DNA, Person B's DNA will be cut into only 4 pieces of different lengths. This means that when run on a gel, there will be a different distribution of DNA for Person A vs. Person B. We can then use a DNA probe for a tandem repeat sequence chosen to detect a specific region of the genome that determines the length of the fragment (which can vary between individuals). The differences in position of the DNA bands on the blot tell you the size difference in the DNA. Each fragment length is considered an allele. Remember you have two alleles that get inherited (one from mom and one from dad). We can probe for multiple alleles on any one Southern blot. A high degree of discrimination can be achieved by using a number of different or longer probes and combining their frequencies and/or bands. If you use 4 different probes, each with 1/100 probability of occurring in the population, then the 4 probes would have a combine frequency of 1/100,000,000:

That is to say 1/100 * 1/100 * 1/100 * 1/100 = 1/100,000,000

Remember that almost all cells contain all your DNA sequences – your entire genome. By cutting the DNA extracted from saliva, you didn't just cut 1 or 2 genes – you cut all the DNA in the genome. Because you've cut the entire genome's DNA per person, you'd most likely see a smear on your agarose gel rather than the clear bands often shown right away on TV. *[Had you amplified just a gene or two by PCR and cut them, you'd be able to see distinct bands. As you'll discover in a future lab, however, there are easier methods to look at genetic difference just through PCR, so sometimes the restriction enzyme step is not necessary.]*

In order to see distinct different bands for your restriction digests, we needed to transfer the DNA from the gel to a solid membrane then probe the membrane with a specific DNA sequence. This process is called a DNA-gel blot or a Southern blot [after E.M. Southern, who originated the process.] The probe sequence can be attached to & detected with:

- A radioactive molecule – detected with clear X-Ray film or a radioactivity-sensitive screen OR
- A fluorescent molecule – detected with light & photography OR
- A molecule that precipitates a colormetric product when developed with detection fluid directly on the blot [you instructor used this colormetric product detection method

When you see a CSI person on TV holding an opaque, white membrane that looks like a piece of paper with bands on it, it is usually a blot that used a colormetric probe and development system. While thought not to be as sensitive as radioactivity, colormetric probes are considered far safer and are certainly easier to handle!

In this exercise, you will analyze "virtual blots" based on a possible "virtual" probing with 4 different tandem repeat probes of blots that could have been created from the cut DNA in Lab #3. An instructor has run the gel, set up the Southern transfer, probed and "finished" the blot so you can analyze the results in today's lab. You will use the resulting DNA fingerprints to judge whether or not there is a match between the DNA found at a "crime scene" and the DNA from the members of your lab group.

Objectives

- To see how meiosis affects genetic inheritance
- Be able to understand what happens during the stages of meiosis using pop beads
- To understand how offspring with different genes can develop from the same two people
- To perform an RFLP analysis

Materials:

For Students:
Pop bead chromosomes
Label tape
Colored pencils
Virtual blots
Tandem repeat probes handout
Rulers

For Instructors:
pop bead chromosomes
webcam for projection
virtual blots

Procedures:

Part 1. Simulate Meiosis in the production of gametes & the recombination of genetic material

1. Use pop beads to follow along with the instructor to re-create meiosis at your bench (see projection at the front of the room). Our parental cell will start with 4 chromosomes (the organism this represents has a total of four chromosomes that defines it as an organism. Remember that humans have 46 chromosomes so imagine this process with 46 chromosomes and the resulting genetic possibilities!). Each pair of students will simulate meiosis. You will be

using the Tandem Repeat Probes handout and adding label tape to your chromosomes to represent tandem repeat numbers to show additional genetic variation and how they are passed on from the parental cell to the daughter cells.

2. Use the colored pencils to represent maternal and paternal chromosomes and draw the stages of meiosis in your notebook.

3. Each pair should now have 4 gametes. Make sure you have sketches of these 4 gametes in your notebook. Combine one of your gametes (if you are using the blue and green chromosomes, imagine these are the sperm) with one of the gametes the other pair in your group created (should be with a red and yellow gamete which can simulate an egg). Repeat this with the other 3 gametes you created. You now have 4 offspring with a full set of 4 chromosomes each, each genetically different. Sketch the resulting offspring in your notebook. Be sure to include the tandem repeat numbers that are on each chromosome.

   Question 1. Are any or all of the 4 combined cells the same?

   Question 2. What relationship would these 4 cells have if they grew into people?

Part 2. RFLP DNA Fingerprinting Analysis

Remember that a Southern blot will be a mirror image of a gel.

Follow along with your instructor as she or he shows a "virtual" blot detailed tandem repeat number analysis procedure on the projector. You will then receive your group's virtual DNA-gel (Southern) blot of last week's EcoRI digest probed with tandem-repeat type probes. An instructor has created this for you over the past week.

Initial Assessment: Analyze your virtual Southern Blot DNA fingerprinting results from last week. Compare the fragments of each group member's DNA (the suspects' DNA samples) to those of the "crime scene" DNA. Is the overall pattern the same? Is the pattern different? How? First decide whose DNA matches the crime scene DNA pattern based on an overall pattern. Make sure to note this in your notebook.

Detailed Analysis I: Complete a detailed analysis of your group's virtual Southern Blot. Using your probabilities handout, make sure to include both your blot and your DETAILED analysis in your notebook.

Detailed Analysis II: After you completed Detailed Analysis I, you will receive a second blot from a simulated Missing Persons case. Tape this blot in your notebook also and complete both an Initial Assessment and a Detailed Analysis of this blot. You must label all bands on the blot in your notebook and create a table of the tandem repeat numbers that you assign to the blot.

Part 3. Clean-up and Notebook Signing

Remember to put back the chromosomes the way you found them – before "crossover" occurred.

Have you answered all of the questions and included both tandem repeat number analysis in your notebook? Did the instructor sign your notebook?