Molecular Biology
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Module - 03
Basics of Biomolecules
Lecture-11 Biomolecules (Part 1: DNA)

Hello everyone! This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different aspects of the molecular biology. And so far what we have discussed? We have discussed about the basic properties of a biological system. In this context, we have discussed about the structure of the biological cells. So we have discussed about the structure of the Bocaryotic cell and we have discussed about the structure of Eukaryotic cell.

While we were discussing about the structure of Eukaryotic cell, we have also discussed about the structure of the different organelles and their functions and their contribution into running the different types of functions or the activities within the cell. In addition to that, in our previous lecture, we have also discussed about the cellular metabolism and how the cell is producing the energy with the help of the different types of catabolic reactions and anabolic reactions. So we have discussed about the anabolic reactions also which are responsible for production of the different types of biomolecules or the proteins. And then we also discussed about how the cell is actually dividing and how what are the different stages it is actually going through.

So we have discussed about the cell cycle and how the cell cycle is tightly being regulated. While we were discussing about the cell cycle, we have also discussed about the some of the technical and as well as the experimental aspects, how you can be able to study the cell cycle with the help of the flow psychometry. In addition to that, we have also discussed about how you can be able to study the mitosis and meiosis with the help of the different types of microscopy techniques. And then at the end, we have also discussed about the apoptosis and cell death. So and there also we have discussed about the different types of pathways.

So we have discussed about the intrinsic pathway and as well as the extrinsic pathway, what are different molecular players which are actually participating into these type of different pathways. And we have also seen how we can actually be able to study these events in the DNA cell with the help of the flow psychometry. So now it is clear that there are so many functions which are happening or there are so many activities what is happening within the cell. And all these activities, if you summarize, you will summarize

that these activities can be classified into three different types of activities. And all these activities can be sum up within the life related activities.

So you can have the life related activities. So first is the production of energy which means the catabolic reactions. We have discussed about the carbohydrate metabolism. And we have also discussed about the lipid metabolism and how the glycolysis and crepe cycle is contributing into the production of energy and how the beta oxidation is taking place in the lipid and how it is actually producing the stile coA and then the stile coA is getting into the crepe cycle and producing the energy. The second aspect is about the growth.

And as you remember that when we were using the energy that energy can be used for producing the growth with the help of running the anabolic reactions. And the third aspect is about reproduction. So growth is different from reproduction where the cell is actually going to perform the different types of divisions. And that is how the single cell is actually going to multiply and becomes double two cell, four cell, eight cell like that. Now what you see here is that if you want to go with the catabolic reactions or the anabolic reactions or the reproduction of the reproduction where you are going to do a division.

In this also there will be anabolic reaction what is going to involve in the production of different types of biomolecules. So all these activities are completely being regulated by the biomolecules at the molecular levels. And in this particular module, we are actually going to go through with some of the basic properties of these biomolecules. So what are the different biomolecules we are going to cover in this particular modules. So we are going to discuss about the DNA or DRC ribonucleic acid.

Why it is important to study the DNA because DNA is a source of the genetic information. This is anyway we are going to discuss when we discuss about the genomic DNA and other kinds of aspects of molecular biology. But for time being you can imagine that the DNA is actually the genetic material. Because when we were discuss about the genetic material, you will understand that that time we are going to discuss about the different types of experiments. Which are actually going to give you the idea that why the DNA is a genetic material.

Then DNA will go through with the activity which is called as replications. And with the help of the replications, one DNA copy will actually go into form the two DNA copies. And remember that when we will discuss about the replications, we will discuss about the replications in the poropyratic system and as well as replication in the eukaryotic systems. And then DNA is also going to participate into the activities which are called as transcription. So, from DNA it is actually going to generate the RNA and RNA is going

to have the same amount of information what the DNA is.

And this process is known as the transcription which is actually going to perform by the RNA polymerase. DNA to DNA is actually going to be catalyzed by the DNA polymerase. Now, once from DNA, you are actually going to synthesize the RNA. RNA or the ribonucic acid, it is actually going to participate into translation. Translation which is actually going to be responsible for production of protein.

So, RNA alone is not responsible. You are going to have the help of the other protein molecules to form the ribosomes and other machinery and then ultimately it is actually going to produce the protein. From the RNA, once you produce the protein and protein is very important because it is a building block for most of the biological system. For example, we have the different types of proteins. So, we can have the protein which is building blocks like collagen.

We can have the proteins as building block like actin, myosin which is actually being responsible for production generation of muscles. And because of these actin and myosin fibers, you can be able to walk and you can be able to run. So, it is not only that it is actually going to be a building block. For example, the collagen is actually the main fiber responsible for production or for the generation of the different types of bones because collagen is going to be calcified. And that is how it is actually going to produce the different types of bones.

Hairs, for example, the keratin and other things. Now apart from that, the protein can also be an enzyme. So, enzyme is actually I am keeping in a different category. So, enzymes are within the protein, they can also be an enzyme. And what is the function of the enzyme? Enzyme is actually going to participate in running the metabolism of the organisms.

What is mean by the metabolism is that it is actually going to run the catabolic reactions and the anabolic reactions. All these anabolic and catabolic reactions are actually going to be catalyzed by the different types of enzyme. Apart from that and what is the purpose of these? It is actually going to be responsible for the energy production. Apart from that, the enzymes are very actively being participate into the three major processes what are happening in the cell. What are these processes? These processes are called as the replication followed by transcription followed by translation.

Now all these are central pathways are actually being governed by the enzyme. Apart from that, the enzymes are also being playing crucial role into the molecular cloning. So, in this particular module, when we talk about the enzyme, we are actually going to discuss about the basic properties of the enzyme. And then we are not going to talk about the

enzyme kinetics and other kinds of aspects of enzyme. Because that you can easily be able to go through or that you can actually be able to study in other kinds of MOOCs courses.

Like for example, I also have another MOOCs course called enzyme science and technology. And if you want to study that part, then you can actually be able to study using this particular module MOOCs course. So, there is a MOOCs course called enzyme science and technology, which deals in detail about the enzyme related kinetics, how you can be able to solve the structures and blah, blah, blah like that. So, that we are not covering in this particular module. What we are covering is what basic properties of the enzyme.

And then we are going to talk about the specific properties of the enzyme which are going to play crucial role in the molecular biology related activities like replication, transcription and translation, and enzymes which are part of the molecular cloning. So, let us first start our discussion about DNA. So, DNA is a nucleic acid, which is a deoxyribonucleic acid. So, it is a deoxyribonucleic acid. You can have the two different types of nucleic acids.

So, and as I said, DNA is the major molecule which is responsible for carrying the genetic information from the one generation to another generation. And this all you are actually going to learn when we are going to discuss about the genomic DNA and genetic information, genetic material actually. So, most of the organisms whether it is a prokaryotic organisms or a eukaryotic organism has DNA as a genetic material, whereas minor fractions such as some of the viruses like the coronavirus or other kinds of HIV and all that has RNA as a genetic material. So, mostly the nucleic acid is going to serve as the genetic material. So, whether it is the DNA or RNA.

DNA or RNA is a biopolymer and it is acidic in nature. And that is why the nucleic acid is acidic in nature. In eukaryotic cell, animal or the plant, the nucleic acid is present within the nucleus, whereas in prokaryotic cell, it is present as a free from into the cytosol. And that is why we have discussed about the differences between the prokaryotic cell and the eukaryotic cell. And one of the major differences that the nucleic acid which is present within the nucleus in the case of eukaryotes, whereas it is going to be present outside as a free form into the cytosol.

The first nucleic acid was isolated by a scientist known as Vergic Mestre in the year 1868. And since then, we will actually discovering the new and new properties of this particular molecule. Now, what is the composition of the DNA? So, the nucleic acid is composed of the three components. You can have the phosphoric acid, you can have the base and you can also have the sugar. The phosphoric acid provides the backbone to the polymer, whereas the sugar work as the anchoring point for the nitrogenous bases.

The nine-membered nitrogenous bases give the diversity in the sequence of the nucleic acid. So, these are the three different components. You can have the sugar, right. So, you can have the two different types of sugar. So, we can have the ribose in the case of RNA, right.

So, we can have the ribose in the case of RNA or we can have the two deoxyribose in the case of DNA, because these are the, and remember that there is only one difference that 2 prime OH is present in RNA, whereas that 2 prime OH is missing in the case of DNA. And then you can also have the phosphate, right. So, the phosphate is actually working as a backbone, right. So, phosphate is running throughout, right. And in this phosphate, you are actually having the, you know, the sugar molecule which is attached to this.

And that sugar molecule is working as an anchorage point for the different types of nitrogenous bases. So, you can have the nitrogenous bases which are belonging to the pyrimidine or you can have the nitrogenous bases which are belonging to the purines. So, forget talking about the components, so phosphate backbone. So, phosphoric acid serves as the backbone of the molecule. So, you can have the two different chains, one is phosphate backbone on this side, the other is having the phosphate backbone on this side.

Then you can also have the sugar. So, you can have the five membered sugar, either it can be a ribose or the deoxyribose. So, in the DNA, you can have the deoxyribose where the 2 prime OH is missing. So, the five membered cyclic reducing sugar is present in the nucleic acid. These are the two different variants, the sugar molecule which contains the hydroxyl group at 3 prime as known as ribose, whereas it is deoxyribose if it is absent, okay. Based on the sugar, the nucleic acid is classified either the RNA or the DNA.

Ribose sugar is present in RNA whereas deoxyribose sugar is present in DNA. The purpose of sugar in the nucleic acid is to provide the attachment point for the nitrogenous bases. So, this is the sugar and it is actually going to have the attachment point so that you can have the nitrogenous bases attached to sugar. So, this is the phosphate backbone on this you are going to have the sugar molecule and on this sugar molecule, you are going to have the base, right. And that is how it is going to be have the same way, it is going to be on this side.

And that is how they actually interact with each other. Is anyway we are going to discuss in details when we discuss about the base pairing and other kinds of things. And then the nitrogenous base, so there are two variants. There are nine-membered conjugated double bond system, right.

And there are two variants. One is purine, the other one is called as pyrimidine. So, purine

such as adenine and guanine, the six-membered single-ringer system which is called as pyrimidine. So, these are called thymine, uracil and the cyclosilane. So, this is the adenine which is a nine-membered ring. And then you also have the another nine-membered ring which is called as guanine.

So, these are the nine-membered ring or I will say two-ring base, right. So, if you have two rings, it is going to be purine. If it is a single ring, then it is going to be pyrimidine, okay. So, pyrimidine is going to be either the thymine, cytosine or the uracil, okay. Whereas for the purines, it is going to be two-ring and pyrimidine, it is going to be one-ring, okay.

And the presence of nitrogenous base in DNA, RNA is predetermined. For example, the DNA has adenine, guanine, thymine and cytosine. And it does not contain the serosol, okay. Whereas the RNA has adenine, guanine, uracil and cytosine and strictly no thymine, okay. Now, the question comes, if you have the adenine, thymine, guanine and cytosine, why there is a strict or what is the base pairing? Which one will actually going to make the base pair with others and so on.

So, what is the rule about having the base pairing between these nitrogenous bases, right. So, the DNA is double-stranded where RNA is single-stranded, right. In most of the cases, RNA can be double-stranded in some of the plant viruses and some of the other special organisms who are actually going to have the RNA double-stranded, but mostly the double DNA is actually going to be double-stranded. The individual monomer responsible for making DNA or RNA is nucleotide and as a result, the DNA or RNA can be considered as poly nucleotide molecules.

So, they are polymers actually. Similarly, just like you know, the sugar is the polymer of the glucose molecule. Similarly, you can actually have the DNA which is a polymer of the nucleotides and that is why it is called as the poly nucleotides. In a poly nucleotide, you can and what is there in the individual nucleotides. So, individual nucleotide is having a nucleoside attached to one or more phosphate group and can be termed as the, if it is attached to the one phosphate group, then it is called as monophosphate nucleoside. If it is attached to the two phosphate group, then it is called as diphosphate nucleoside and if it is conducted to the three phosphate group, then it is called as triphosphate nucleotides.

This is what it is actually going to show. So, if the sugar is attached to the base, if the sugar is attached to the base, then it is called as nucleoside. So, if the sugar is plus base, then it is called as nucleoside. Now, if nucleoside is attached to the one molecule of phosphate, then it is going to be called as nucleoside monophosphate or I will say nucleoside. Then if it is attached to the two phosphate groups, then it is going to be called

as nucleoside diphosphate. Or if it is attached to the three phosphate group, then it is going to be called as nucleoside triphosphate.

And the base is attached to the sugar molecule with the help of the glycosidic bond. If the two prime OH is present, then it is going to be called as ribose. If the two prime OH is absent, then it is going to be called as DRC ribose. Each nucleoside is composed of the nitrogenous base attached to the sugar to the glycosidic bonds. So, when the nucleoside is going to be attached to the phosphate, then it is also going to be called as nucleotide.

So, when the attached with phosphate, then it is actually going to be called as nucleotide. So, nucleotide monophosphate, nucleoside diphosphate, nucleotide triphosphate. Now, nucleotide has the free hydroxyl group at the, if the nucleotide has a free hydroxyl group at three prime carbon and a phosphate group at the five prime of the sugar. This is what is going to show. So, it has actually the five prime phosphate and actually going to have the three prime OH.

And that actually provide some kind of the orientation or polarity into the molecule. So, the first nucleotide has the free phosphate group and the three hydroxyl group which are going to make the bond with the phosphate group at the five prime of the next nucleotide. So, this one is actually going to make a bond with the phosphate group by the subsequent nucleotides. And that will continue. So, that is why you are actually going to have the five prime free phosphate group on one end.

And when it ends, then you are actually going to have the three prime free OH groups at the other end. And that is why it actually provides the polarity into the molecule. So, the propagation of the nucleotide along the length of the chain give rise the poly nucleotide. As a result of each poly nucleotide chain has the free five prime phosphate group on first nucleotide and the free three prime hydroxyl group on the last nucleotide. So, this is the first nucleotide on which you are actually going to have the five prime free phosphate group because it is not attached to anybody.

So, this phosphate group is free. And on the last phosphate nucleotide, this end, this phosphate, the sugar what is attached, it is actually going to have the free phosphate OH group on the three prime OH. It gives the polarity to the poly nucleotide chain and it runs in the direction from the five prime to the three prime. So, because of the simplicity and to make the things more systematic, we actually call this as running from the five prime to the three prime. Because five times you are actually going to have the phosphate group and on the three prime you are actually going to have the OH. Because so basically if you want to extend this growth, you are actually going to extend on this side not on this side because this side it is already been blocked by the five prime phosphate group.

So, DNA is a double standard where RNA is a single standard. This is there are exceptions that RNA could be double standard like some of the plant viruses and the animal viruses. Both strands of the DNA are held together by the hydrogen bonding between the bases attached to the sugar. So, you can have the A, you can have the G, you can have the T, you can have the C and all these are actually having the hydrogen bonding between them. So, adenine of one chain is always making the two hydrogen bonding with the thymine of the next chain. So, on one side if you have the adenine and the other side if you have the thymine, then it is actually going to make the hydrogen bonding of the two hydrogen bonding.

Whereas in the case of the same way if you have the guanine on this side, you can actually have the cytosine on this side. So, it is actually going to make the three hydrogen bonding. So, similarly the guanine of the one chain is making the three hydrogen bonding with the cytosine of the next chain. Now, the question comes why the adenine is making a pair with thymine and why the guanine is making a pair with cytosine. What is unique about this base pairing? It is possible that adenine can make a pair with cytosine and guanine is making a pair with thymine.

But that does not happen because of that there is a strict base pairing that adenine is always making a pair with thymine and guanine is always making a pair with cytosine. So, the question comes why there is such a strict base pairing and that strict base pairing the answer to this strict base pairing comes from their structure itself. So, you know that the adenine is adenine and guanine are the two ring structure and the thymine and cytosine is one ring structure. This means they are small, these are big. Now, you actually so and apart from that the groups what are attached to the adenine and guanine are actually different than the group what is present on the thymine and cytosine.

And you know for hydrogen bonding, it is these kind of stoichiometry and the distances are very important. So, why there is a base pairing of such a strict base pairing that adenine is going to make a pair with thymine and guanine is always making a pair with cytosine. So, question lies within their structure and as well as the groups what are present on to their rings actually. So, adenine or guanine is a purine and it is a 9 membered ring, 9 membered means it is actually having the 2 rings whereas, the thymine or cytosine is a pyrimidine which is a 6 membered ring.

So, this means a 1 ring structure. So, presence of both purines such means which means adenine and guanine right which are bulky actually. So, there are 2 rings right. So, they are actually bulky. So, if you put a peak adenine on one side and guanine on other side, it is actually going to have the steric hindrance. It is actually going to having the not enough

space between the DNA strands right and because of that they will be actually going to be too wide actually.

So, they are actually going to be too wide to get accommodated within the DNA structures right because the distances between the 2 strand of the DNA is going to be very strict right. So, they are not going to vary. In comparison to that for example, if you have 2 pyrimidines for example, the cytosine and thymine, then the cytosine and thymine are very small. So, they will not they will be placed very far away and then they will not actually going to have any kind of hydrogen bonding. So, hydrogen bonding is not going to be possible if the pyrimidine in pyrimidine is present because the pyrimidine is actually going to be with small because it is a single ring structure.

So, they will not be able to interact with each other and because of that the DNA structure is going to be unstable at this particular region right. So, if they will not be able to interact, this region can be broken very easily and it is actually going to form the loops and other kinds of bulbs right. The only combination what is possible is if you have the purine on one side, then you should have the pyrimidine on the other side, which means if you have the adenine on this side, then you can actually have the thymine on this side. So, in that case, the distances are also fine. So, if you have this combination, it is actually going to manage the distance because then the distance between the groups are going to be consistent.

Whether you have the bulky on this side and small one on this side or you have the bulky on this side and the small one on this side does not matter. The distance is going to remain the same. So, that is it is not going to distort, it is not going to destroy the DNA structure. Apart from that, the groups what are going to be present on to the purine is actually going to place the groups in such a way that they are actually going to interact and they are going to form the hydrogen bonding with the groups what is present on to the pyrimidines. So, this way, the most possible combination is that the purine on one side and the pyrimidine on the other side.

This means the amount of purine could be or is actually equivalent to the amount of pyrimidine. This way anyway we are going to discuss in detail. So, if you have the two purines, it is difficult because they are bulky. If you have two pyrimidines, they are small.

So, it will be too small to form the hydrogen bonding. And so the only combination would be that if you have the purine on one side and the pyrimidine on the other side and then it is actually going to have the perfect match for hydrogen acceptor and the donor side, which are present on to the purine and pyrimidine. So, that is why there is a strict base pairing, two chains and that is why the two chains are called as complementary to each other, which

means if you know the sequence on one chain, you can actually be able to predict the structure on the second chain. And that is why they are actually complementary to each other. Now, the question comes, what is complementary mean to you? What is complementary? So, it means that if I will provide you the sequence of nucleotide on the one strand, if I provide you this information about the nucleotide sequence on the strand one, it will let you to predict very precisely the sequence of the nucleotide on the other hand, because A, wherever you have A, it is actually going to interact with T and wherever you have G, it is actually going to interact with C.

Let us take this with an example. So, for every appearance of A, you will actually going to give the T and for every appearance of G, you are going to take the C. Let us take an example. For example, this is the strand one on which you are actually know the sequence, right. And if you want to know the sequence of the complementary strand, so this is going to be the strand number two. Remember that the strands are not only going to be complementary in terms of the sequence, they are also going to be complementary in terms of the polarity.

For example, strand one is running in the direction of 5 prime to 3 prime. This means it is running in this direction. This means the complementary strand should run in this direction. This means the 5 prime is going to be on this side and it will go run in this direction. This kind of information is very important and this aspect and concept is very important for you to understand because it is actually going to be used extensively when we are actually going to discuss about the replication and transcription because that time this complementary information and the concept of complementary is very, very important to understand.

So, let us see. So, strand one, you have the adenine, you have thymine, you have guanine, guanine, cytosine, cytosine. So, the first nucleotide, if you go to the first nucleotide, it is adenine. So, what I will do is I will put the thymine.

If it is thymine, then I will put the adenine. If it is guanine, I will put the cytosine. If it is guanine, I will put cytosine. Cytosine, I will put guanine like that. And that is how you can be able to have the generation of the second strand. And since it is starting from the 5 prime, I will put the 3 prime because as I said, the strands are complementary to each other not only in terms of sequence, but also in terms of the polarity. So, the individual strand of the DNA runs in the direction of 5 prime to 3 prime and on the other strand runs in the direction of 3 prime to 5 prime.

Hence, both strands are running in an anti-parallel direction to maintain the base complementarity. The presence of complementarity in base pairing and running of strand

in the anti-parallel direction allows the precise duplication of DNA through a process known as replication. And this is all we are actually going to discuss in detail when we are going to talk about the replications. So, remember that this is the 5 prime of sugar and this is the strand 1.

And all these are the nucleotides. So, this is the base what is attached and then it is actually interacting with the, so this is adenine interacting with the thymine and this side this is strand 1. So, this is the strand 1 and this is running in from in this direction and whereas this is a strand 2 where it is running in the opposite direction. And remember that if you have adenine on this side, you are going to have thymine on this side. Now, let us talk about some of the rules what is related to this complementarity. So, understanding the base pairing requirement, the Chargaf has proposed a rule about composition of DNA.

Summary of this rule is as follows. Point number 1, the purines and pyrimidines are always going to be in an equal quantity, which means the amount of purines is going to be equal to the amount of pyrimidines. And this is understandable because whatever the amount of purine is going to be present on strand number 1, it is actually going to be present on the same amount of pyrimidine on the other strand. And that is why if you take the composition of the total DNA, the amount of purine is going to be equal to the pyrimidine. Because A is making a pair with T and G is making a pair with C. The amount of adenine is equal to the thymine and the amount of cytosine is equal to the guanine, which means A is going to be equal to the T and G is going to be equal to the C.

Not only that, the base ratio, which means A plus T divided by G plus C may vary from one species to another, but it will remain constant for a given species. And this is a very, very important information because if you calculate the AT by GC ratio, you can be able to say very precisely what is the species of, you can actually be able to identify the species of that particular organism. Because as I said, it will vary from one species to another species, but it will remain constant for a given species. So, he proposed that these ratio can be used to identify these species and you can actually be able to use this information to classify them.

Now, number 4, the deoxyribose sugar and the phosphate component occurs in the equal proportions. Now, the question comes if the DNA is double stranded, how it can be denatured to access the information of the nucleotide sequence. So, DNA double helix can be broken open if it is exposed to the high temperature or titrate with the acid or alkaline. Remember that the DNA strands are attached with each other with the help of the hydrogen bonding. So, this hydrogen bonding can be broken by two things, either you add something which is more polar, such as you change the pH or you add salt.

If you add the salt, the salt will actually interact with the base pairs and it is actually going to break. Other point is if you heat, if you increase the temperature, heat is actually going to break the hydrogen bonding between the bases. During this process, the hydrogen bonding between the two strand breaks and this process is known as the melting or the denaturation of the DNA. When the denatured DNA is incubated at low temperature, the separated strands re-associate to form the duplex DNA. This process is known as the re-naturation and this is very important concept to understand that when you are going to heat in the DNA, the two strands are actually going to be get depart, right, because the hydrogen bonding between the bases is actually going to be broken. So, they will actually going to get separated and when you are actually going to lower down the temperature, it is actually going to re-naturate, right.

And this concept is very effectively being used when you are talking about the technique which is called as polymerase chain reaction. So, the denaturation or re-naturation kinetics is used to understand the complexity of DNA and it has a wide application in amplifying the strand using a technique which is called as polymerase chain reactions. So, DNA denaturation and stability. So, if you do that, what you are going to see here is that it is actually going to give you the fraction of DNA what is present as the double standard versus single standard.

And if you plot this denaturation curve, you are going to get a sigmoidal curve like this. And this actually is actually going to give you the information when the 50% DNA is actually being denatured, which means when the 50% DNA is present in the double standard form versus single standard form. So, that is actually going to give you the TM of that particular DNA and that TM of that particular DNA is actually going to be a very, very characteristic to that particular species. It varies between the species and it also varies, where if the DNA is more complex or the, so it varies in terms of when the complexity of the DNA will actually go up, right. And that is why this particular type of denaturation curve can be used to understand the complexity of DNA without even going through the process of sequencing.

Now, let us talk about how you can be able to isolate the genomic DNA from the cell, right. Because you are actually going to use this information if you want to perform these kinds of experiments like where you are actually going to understand the complexity of DNA and all other kinds of things. So, what you are going to do is you are going to, so we are not getting into the detail of the protocol, like how you are going to put the different types of reagents and all that. What you are going to do is first you are going to lyse the cell with the different detergents containing so that it will actually going to prepare the, lyse the cells. So, you are going to lyse the cells and once you have the lysate, right, so it is actually going to contain DNA, it is actually going to contain the DNA and also going

to contain the protein. And it is also going to contain the minor quantity of lipids, right, because it is going to have the lipids from the plasma membrane.

So, these are the three biomolecules what is going to be present in this particular lysate, right. Then what you are going to do is, and DNA is actually going to be present in complex with protein because you know that the DNA is always making a pair, making a complex with protein because DNA is negatively charged. So, it binds the positively charged histones and that is how it is actually going to be packed within the nucleus. This all we are going to discuss in detail when we talk about or when we discuss about the genomic DNA in detail, right, when we are going to talk about the genetic material. So, then the second step is you are going to do enzymatic digestion. So, you are going to treat the cells with the digestion buffer, right, and the digestion buffer is actually going to contain the protease which is called as protease K and the SDS and it is actually going to release the genomic DNA from the DNA protein complex.

Then you are actually going to precipitate or isolate the genomic DNA by the alcohol precipitation. So, you are going to in the third step, you are going to precipitate the genomic DNA by the absolute alcohol. And after that, you are actually going to get the DNA and as well as protein and as well as the lipids. So, then you are actually going to do the purification step. So, you are going to extract the things with the help of the chloroform and phenol chloroform isomer solutions.

And when you do that, you are actually going to get the two phases. You are going to get the aqueous phase and you are actually going to get the organic phase. In the organic phase, you are actually going to have the proteins whereas the lipids, whereas in the aqueous phase, you are actually going to have the DNA or the genomic DNA actually. And then you can collect this and again, you are going to precipitate the DNA with the help of the absolute alcohol and that is how you are actually going to get the pure mammalian DNA. If you analyze this genomic DNA onto agarose gel, we are not discussing about the agarose gel in this particular course.

What you will see here is that it is actually going to run as an intact band and it will run very close to well actually. This is a well where you have loaded actually. Why it is so because the genomic DNA is very big and it is actually quite. So, it is actually going to be slow, run very slowly. So, genomic DNA is actually going to be analyzed on 0.

28% agarose and a good preparation of genomic DNA gives an intact band with no visible spheres. Now, once you isolated the genomic DNA, you isolated the genomic DNA, you are actually should do the estimation. You should know what is the amount of DNA what you have isolated. So, if you want to isolate the genomic DNA, you have two choices.

One, you can actually have the absorbance at 280 nanometer or the other is you can actually be able to do the colorimetric method.

So, you can actually do the absorbance at 280 nanometer. So, what you can do is you can take the small amount of DNA and then you actually add the buffer. So, you can actually be able to. So, what you do is and you know that the DNA is absorbing very strongly at 260 nanometer and RNA also. So, RNA and DNA both absorbs very strongly at 260 nanometer. So, what you can do is you can take the buffer and first take the absorbance at 260 nanometer and that is going to be the control reading or I will say blank.

So, it is actually going to be considered as zero reading and then what you do is you add the buffer and you add the small amount of DNA. For example, add the 2 microliter of DNA and again, I will take the absorbance. So, it is actually going to show me the absorbance of for example, 0.

15. So, this is absorbance what I got at 260 nanometer and I can thus convert this and get the concentration of DNA. So, that you can do in a spectrophotometer or you can actually be able to use the nanotopes. So, we have prepared a small demo clip and where the students are actually going to show you a small demo clip. So, that you can be able to determine the DNA concentration with the help of the absorbance at 280 nanometer. Today, we are going to estimate DNA concentration using UV visible spectroscopy. One of the most common methods for DNA concentration detection is the measurement of solution absorbance at 260 nanometer due to the fact that nucleic acids have an absorption maximum at this wavelength.

For this experiment, we need DNA for standard curve preparation, distilled water, DNA sample of unknown concentration, micro pipettes, tips, cuvette and spectrophotometer. According to this table, we will prepare different concentrations of DNA solution for the standard curve. After preparing different concentration of DNA solution, we will measure the absorbance at 260 nanometer using spectrophotometer.

We will take the absorbance of the blank. Now, we will take the absorbance for the 5 microgram per ml concentration. These are the absorbance values. From the absorbance value, we have plotted the graph and we have got the regression equation. Our absorbance for unknown sample was 0.478 and this value is the value of the Y.

Substituting the Y value in the regression equation and solving it will give the X value which is our unknown concentration that is 22.319 microgram per ml. Now, the second method is you can actually be able to do the DNA estimation with the help of the chloromyrtic method and that method is called as estimation of DNA by the diflamine

reactions. So, diflamine is a chloromyrtic reagent when it reacts with DNA. So, if it reacts with diflamine, it is actually going to give you the blue colored complex and that blue colored complex you can actually be able to give you the absorbance and that is actually can be used for determining the total DNA content what is present.

Now, the question comes why we are doing this instead of the absorbance at 260 nanometer. The answer to this question is that the absorbance at 260 nanometer is a quick method and it actually gives you the quite reliable results, but it is not very, very quantitative. It will not going to give you the absolute correct answer and that is why there is a chloromyrtic method in case you want to verify because if you are doing at 260 nanometer absorbance, there are other molecules which also can contribute into the reactions. So, what is the principle of the diflamine reactions? So, the deoxyribose, the sugar part in DNA is in the presence of acids forms the beta hydroxy linoleumaldehyde, which reacts with the diflamine to give a blue colored complex which is shot of absorbance at 595 nanometer. In DNA only the deoxyribose of the purine nucleotide reacts.

So, the value what you are going to obtain represent the half of the total deoxyribose. So, what you have is you have a DNA, DNA has pyrimidines and the purines also. So, when you are going to put them into the acidic reactions, it is actually going to have the purines and then you also going to have the sugar part. This sugar in the presence of sulfuric acid, it is actually going to react with the diflamine to form the beta hydroxy linoleumaldehyde. And then the beta hydroxy linoleumaldehyde is actually going to react with the diflamine and the diflamine is actually going to give you the blue colored complex and this blue colored complex is going to absorb very strongly at 595 nanometer.

What are the metal logic? The material requires, you actually requires the spectrophotometer and a water bath. So, you require a boiling water bath. Remember that you actually require a water bath which actually can maintain 100 degree Celsius. Then you require the chemicals, you require the standard DNA solutions, diflamine reagents, DNA sample. You require the citrate buffers, you require the acetic acid, concentrated sulfuric acid and ethanol.

The glassware, you require a test tube, pipettes and graduated cylinder. Then the procedures, so you are actually going to prepare the reagents. So, you are going to prepare the diflamine reagents and while you are preparing the diflamine reagents, remember that these are the reagents where you are actually going to have the glycolytic acid and you are also going to have the concentrated sulfuric acid. So, you should be taking very careful, very lot of cares and the reagent has to be stored in a dark glass bottle. So, on the day of use, prepare a fresh solution of methanol and you are going to add the things into like 1 ml of methanol in the 50 ml of water and you add the 0.

5 ml of the solution to each 100 ml of the diflamine reactions. You have to always be very cautious because you are actually going to deal with the concentrated sulfuric acid and concentrated glycolytic acid. So, you always use the eye wear protection and you use the fume cupboard for these reagents. And diflamine reagent is also very harmful.

So, if ingested or inhaled, may irritate the skin or eyes and it comes out into the contact with them. Now, we are going to set up the assay. So, you are going to prepare a series of dilution of a standard DNA starting from the 0.25 mg per ml stock in a saline-seated buffer to give a concentration of 50 to 500 microgram per ml. You prepare all the sample in duplicates, then to 2 ml of each of these blank, standard and unknown, you add the 4 ml of diflamine reagent and mix. Tube 1 is used as a blank and tube 2, 2, 7 are used as a concentration of a standard calibration curve, whereas tube 8 to 11 are for the unknown samples.

This anyway you are going to see in the table, right. Then you incubate all the tubes into a boiling water bath for 10 minutes, cool the temperature and read the absorbance at 500 nanometer. Then you can actually be able to make a calibration curve of the absorbance at 500 nanometer versus the consideration of the DNA. And this is a table, this is the recipe table what you are going to use.

So, the first reaction is actually the blank reaction where you have not added the DNA. So, this is the minus DNA reactions. So, this is actually going to be the blank. So, whatever the reaction you are going to, whatever the values you are going to get that has to be subtracted from this value, right. So, this is the value what you are going to get, right. This is the average of this and this value has to be subtracted and when you do that, you are actually going to get, this is the corrected absorbance value at 525 nanometer.

And these are the standard reactions what you are going to use, right. And these are the unknown samples. So, DNA what is present in the unknown samples. And then using these reactions, using these values, you can be able to make a calibration curve. And using this calibration curve, you can be able to determine the DNA concentrations into the unknown samples. So, this is the standard curve what you are going to prepare where you are going to have the corrected absorbance value onto the y-axis, right. This is the y-axis and the concentration of the DNA or amount of DNA onto the x-axis. And then you are going to get the calibration curve and you can actually be having two options, either you use the equations and you can be able to determine the concentration of the unknown samples, right.

Or you can actually be able to use this calibration curve to determine the unknown

samples. So, to show you all these, we have prepared a small demo and with this small demo, you can be able to understand how to prepare these reactions and what are the different places where you are supposed to take the precautions. Because you are dealing with the corrosive samples. So, you should be very, very careful.

Hello everyone. In this video, we are going to demonstrate how to estimate the concentration of DNA using the diphenyl amine method. The basic principle in this method is that the deoxyribose in DNA of the purine nucleotide in the presence of sulfuric acid going to form beta-hydroxy-levinol-dehyde. That in turn going to react with the diphenyl amine forming a blue colored complex with absorbance at finite different nanometer. So, here the material which will be requiring are the standard DNA solution prepared in citrate buffer of 250 microgram per ml. The diphenyl amine reagent, the saline citrate buffer, the test tubes for the standard curve preparation of 6 standards, 3 unknowns as well as the buffer blend.

And also we have been needing the hot water work as well as the spectrophotometer for the absorbance. So, coming to the procedure to prepare the standard curve, we need to add the known concentration of DNA in each of the standard tube. So, in standard 1, we will be adding 50 microgram of DNA, in standard 2 100 microgram of DNA, in standard 3 200 microgram of DNA, in standard 4 200 microgram of DNA, in standard 5 400 microgram of DNA and in standard 6 500 microgram of DNA. So, to add this particular concentration of DNA in each of the test tube we need to we have already have the standard DNA solution of 250 microgram per ml. So, for 50 microgram of DNA to be added in standard one we need to add 200 microliter of the standard DNA solution with 1800 microliter of water into the standard one test tube. So, likewise for 100 and 200 and for 100 200 200 like that we will be adding the DNA non DNA concentration along with the distilled water to make up the volume 2 by ml in each of the standard test tube.

And now we will be adding the known concentration of DNA into each of the standard test tube. So, for the standard one we will be adding 200 microliter of standard DNA solution of 250 microgram per ml to make it into a a known concentration of 50 microgram. And we will be adding the distilled water to make up the concentration of 2 ml. Likewise for other standard tubes with the known concentration of DNA we will be making up to 2 ml in each of the standard tubes. Now, after preparing the known concentration of the DNA in each of the standards along with the unknown now we will be adding the 4 ml of DPA reagent to each of the test tube including the gland.

Likewise we are going to add to all of the standards as well as the unknowns. So, we will be adding the DPA reagent to each of the test tubes to make up a total volume of 6 ml in each of the test tube. Now, we have added the DPA reagent of 4 ml each in all of the test

tubes. Now, after adding we are going to incubate all of the test tubes in hot water bath that we are going to put it for 10 minutes. After incubating the samples for 10 minutes at 100 degree centigrade now we need to let the samples to cool down to room temperature.

Now, we could see the blue coloured complex formed in the standards as well as in the unknowns. So, taking from the standard 1 to 6 we could see as the concentration was increasing the intensity of the blue colour was also increasing. Now, to record the absorbance we need to check at finite defined nanometre in the spectrophotometer. Now, we are going to measure the absorbance at finite defined nanometre using the spectrophotometer. So, this is the spectrophotometer device where we are having two cuette holders one is for the blank other is for the test samples.

So, first we need to set the absorbance at finite defined nanometre and I am going to take the blank in one of the cuette and place it in the cooler. So, we are going to take the standard one in another cuette and place it in the another cuette holder. This would be constant and for the standards we would be changing from second third to third is the unknown samples. Now, it is been measuring. Here are the absorbance values taken twice for each of the sample including the unknowns to reduce the error percentage by taking the average of two values.

The last column gives us the corrected OD after nullifying the blank from each of the cuette and unknown samples. Now, by plotting the standard curve with absorbance on y axis and quantity of DNA on x axis we have determined the unknown concentration of DNA using the equation of slope. Here the obtained concentration is usually half since the purine nucleotides only forms the blue coloured complex after reaction with the diphenyl amine reagent in the presence of strong acid.

So, doubling the obtained concentration for each unknown gives us the actual concentration of DNA. Like for unknown one it is around 0.4 microgram. So, by doubling it you get around 0.8 microgram. So, this is all about the DNA. We have some more aspects what is related to the deoxyribonucleic acid which we are going to discuss in our subsequent lectures. Thank you.