LAB 9 – DNA and Mitosis

Section 1 – DNA Introduced

[2] DNA, once suspected to be the powerful and mysterious force behind inheritance as well as a great prize at the end of the race for discovery of its structure, is a now a daily headline in news reports and a subject introduced as early as the elementary school level. Surprisingly, as common a topic as DNA is, few individuals comprehend how we arrived at our understanding of its heritable powers or how its structure was finally revealed. What a story! What a structure! What a remarkable subject to investigate! Let's start at the molecular level and get to know the structure of DNA!

[3] Deoxyribonucleic acid, or DNA, is the genetic material that an organism inherits from its parents. Does that mean it comes from a lawyer in the form of a will? No! When we say you inherit your DNA from your parents it is an inheritance you receive that enables you to come into existence, develop and be born! Record the name that DNA represents in your lab manual now.

[4]You inherited 23 molecules of DNA from your father's sperm and 23 molecules of DNA from your mother's egg. The sperm and egg fused and you had all of the information you needed to direct the development of you. Fill in the blanks and answer the questions following the diagram before you continue.

[5] Each molecule of DNA you received is shown here as an individual chromosome. Each chromosome represents one molecule of DNA.

[6] The egg that carries the 23 chromosomes is called a haploid cell. The sperm is also a haploid cell. A haploid cell contains only 1 set of chromosomes. Sperm or egg can be called gametes. Gametes are haploid reproductive cells. Record these definitions in your lab book.

[7] Compare these chromosomes that are found in haploid cells. Can you see a difference? Click on the chromosome that is available in the sperm and <u>not</u> the egg. Record your discovery by putting an arrow to the unique chromosome in your lab book and answer a couple of questions. We will discuss this important difference later.

[8] The chromosomes in a sperm or in an egg are composed of DNA and proteins coiled up like springs. The complex of DNA and proteins is called chromatin. The proteins found in chromatin help to organize the DNA. Label the protein and DNA components on the diagram in your lab book.

[9] Whether a molecule of DNA is coiled up with proteins and called a chromosome or relaxed and often referred to as simply chromatin, it is still the same molecule of DNA in a different form ready to perform a different function. In its coiled form chromatin is called a chromosome and is ready to go through cell division. Answers the questions regarding chromatin before you continue.

[10] Form dictates function and relaxed chromatin is diffuse. This is perfect for producing an exact copy of the DNA to pass on to a new cell during cell division. Relaxed chromatin appears as a mass of long fibers that would not be visible with your light microscope.

[11] Look at these two forms of chromosomes. Drag the word chromatin to DNA and proteins in their relaxed state**.** Now, drag the word chromosome to DNA and proteins in its coiled state. Record this information in your lab book.

[12] In sexually reproducing organisms, when the chromosomes of the haploid sperm cell fuse with the chromosomes of the haploid egg cell we call the resulting cell diploid. This fusion is called fertilization and it produces a zygote which is a fertilized egg. Record these important definitions in your lab book.

[13] Do you think only humans have DNA? Click on your answer.

[14] Did you realize that all known forms of life carry their hereditary information within their molecules of DNA? Humans have 46 chromosomes, but a dog has 78 chromosomes! A Koala has 16 chromosomes! The number of chromosomes in a eukaryotic cell depends on the species. Here you see a table listing the chromosome numbers of various species. Take a look at that now and answer the questions next to the table.

[15] Let's examine the components of the molecule of DNA more closely. Each molecule of DNA consists of a double-stranded helix. This is a common image you will see representing DNA.

[16] The "blue ribbon" backbone is actually made up of repeating molecules of sugar and phosphate. The letters in between the ribbons represent the hereditary instructions contained in the DNA molecule that produce organisms like you. Each letter (A, T, C and G) represents one of four nitrogenous (nitrogen-containing) bases. They are called *bases* because they act as a base and accept hydrogen ions. Record this information near the double-helix in your lab book.

[17] Let's take an even closer look at part of a molecule of DNA (because a molecule of DNA is MUCH longer than what you see here). This picture still shows the sugar phosphate backbone as a blue ribbon, but it shows dots between the letters of DNA. The dots represent hydrogen bonds. The two strands of the double-helix are held together by hydrogen bonds formed between the nitrogenous bases A, T, C, and G.

[18] What do you remember about hydrogen bonds? Do you remember if they are strong or weak? Click on the answer you think is correct. Record the information about hydrogen bonds in your lab notebook.

[19] Compare these two images. The image to the left should be familiar to you now. To your right is a more detailed image of the chemical structure of a molecule of DNA. The sugar phosphate backbone is represented by lavender deoxyribose sugar molecules and yellow phosphate molecules. You can now see the basic chemical structure of the nitrogenous bases and the hydrogen bonds formed between them.

[20] Before we go on, let's make sure that you know the different parts that make up DNA. Drag the labels to the correct part of the molecule. Record the same labels in your lab book when you get them all correct.

[21] DNA is composed of monomers called nucleotides. Each individual DNA nucleotide consists of three parts: a deoxyribose sugar, a phosphate and a nitrogenous base. Record these in your lab book and remember a monomer is a chemical subunit that is a building block of a polymer.

[22] A polymer is a larger unit consisting of many similar molecules *covalently* joined in a chain. Look at this longer sequence of DNA nucleotides. In DNA, the chain of covalently joined monomers of nucleotides is one strand of the double-stranded helix. Answer the questions about the DNA polymer before you continue.

[23] Take a closer look at these nucleotides and note that all have identical 5-carbon deoxyribose sugars and negatively charged phosphates. We have so far abbreviated the bases as A, G, C, and T. These letters represent the double-ring structures of bases adenine and guanine. Cytosine and thymine are single-ring structures. Take a moment to finish the questions in Section 1 before we move on.

Section 2 – DNA Identified as the Molecule of Heredity

[24] We will talk more about the amazing structure of DNA, but first, let's ask some questions; "How did we find out that DNA was the chemical that controlled a cell's characteristics and functioning or cause genetic disease?" "How did we find out DNA was the hereditary material?" All scientific discoveries are made by the blood, sweat and tears of countless hard working, devoted scientists, researchers and lab personnel. To illustrate DNA's discovery, let's take a look at some of the notable experiments that played a role in identifying DNA as the molecule of heredity.

[25] In 1928, a scientist and British medical officer named Frederick Griffith was studying the relationship of two strains of bacteria to the disease pneumonia. One of the strains was called "smooth" because it was surrounded by a *polysaccharide capsule* and formed smooth colonies on a culture dish. The capsule allowed the bacteria to be pathogenic which means disease-causing, by protecting it from the mouse's immune system. When Griffith injected the smooth or "S" bacteria into a mouse, the mouse died. Make note of this in your lab book.

[26] The other strain of bacteria studied by Griffith is seen here. This bacterium is not surrounded by a polysaccharide capsule and is nonpathogenic, and did not cause disease when injected into a mouse. It was labeled the "R" strain for its rough appearance on a culture dish. Can you explain why this mouse would live?

[27] If you answered that the mouse lived because its immune system identified and destroyed the "R" strain of bacteria, you are correct. It will help you to take notes in your lab book under the pictures of the mice to summarize this experiment.

[28] Griffith then heat-killed the smooth pathogenic "S" bacteria and found that they were no longer pathogenic. When injected with the heat-killed "S" bacteria, the mouse would live!

[29] Being meticulous as good researchers are, Griffith mixed heat-killed bacteria with live nonpathogenic bacteria as shown here. Griffith injected the mouse with the mixed strains of bacteria and the mouse died. He removed blood from the dead mouse and discovered live, pathogenic "S" bacteria with a capsule. How could this happen?

[30] Griffith reasoned that there must have been some factor from the pathogenic “S” bacteria, even after it had been heat-killed, that could transform or alter the nonpathogenic bacteria into a pathogenic form. Griffith called the event transformation. Copy this definition down in your lab book.

[31] We now understand transformation to be a change in the genes and physical characteristics of an organism by the incorporation of external DNA into a cell. Griffith did not discover the transforming factor, but laid the groundwork for other researchers. Summarize Griffith’s findings in your lab manual before you go on.

[32] For 14 years American bacteriologist Oswald Avery and his colleagues McCarty and MacLeod continued Griffith’s search by asking the question “what chemical component of the heat-killed ‘S’ cells could cause this transformation”?

[33] When Avery and colleagues destroyed the DNA of the heat-killed “S” strain bacteria, transformation did not occur and the “R” strain bacteria did not become pathogenic. The scientists announced that the transforming agent was DNA. Even so, biologists were still not convinced. Note this discovery in your lab book.

[34] In 1952, additional experiments were done by two scientists named Hershey and Chase that confirmed DNA as the genetic material. To accomplish this, they used bacteriophages. Bacteriophages are viruses that attack and infect bacteria. You can see the tiny space-ship shaped bacteriophages infecting the oval shaped bacteria. Label the bacteriophage and the bacterium in your lab book before you continue.

[35] Viruses consist of only DNA and protein. The viruses head and body are made of a protective protein coat that encloses the DNA in the head or top portion of the virus. Hershey and Chase took advantage of the fact that DNA contains the element phosphorus but not sulfur and protein contains sulfur but not phosphorus. To follow the activity of the virus’s DNA and proteins, they attached a radioactive signal to the phosphorus of DNA and a radioactive signal to the sulfur of proteins so that they could track the destiny of both molecules. Label the location of the DNA and protein before infection in your lab book using the colored pencils provided.

[36] Hershey and Chase allowed the two different radioactively labeled bacteriophages to infect the bacteria. The radioactive signal attached to the sulfur in protein was found on the outside of the bacterial cells in the phage parts. When the radioactively labeled phosphorus in DNA was inspected, the signal was found inside the bacterial cells. Label the correct location on the diagram in your lab book of the two radioactive signals after infection of each bacterium.

[37] Hershey and Chase’s conclusion was that bacteriophage DNA entered the cell, but phage protein did not. They further concluded that DNA, not protein, was the genetic material of the bacteriophages.

[38] Later, scientists used electron microscopes to confirm that a bacteriophage attached itself to a bacterial cell by its tail and injected its DNA into the cell, leaving the empty protein coat on the outside. We now know that to reproduce, the virus must hijack the bacteria’s reproductive machinery to reprogram it with viral DNA.

Section 3 – Chromosome Theory of Inheritance, Karyotypes

[39] What prompted scientists to investigate chromosomes in cells as being the hereditary material? Chromosomes were the molecule of interest because, as you know, they are made up of DNA and proteins.

[40] Earlier experiments were performed by Sutton, Boveri and other scientists. In 1902, they had accumulated information that formed the *chromosome theory of inheritance.* The theory was based on the similarities between the behavior of chromosomes and theories of inheritance. Information about chromosomes supported the idea that DNA was the cell's hereditary material.

[41] Let's consider some of the supporting information. First, pictured here you see the 46 chromosomes of a human male. This is called a karyotype. A karyotype is a display of the chromosome pairs of a cell arranged by shape and size. Record the definition.

[42] Here is a karyotype of a human female. You have already reviewed the table that lists unique numbers of chromosomes in a cell depending on the species. Fill in the characteristic number of chromosomes for humans in your lab book.

[43] Let's summarize the supporting information for the chromosome theory of inheritance. First, remember that chromosomes are present in almost every cell of the trillions of cells that can make up an organism.

[44] Second, the presence of a unique and constant number of chromosomes in practically every cell indicated their importance and hinted at their role in heredity.

[45] Point number 3: chromosomes are present in PAIRS in somatic cells. Somatic cells are any cell of a multicellular organism except a sperm or egg.

[46] Why would there be pairs of each chromosome? Think back to how we began this lab…talking about what you inherited from each of your parents. Your parents each passed on a gamete to you – the sperm or egg you received each had HALF the amount of your total DNA. The gamete is a haploid cell…a specialized cell that contains one complete set of chromosomes. To maintain the characteristic species chromosome number present in cells, each parent can only contribute half of their genetic material to offspring and this is accomplished through a gamete.

[47] Last point. Each gamete contains a haploid number of chromosomes from each parent. Fertilization restores the diploid chromosome number unique to each species. In humans we know that fetuses that inherit more or less than 46 chromosomes rarely survive.

[48] More knowledge only leads to more questions! How, when and why do organisms produce diploid or haploid cells? We will investigate these processes called mitosis and meiosis in this lab and the next. Make sure you have completed Section 3 before you continue.

Section 4 - The Discovery of the Structure of DNA

[49] By the early 1950's it was a generally accepted fact that DNA was the cell's hereditary material and could be isolated as a clear, sticky substance. Though it was already known that DNA was a polymer of nucleotides, the race was on to discover the three-dimensional molecular structure of DNA. If you did not know it was a double-stranded helix – what would you suspect it looked like?

[50] The two men who won the very competitive race to discover the structure of DNA are pictured here; Dr. James Watson on the left and Dr. Francis Crick on the right. In 1953, at the Eagle Pub in Cambridge, England, Francis Crick proclaimed to a lunch-time crowd that they had "discovered the secret of life". They received the Nobel Prize for their work with DNA in 1962.

[51] How did Watson and Crick determine the three-dimensional structure of DNA? The two scientists never performed any experiments on DNA. Watson and Crick THOUGHT about what many researchers had already discovered about DNA. They built models, asked questions and argued with each other about DNA.

[52] One of the scientists whose work contributed to Watson & Crick's discovery was Erwin Chargaff of Columbia University. In the 1940's he analyzed the amounts of the four nucleotides in DNA from organisms as diverse as bacteria, fish and humans and found that the DNA of any given species contains **equal amounts of adenine as thymine, as well as equal amounts of guanine and cytosine.** Make note of this in your lab book.

[53] Note the percentage of bases you see here in human DNA. This discovery became known as Chargaff's rules: In an organism's DNA sample, the base amounts of A = T and C = G. This information strongly influenced Watson & Crick's thinking about the structure of DNA. What does it suggest to you?

[54] To make sense of this information, let's add to what we know about the bases of DNA. Nitrogenous bases are divided into two different groups, purines and pyrimidines. Purines are the larger of the two, having a two-ring structure. Adenine and guanine are purines.

[55] Pyrimidines are smaller having a single-ring structure. There are three different pyrimidines: thymine and cytosine which you see here. Uracil, a third member of this group, is part of RNA, an important nucleic acid we will discuss in a later lab. Take a minute to correctly identify the purines and pyrimidines in your book.

[56] Now add to this information the discoveries made by Maurice Wilkins and Rosalind Franklin. Using a technique to visualize DNA called X-ray diffraction; Wilkins and Franklin provided essential information to Watson & Crick that allowed them to deduce the helical nature of DNA.

[57] This is a molecule of DNA that has been crystallized, bombarded with X-ray which then bounced off of the DNA to create a picture of diffraction patterns. *This is not a picture of the double-helix of DNA!* Researchers such as Franklin used mathematical equations to translate such patterns into information about the three-dimensional shape of molecules. I am sure you are relieved to know we will not be attempting those mathematical equations today!

[58] The information presented by Franklin's x-ray diffraction provided further evidence for Watson and Crick that DNA was a double-stranded helix, and that the sugar-phosphate backbone was on the outside of the molecule. This did not answer every question. Watson and Crick did not know how the bases came together in the middle of the helix.

[59] The solution was found after further study of the x-ray data. Measurements of the width between the two strands of DNA were consistent with the pairing of a purine and a pyrimidine. This should help you answer the questions about Franklin's work in your lab book.

[60] Remember Chargaff's rules? A = T and C = G. Not only would a purine always pair with a pyrimidine, Chargaff's research allowed Watson and Crick to recognize that A would always pair with T and C would always pair with G. Look at the DNA molecule in your lab manual and start to answer the questions about DNA pairing.

[61] Continue inspecting this DNA double-stranded helix. This picture clearly illustrates that the bases C and G form three hydrogen bonds between them and the bases A and T form two hydrogen bonds between them. Did you notice that each strand of the double-stranded helix runs in an opposite direction? This orientation is termed anti-parallel. Record this term in your lab book.

[62] To finish up Section 4, demonstrate your understanding of base-pairing by completing the missing bases in the double-helix and have the instructor sign your diagram. Continue when you are ready to start Section 5.

Section 5 – Constructing a Virtual DNA Molecule

[63] You will now construct a virtual DNA molecule. Remember, you can return to any previous slide in the program to review if you are having trouble. Let me give you a few hints before you begin.

[64] As you build your molecule, remember what we have learned so far: 1. DNA is a polymer of nucleotides. 2. Remember what we learned from Chargaff's rules! And 3 – remember how many hydrogen bonds are formed between certain nucleotides and the antiparallel orientation of the molecule. Make sure you get your lab book signed off before you continue.

[65] Now that you have constructed a DNA molecule you understand how it is composed of two strands of nucleotides connected by hydrogen bonds between specific bases. Your virtual model was two-dimensional, but keep in mind that actual DNA resembles a "twisted rope ladder", it is a three-dimensional, double-stranded helix. Take a moment to answer the questions at the beginning of Section 5 and observe the model of DNA on the demonstration table.

[66] By now you are very familiar with the rules of base-pairing. You know that between the double-strands of DNA, A must pair with T and C must pair with G. What about the order of letters along each strand of DNA? The strand of DNA, or the SEQUENCE, is where the message of DNA is found. Stop now to copy the sequence from the right-strand of your virtual DNA molecule in your lab book.

[67] The sequence or ordering of bases along the length of each strand of DNA is exclusive to each chromosome and unique to an organism. It is the sequence of DNA that provides the instructions for the development, characteristics and functioning of the organism.

[68] The sequence of each strand of DNA represents the genetic code – a code that was deciphered in the 1960's. You will learn to read the code in an upcoming lab. Every chromosome has thousands to millions of bases arranged in a specific sequence that is unique for that chromosome. For example, chromosome 15 in all human beings should generally have the same sequence along each strand, but that sequence will be different on chromosome 18.

[69] This image shows less than 1/10,000th of the sequence of human chromosome number 18 starting near the thirty-nine-millionth nucleotide along one strand. Human chromosome 18 has approximately 78 million base pairs and each strand of the double-helix is composed of a unique sequence using nucleotides A, T, C, and G. Thanks to the Human Genome Project we know the sequence of the approximately 3 billion base pairs that make up one set of human chromosomes.

[70] This is an ideogram of the human chromosomes found in one cell. Ideograms are graphic symbols here representing actual human chromosomes. You can see that human chromosomes have 22 pairs of numbered chromosomes called autosomes. You can also see the sex chromosomes – an X and Y chromosome if you are male or two X chromosomes if you are female. Finish answering the questions in section 5 before we look at how new cells are produced.

Section 6 – DNA Replication

[71] Now that you know so much about the structure of DNA, you can learn how hereditary information is copied and passed on to new cells. This is accomplished through two processes called DNA replication and cell division.

[72] Cell division is the reproduction of a cell. It is a process organisms utilize for three reasons: The growth of a new organism, the replacement of old or damaged cells or to accomplish asexual reproduction. Record these functions in your lab book along with the definition.

[73] Here you see a human embryo at the 6-8 cell stage of growth. This was accomplished by cell divisions starting with the original fertilized cell and ending in a multicellular organism. Cells, whether old or new, possess a complete set of genetic information in the form of chromosomes.

[74] To insure that each cell has a complete set of genetic information, the original parental DNA must go through a process called replication. DNA must be copied or "replicated" so there is a copy of each chromosome to pass on to what biologists refer to as the "daughter" or offspring cell.

[75] How is DNA replicated? The general mechanism of DNA replication can be described as a simple process as you will see in this lab. In reality, replication is a complex procedure that requires dozens of enzymes and other proteins. Let's concentrate on the general mechanism.

[76] DNA replication begins with the original, “parental” double-strands of the DNA molecule. In this simplified illustration, the helix is not twisted and the base-pairs are not shown. The DNA parental strands unwind to open the molecule at an origin of replication by breaking the hydrogen bonds. Each parental strand will act as a template for the synthesis of a new “daughter” strand of DNA. Free nucleotides will base-pair to the open parental strand creating the two new daughter strands. Replication of the molecule is then complete. Each of the two new DNA molecules consists of one parental strand and one daughter strand.

[77] This image shows a diagram of a piece of the DNA molecule composed of two parental strands making up the double-stranded helix. The parental strands are what will be replicated. Record these bases in your lab book to help you keep track.

[78] This image shows the same molecule of DNA. Here, the hydrogen bonds *between* the bases of the double-stranded helix are breaking as the molecule unwinds. It comes apart along the length of the double helix. Can you see which bases have three hydrogen bonds and which have two? Record these bases in your lab book as well.

[79] Free nucleotides will base pair to the parental strands of DNA to form the new daughter strands. The free nucleotides are found in the environment of the cell just as molecules of oxygen are available to you in your environment. The organism has produced the nucleotides from its nutrients. Drag two of the nucleotides to their correct base pairing position.

[80] Here is the replication a little further along. Record all of the base pairs in your lab book when you have placed them on the screen correctly.

[81] The key to the remarkable accuracy of DNA replication is base-pairing. “A” always pairs with “T”. “C” always pairs with “G”. This is also called “complementary” base pairing. Based on the base-pairing rules that you know, complete your replication in the lab book.

[82] Here is a summary drawing of replication. Two new strands of DNA have been synthesized. Note that the synthesis of the new daughter strand was directed by the parental strand by following the base-pairing rules. Drag the word parental to the template strands. Drag the word daughter to the new strands. Make note in your lab book which is which.

[83] The daughter molecules are identical in base sequence but are formed from an old strand of DNA and a new strand of DNA. This is called semi-conservative replication because each newly formed DNA molecule consists of one-half conserved “parental” DNA and one half new “daughter” DNA. Answer the questions at the end of Section 6 and have the instructor sign off on your completed molecules.

Section 7 – Cell Division: Mitosis and Cytokinesis

[84] In this section of the lab you are going to investigate cell division...a process called mitosis and cytokinesis. We introduced cell division when talking about DNA replication. Mitosis cannot take place until DNA replication provides a copy of each chromosome for the new cell. Mitosis is the division of a single nucleus into two genetically identical daughter nuclei. Record this information in your lab book.

[85] Mitosis is followed by cytokinesis. Cytokinesis is the division of the cytoplasm to form two separate daughter cells. Remember, the term “daughter” does not indicate gender, but represents new cells. Make note of this information before you go on.

[86] Cell division is essential for the growth of a multicellular organism, even those that reproduce sexually. Multicellular organisms begin development as a zygote which you remember is a fertilized egg. That includes you! You did not remain a single-celled organism and you have mitosis to thank! New cells for the growth of an organism are provided by cell divisions.

[87] You see here polar bears and stalked eyed flies. Both are in heated contests, likely over a female. They are similar in that they both fight over mate selection, but they are different in their size as organisms. Are bears larger because their cells are larger? No! Bears and flies are different in size because the NUMBER of cells they have are different. Both organisms’ cells have been produced through cell division, but the number they each possess is very different.

[88] Cells do not live forever! Cell division provides new cells to replace those that die or are damaged during the lifetime of the organism. Every second that you sit here in lab, learning about cells, your surface layer of skin cells are being sloughed off. But don’t blame Bio 3! Thanks to cell division the surface layer of your skin is replaced every 2 weeks. What happens to the old skin? It is a main component of house dust and a favorite food for dust mites.

[89] You can appreciate how cell division provides a 24/7 replacement mechanism. Regardless of how you or any other organism is using the new cells produced by mitosis and cytokinesis, the process must be precise! If the chromosomes in a cell were to divide randomly, the two new cells produced would likely get an incorrect complement of chromosomes.

[90] Are there serious consequences to incorrect cell division? One result is seen here. These are the chromosomes from one human cancer cell. Note that this does not look like a normal karyotype which should have 23 pairs of chromosomes. Cancer cells divide uncontrollably, causing tumors, which can cause death.

[91] Therefore, in the interest of survival of cells or whole multicellular organisms, cell division must involve precise replication of each chromosome followed by the accurate division of the nucleus. Replication and mitosis ensure that each new daughter cell gets a set of chromosomes identical to those in the parent cell. Complete Section 7 in your lab book before you go on.

Section 8 – Chromosomes Ready for Mitosis

[92] Form dictates function and chromosomes before and after mitosis have a special form. Before mitosis, when a cell is not dividing, the molecules of DNA and associated proteins are long thin fibers that you remember we called chromatin. Even as the cell goes through replication the chromatin remains diffuse or relaxed.

[93] After DNA replication, as the chromatin becomes densely coiled and forms a chromosome, we can begin to see each duplicated DNA molecule. The two identical copies of the DNA molecule are now *each* called a “sister chromatid”. This is to differentiate replicated DNA from non-replicated DNA.

[94] Though there are two sister chromatids, this replicated molecule is still considered one chromosome. The two identical DNA molecules, the sister chromatids, are joined at an indented area called the centromere. The centromere will play an important role in cell division when it comes time to divide the sister chromatids and form a daughter cell. Answer the questions in your lab book next to the chromosome illustration.

[95] The separation of the sister chromatids will occur during mitosis. Make sure you have completed Section 8 in your lab book before you move on.

Section 9 – Phases of Mitosis

[96] This is an illustration of the cell cycle. The cell cycle represents each stage in the life of a cell. A cell has two general stages in its life; interphase and the mitotic phase. Write these two stages on the cell cycle illustration in your lab book.

[97] Interphase accounts for approximately 90% of the life of a cell. Interphase is the period of cell growth and replication. There are two growth phases, named G_1 and G_2, divided by the “S” phase. “S” stands for synthesis of DNA, the phase where DNA is replicated. Add these stages to your cell cycle illustration in your lab book and make sure you’ve answered all the questions regarding the cell cycle before going on.

[98] During interphase, the chromosomes exist as long thin threads of DNA and proteins tangled together in the nucleus. This material which appears granular you already know to be called chromatin. You cannot see the chromosomes as discreet units, but the nucleus is visible. Also visible is the nucleolus and sometimes you will see more than one. You can also distinguish the cell wall clearly. Drag the names of these cell structures to the correct location on the cell and when correct, record the information in your lab book.

[99] Though mitosis is a continuous and fairly swift process...for the sake of order and understanding biologists divide mitosis into four main stages or phases: prophase, metaphase, anaphase and telophase. In reality, one phase merges into the next as a continuum, but there are characteristic events of each phase that you should become familiar with. As we review each phase, drag the structure that is being described to its correct location in the cell and then record the main events in your lab book.

[100] The first phase of mitosis is called prophase. During prophase, the sister chromatids that are made up of chromatin shorten and thicken by coiling, looping and folding around proteins. As the chromatin condenses, you will begin to be able to visualize chromosomes. Here you see only 4 chromosomes going through mitosis for the sake of simplicity. In a human cell, there would be 46!

[101] The nuclear membrane and nucleoli dissipate during prophase, allowing the chromosomes to move freely. Though it cannot be seen under a light microscope, fibrous protein material called spindle microtubules form to eventually attach to the centromeres of the chromosomes. Label these parts in the cell.

[102] The second stage of mitosis is called metaphase. The microtubules, now attached to the centromeres, move the chromosomes so that they are arranged along the equator of the cell. This position is often called “the metaphase plate”. You can see the chromosomes but will not be able to distinguish which chromatid belongs to which chromosome. It is not possible to see the centromeres, but each chromatid has its own.

[103] The third stage of mitosis is anaphase. The centromere of each attached sister chromatid are pulled to opposite poles of the cell. As the chromatids are separated, they are now each referred to as an individual and complete chromosome as they no longer represent attached replicated DNA molecules.

[104]The centromeres are pulled toward the poles by the microtubules and the ends of the chromosomes are dragged along behind, making the chromosomes appear “V” shaped. By the end of anaphase, two identical, complete sets of chromosomes have reached opposite poles of the cell.

[105] Telophase, the last stage of mitosis, begins with the two groups of chromosomes at the opposite poles in the cell. The chromosomes will begin to uncoil and relax so that eventually the individual chromosomes will once again be visible and will appear granular. The nuclear envelope begins to re-form and becomes visible once again. It is at this point in telophase that the division of the cytoplasm begins. The division of the cytoplasm is called cytokinesis and often occurs simultaneously with telophase.

[106] In a plant cell, such as the one you are looking at, cytokinesis is accomplished by the formation of a cell plate. Membranous vesicles filled with cell wall material are deposited in the middle of the cell to form the cell plate. The vesicles gradually accumulate until they fuse with each other and join the plasma membrane of the parental cell wall. At this point, the cell plate forms a new cell wall resulting in complete separation and formation of two new daughter cells.

[107] Make sure that you are familiar with the various stages of interphase, mitosis and cytokinesis and that you have copied the structures and made notes of the important events of each phase in your lab book. Answer the last few questions of Section 9 before we get out our microscopes.

Section 10 – Identifying Mitotic Stages Under a Microscope

[108] To study the phases of mitosis we have selected plant material in which cell division is taking place rapidly. In plants, growth through cell division is most rapid in the root or stem tips. Let’s turn our attention to the chromosomes in the cells that make up the root of an onion.

[109] In this high-power view of an onion root tip section, you can see many cells at different stages of cell division. In some of the cells, the chromosomes appear as dark thick threads. In other cells, the chromatin is relaxed and appears as dark granular areas.

[110] Now that you have become an expert in identifying the stages of mitosis, it is time to identify the phases in actual cells using a prepared slide of onion root tip. Your goal is to find each phase of mitosis for yourself using your microscope and slide and then sketching a

representative cell in your lab book. You do not need to be an artist to produce scientific drawings. It should be an accurate representation of what you have seen, not a work of art!

[111] Have you completed your drawings? If so, locate one onion cell in your microscope in anaphase and put the pointer on that cell. Have the instructor initial your lab book, then you can move on to Section 11 and finish up lab 9. Remember the instructor is available to help you if you run into any problems.

Section 11 – Mitotic Stages in Animal Cells; Different than Plant Cells?

[112] You have investigated and drawn cell division in a plant cell, specifically an onion. Had you thought about onions having chromosomes? What about animal cells? Are they different?

[113] Let's see how animal mitosis might look in animal cells with this animation. Remember as we view the animation that whether these phases occur in a plant or animal cell, the goal of mitosis and cytokinesis remains the same - to produce genetically identical daughter cells. But, there are differences worth taking note of.

[114] Though you would not be able to see centrioles in animal cell slides in your microscope, you can see them in the still slides from the animation. Centrioles are present at the pole of each dividing animal cell. Centrioles physically anchor one end of each microtubule. Most plant cells anchor their microtubules, but not with centrioles.

[115] You may have already wondered…where is the cell wall? Animal cells do not have cell walls so do not get that question wrong on a quiz! In animal cells, cytokinesis is accomplished through a process called cleavage. The first sign that cleavage is occurring is seen here as a wrinkled indent at the metaphase plate of the cell. This is called the cleavage furrow. It looks as though someone is pulling the drawstrings of a trash bag. No cell wall means greater flexibility for animal cells. Complete the section on mitotic phases in animal cells before going on.

[116] We began this lab talking about what you inherited from your parents. You began life as a zygote, the result of the fusion of sperm and egg. You inherited one set of chromosomes from each of your parents. As a single-celled, diploid zygote, you then divided by mitosis into 2 cells, 4 cells, 8 cells and so on until your cells became tissues and organs. From the moment fertilization occurred, mitosis accomplished the task of producing you, a multicellular organism.

[117] Does it always produce just one organism? Not if you are an identical twin! Identical twins are referred to as monozygotic twins. Monozygotic twins develop from one zygote which makes them genetically identical. At an early point of mitosis the cells of an embryo divide up and each group of cells develop into a complete individual. The end result… two human beings who carry the same genetic information. Approximately 1 in 250 pregnancies result in identical twins.

[118] How do non-identical twins occur? Non-identical twins are referred to as dizygotic twins. This is no trick of mitosis! Very simply, 2 eggs are available from the female at the time of fertilization and each fuse with an available sperm. The embryos which develop together are genetically no more similar than a brother or sister born 10 years apart.

[119] Where do we get our eggs and sperm? That is another fascinating subject called meiosis and you'll learn about that next week. Make sure you have completed Section 11…you did great work!