Cell and Molecular Biology
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Week 09
Central Dogma of Molecular Biology (Part 1)
Lecture - 31
Structure of DNA (Part 1)

Hello everyone, this is Dr. Vishal Tevedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And what we were discussing was the basic properties of a biological system. So in this context, we have discussed the structure of the biological cells. So we have discussed the structure of the prokaryotic cell and the structure of the eukaryotic cell. While we were discussing the structure of eukaryotic cells, we also discussed the structure of the different organelles, their functions, and their contribution to running the various activities within the cell.

And then we also discussed how the cell is actually dividing and what the different stages it is actually going through are. So we have discussed the cell cycle and how the cell cycle is tightly regulated. While we were discussing the cell cycle, we also discussed some of the technical as well as experimental aspects, and how you can study the cell cycle with the help of flow cytometry. In addition to that, we have also discussed how you can study mitosis and meiosis with the help of different types of microscopy techniques.

And then at the end, we have also discussed apoptosis and cell death. And there we also discussed the different types of pathways. So we have discussed the intrinsic pathway as well as the extrinsic pathway; what are the different molecular players that are actually participating in these types of different pathways? And we have also seen how we can actually study these events in the limit cell with the help of flow cytometry. So, let us first start our discussion about DNA, right? So, DNA is a nucleic acid, right? It is deoxyribonucleic acid. So, it is deoxyribonucleic acid.

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

DNA or RNA is a biopolymer, and it is acidic in nature; that is why nucleic acid is acidic in nature. In eukaryotic cells, whether animal or plant, the nucleic acid is present within the nucleus. In prokaryotic cells, it is present as a free form in the cytosol, and we have discussed the differences between prokaryotic and eukaryotic cells, one of the major differences being that the nucleic acid is present within the nucleus in the case of eukaryotes. Whereas it is going to be present outside as a free form in the cytosol, the first nucleic acid was isolated by a scientist known as Frederick Mixture in the year 1868. And since then, we have actually been discovering new properties of this particular molecule.

Now, what is the composition of the DNA? Right, so the nucleic acid is composed of three components. You can have the phosphoric acid, the base, and also the sugar. The phosphoric acid provides the backbone to the polymer, whereas the sugar works as the anchoring point for the nitrogenous bases. The nine-member nitrogenous bases give diversity to 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: we can have ribose in the case of RNA, or we can have 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 actually have 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 that belong to the pyrimidines, or you can have the nitrogenous bases that belong to the purines.

Forget about talking about the components. So, phosphate backbone. So phosphoric acid itself is the backbone of the molecule. So you can have two different chains. One is the phosphate backbone on this side.

The other has the phosphate backbone on this side. Then you can have the sugar, too. So you can have the 5-membered sugar; it can be either ribose or deoxyribose. So, in the DNA, you can have deoxyribose where the 2 prime OH is missing. So, the 5-membered cyclic reducing sugar is present in nucleic acid.

These are the two different variants. The sugar molecule which contains the hydroxyl group at the 3 prime position is known as ribose. Whereas it is deoxyribose if it is present, okay. Based on the sugar, the nucleic acid is classified as either RNA or DNA.

Ribose sugar is present in RNA, whereas deoxyribose sugar is present in DNA.

The purpose of sugar in nucleic acid is to provide the attachment point for the nitrogenous bases. So this is a sugar, and it is actually going to have the attachment point so that you can have the nitrogenous bases attached to the 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's how it is going to be the same way it is going to be on this side, and that's how they are actually interacting with each other. This anyway we are going to discuss in detail when we discuss the base pairing and other kinds of things. And then the nitrogenous base, so there are two variants: there is a nine-membered conjugated double bond system, and there are two variants; one is purine, the other one is called pyrimidine.

Purines such as adenine and guanine are part of the six-membered single-ring system called pyrimidine, which includes thymine, uracil, and cytosine; adenine, on the other hand, is a nine-membered ring. And then you also have another nine-membered ring which is called guanine, so these are the nine-membered rings, or I will say two-ring bases. 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 thymine, cytosine, or uracil, okay? Whereas for the purines, there are two rings, and for the pyrimidines, there is one ring, okay? And the presence of nitrogenous bases in DNA and RNA is predetermined.

For example, the DNA has adenine, guanine, thymine, and cytosine, and it does not contain uracil, okay? Whereas, RNA has adenine, guanine, uracil, and cytosine, and strictly no thymine. Now, the question arises: if you have the adenine, thymine, guanine, and cytosine, why is there a strict base pairing? Which one will actually make the best pair with the others and so on? So, what is the rule about having the best pairing between these nitrogenous bases? The DNA is double-stranded, while RNA is single-stranded, right? In most cases, RNA can be double-stranded in some plant viruses and other special organisms that actually have double-stranded RNA, but mostly the double DNA is going to be double-stranded. The individual monomer responsible for making DNA or RNA is a nucleotide. And as a result, the DNA or RNA can be considered as polymers. Similarly, just as you know that sugar is the polymer of the glucose molecule, you can also have DNA, which is a polymer of nucleotides, and that is why it is called polynucleotides.

In a polynucleotide, you can see what is present in the individual nucleotide. So, an individual nucleotide has a nucleoside attached to one or more phosphate groups, and if it is attached to one phosphate group, then it is called a monophosphate nucleoside. If it is attached to the two phosphate groups, then it is called a diphosphate nucleoside. And if it

is conducted to the 3-phosphate group, then it is called triphosphate nucleotides. This is what it is actually going to show.

So if the sugar is attached to the base, then it is called a nucleoside, okay? So if the sugar is plus base, then it is called a nucleoside, okay? Now, if a nucleoside is attached to one molecule of phosphate, then it is going to be called nucleoside monophosphate, or I would say nucleotide. Then, if it is attached to the two phosphate groups, it is going to be called nucleoside diphosphate, or if it is attached to the three phosphate groups, it is going to be called nucleoside triphosphate. And the base is attached to the sugar molecule with the help of a glycosidic bond. If the 2 prime OH is present, then it is going to be called ribose. If the 2 prime OH is absent, then it is going to be called DLC ribose.

Each nucleoside is composed of a nitrogenous base attached to the sugar through glycosidic bonds. So, when the nucleoside is going to be attached to the phosphate, it is also going to be called a nucleotide. So, when it is attached to phosphate, it is actually going to be called a nucleotide. So, nucleotide monophosphate, nucleotide diphosphate, nucleotide triphosphate. Now the nucleotide has a free hydroxyl group at the 3' carbon and a phosphate group at the 5' carbon of the sugar, right? This is what is going to show, right? So, it actually has the 5 prime phosphate and is actually going to have the 3 prime OH, right, and that actually provides some kind of orientation or polarity to the molecule, right.

So, the first nucleotide has the free phosphate group and the three hydroxyl groups which are going to make the bond with the phosphate group at the 5' prime of the next nucleotide. So, this one is actually going to form a bond with the phosphate group by the subsequent nucleotides, right? And that will continue, right? So, that is why you are actually going to have the five prime free phosphate group on one end. And when it ends, you are actually going to have the three prime free OH groups at the other end. And that is why it actually provides polarity to the molecule. The propagation of the nucleotide along the length of the chain gives rise to the polynucleotide.

So, this is the first nucleotide on which you are actually going to have the 5-prime free phosphate group because it is not attached to anybody, right? So, this phosphate group is free, right? And on the last nucleotide, this phosphate, the sugar that is attached, right? It is actually going to have the free phosphate OH group, right? On the 3-prime OH. It gives polarity to the polynucleotide chain and runs in the direction from the 5' prime to the 3' prime. So, because of the simplicity and to make things more systematic, we actually call this running from the 5 prime to the 3 prime because at the 5 prime you are actually going to have the phosphate group. And on the 3 prime, you are actually going to have the OH because, basically, if you want to extend this growth, you are actually going to extend on

this side, not on this side, because this side is already being blocked by the 5 prime phosphate group. So, DNA is a double strand while RNA is a single strand.

This is because there are exceptions where RNA could be double-stranded, 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, right? So you can have the A, you can have the G, you can have the T, you can have the C. And all these actually have hydrogen bonding between them. So adenine in one chain is always making two hydrogen bonds with the thymine in the next chain. So on one side, if you have adenine and on the other side, if you have thymine, then it is actually going to create the hydrogen bonding between the two.

Whereas in the same way, if you have the guanine on this side, you can actually have the cytosine on the other side. So it is actually going to make three hydrogen bonds. So, similarly, the guanine of one chain is forming three hydrogen bonds with the cytosine of the next chain. Now, the question arises as to why adenine pairs with thymine and why guanine pairs with cytosine. What is unique about this base pairing, right? It is possible that adenine can pair with cytosine and guanine is pairing with thymine, right? But that doesn't happen, right? Because of that, there is a strict base pairing in which adenine always pairs with thymine and guanine always pairs with cytosine.

So, the question is why there is such a strict base pairing, and the answer to this strict base pairing comes from their structure itself, right? So, you know that adenine and guanine are the two-ring structures, right, and thymine and cytosine are one-ring structures. Right, this means they are small; these are big. Okay, so apart from that, the groups that are attached to the adenine and guanine are actually different from the group that is present on the thymine and cytosine, and you know for hydrogen bonding, right? Um, these kinds of stoichiometry and the distances are very important. So why is there such a strict base pairing that adenine pairs with thymine and guanine always pairs with cytosine? So the question lies within their structure as well as the groups that are present on their rings, actually, right? So adenine or guanine is a purine, and it is a nine-membered ring. Nine-membered means it actually has two rings.

Whereas, thymine or cytosine is a pyrimidine, which is a 6-membered ring. So, this means the 1-ring structure is okay. So, the presence of both purines, adenine and guanine, which are actually bulky. So there are two rings; therefore, they are actually bulky. So if you put adenine on one side and guanine on the other side, it is actually going to have steric hindrance.

It is actually going to have not enough space between the DNA strands. And because of

that, they will actually be too wide to be accommodated within the DNA structures because the distances between the two strands of the DNA are going to be very strict. So they are not going to vary in comparison to that; for example, if you have two pyrimidines, the cytosine and thymine, then the cytosine and thymines are very small. So they will not be placed very far away, and then they will not actually have any kind of hydrogen bonding. So, hydrogen bonding is not going to be possible if pyrimidine is present because it is actually going to be small due to its 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 in this particular region, right? So, if they are not able to interact. This region can be broken very easily. And it's actually going to form the loops and other kinds of bulbs, isn't it? The only combination that 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 fine as well. So if you have this combination, it is actually going to manage the distance because then the distance between the groups is going to be consistent.

Whether you have the bulky one on this side and the small one on this side, or you have the bulky one 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 that are going to be present on the purine are actually going to place the groups in such a way that they will interact and form hydrogen bonds with the groups that are present on the pyrimidines. So, this way the most possible combination is that there is a purine on one side and a pyrimidine on the other side.

This means the amount of purine could be or is actually equivalent to the amount of pyrimidine, right? Anyway, we are going to discuss this in detail, right? 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 hydrogen bonds. So the only combination would be that if you have the purine on one side and the pyrimidine on the other side, then it is actually going to have the perfect match for the hydrogen acceptor and the donor side, which are present in the purine and pyrimidine. So, that is why there is a strict best pairing of two chains and that is why the two chains are called complementary to each other.

Which means if you know the sequence on one chain, you can actually predict the structure on the second chain, and that is why they are actually complementary to each other. The question is, what does complementarity mean to you, right? What is complementarity, right? So, it means that if I provide you with the sequence of

nucleotides on one strand, right, if I give you the information about the nucleotide sequence on strand 1, it will allow you to predict very precisely the sequence of nucleotides on the other strand because 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. Right, let us take this with an example. So for every appearance of A, you are actually going to give T, and for every appearance of G, you are going to take C.

Let's take an example. For example, this is strand one on which you actually know the sequence, right? And if you want to know the sequence of the complementary strand, this is going to be 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 1 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 run in this direction. This kind of information is very important, and this aspect and concept are very important for you to understand because they are actually going to be used extensively. When we are actually going to discuss replication and transcription, this complementary information and the concept of complementarity are very, very important to understand. So, let us see. So in strand 1, you have adenine, thymine, guanine, guanine, cytosine, cytosine.

So, if you go to the first nucleotide, it is adenine. So what I will do is put the thymine. If it is thymine, then I will put adenine. If it is gone in, I will put in the cytosine. If it is gone in, I will put in cytosine.

If I see cytosine, I will put it in like that. And that is how you can generate 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 polarity. So, one individual strand of the DNA runs in the direction of 5 prime to 3 prime, while the other strand runs in the direction of 3 prime to 5 prime. Both strands are running in an antiparallel direction to maintain base complementarity.

The presence of complementarity in base pairing and running of strands 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 talk about the replications. So, remember that this is the 5' prime of sugar and this is strand 1, right? And all of these are the nucleotides. So, this is the base that is attached, and then it is actually interacting with it. So, this is adenine interacting with thymine, and on this side, this is strand 1.

So, this is strand 1, and it is running in this direction, right? Whereas this is strand 2, where it is running in the opposite direction, okay. And remember that if you have adenine on this side, you are going to have thymine on this side. Now let's talk about some of the rules related to this complementarity. So understanding the base pairing requirement, Chargaff proposed a rule about the composition of DNA.

The summary of this rule is as follows. Point number one: the purines and pyrimidines are always going to be in equal quantities, 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 one, it is actually going to be present in 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 that of pyrimidine. Because A is pairing with T and G is pairing with C, the amount of adenine is equal to the amount of thymine. And the amount of cytosine is equal to the amount of guanine, which means A is going to be equal to T and G is going to be equal to C, right? 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 very, very important information because if you calculate the AT by GC ratio, you can say very precisely what the species is; you can actually identify the species of that particular organism. Because, as I said, you know, it will vary from one species to another, but it will remain constant for a given species. So he proposed that these ratios could be used to identify these species and that you could actually use this information to classify them. Now, number four, the deoxyribose sugar and the phosphate component occur in equal proportions. Now, the question arises: if the DNA is double-stranded, how can it be denatured to access the information of the nucleotide sequence? So, the DNA double helix can be broken open if it is exposed to high temperature or titrated with acetyl alkylate.

Remember that the DNA strands are attached to each other with the help of hydrogen bonding, right? So, this hydrogen bonding can be broken by two things. Either you add something that is more polar, such as changing the pH, right? Or do you add salt, right? If you add the salt, it will actually interact with the base pairs and is actually going to break. Another point is if you heat, right? If you heat it, increasing the temperature will actually cause the heat to break. Break the hydrogen bonds between the bases. During this process, the hydrogen bonding between the two strands breaks, and this process is known as the melting or denaturation of the DNA.

When the denatured DNA is incubated at low temperatures, the separated strands re-

associate to form duplex DNA. This process is known as renaturation. And this is a very important concept to understand: that when you are going to heat the DNA, the two strands are actually going to depart, right, because the hydrogen bonding between the bases is actually going to be broken. So, they are actually going to get separated. And when you are actually going to lower the temperature, it is actually going to re-nature, right? This concept is being used very effectively when you talk about the technique called polymerase chain reaction.

So, the denaturation or renaturation kinetics is used to understand the complexity of DNA, and it has a wide application in amplifying the strand using a technique called polymerase chain reactions. So, DNA denaturation and stability. So if you do that, what you're going to see here is that it is actually going to give you the fraction of DNA that is present as the double strand versus single strand. And if you plot this denaturation curve, you are going to get a sigmoidal curve like this, and this information is actually going to give you the information about when 50% of the DNA is being denatured. Which means when the 50% DNA is present in the double-stranded form versus the single-stranded form, 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 very, very characteristic of that particular species. It varies between the species, and it also varies in terms of when the complexity of the DNA will actually increase, right? And that's 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's talk about how you can 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're going to do is, we are not getting into the details of the protocol, like how you are going to add the different types of reagents and all that. What you're going to do first is lyse the cells with the different detergents so that it will actually prepare the lysed cells.

So you're going to lyse the cells. And once you have the lysate, it's actually going to contain DNA. It's actually going to contain the DNA and also the protein. And it's also going to contain a minor quantity of lipids, right, because it's going to have the lipids from the plasma membrane. So these are the three biomolecules that are going to be present in this particular lysate, right? Then what you're going to do is that DNA is actually going to be present in a complex with protein because you know that DNA is always forming a complex with protein since DNA is negatively charged. So it binds to the positively charged histones, and that's how it's actually going to be packed within the nucleus.

This is all we are going to discuss in detail when we talk about the genomic DNA and the genetic material. So then the second step is that you're going to do enzymatic digestion, treating the cells with the digestion buffer, and that digestion buffer is actually going to contain the protease called protease K and the SADS, which will actually release the genomic DNA from the DNA-protein complex. Then you are actually going to precipitate or isolate the genomic DNA using alcohol precipitation. So in the third step, you're going to precipitate the genomic DNA with absolute alcohol. And after that, you are actually going to get the DNA, as well as the protein, and the lipids.

So, you are actually going to do the purification step. So you're going to extract the things with the help of chloroform and phenol-chloroform SOML solutions. And when you do that, you are actually going to get both phases. You are going to get the aqueous phase, and you are actually going to get the organic phase. In the organic phase, you will actually have the proteins or the lipids. And then you can collect this, and again you are going to precipitate the DNA with the help of absolute alcohol, and that is how you are actually going to get pure mammalian DNA.

If you analyze this genomic DNA on agarose gel, we are not discussing agarose gel in this particular course. What you will see here is that it is actually going to run as an intact band, right? And it will run very close to well, actually. This is a well where you have actually loaded. Why is it so? Because the genomic DNA is very big and it is actually quiet, right? 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 smears. Now once you have isolated the genomic DNA, you should actually do the estimation. You should know what the amount of DNA you have isolated is, right? So, if you want to isolate genomic DNA, you have two choices. One, you can actually have the absorbance at 280 nanometers. Or the other is that you can actually be able to do the colorimetric method, right? So you can actually do the absorbance at 280 nanometers.

So what you can do is take a small amount of DNA and then actually add the buffer, right? So you can actually be able to, so what you do is, and you know that the DNA is absorbing very strongly at 260 nanometers and RNA also, right? So RNA and DNA both absorb very strongly at 260 nanometers. So what you can do is take the buffer and first measure the absorbance at 260 nanometers, and that will be the control reading, or I would say the blank, right? So it is actually going to be considered a zero reading. And then what you do is add the buffer and add the small amount of DNA.

For example, add the two microliters of DNA. And again, I will take the absorbance. So it is actually going to show me the absorbance of, for example, 0.15, right? So this is the absorbance that I got at 260 nanometers. And I can just convert this to get the concentration of DNA, okay? So that you can use a spectrophotometer or actually be able to use the nanotropes. So we have prepared a small demo clip where the students are actually going to show you how to determine the DNA concentration with the help of the absorbance at 280 nanometers.

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 nanometers. Due to the fact that nucleic acids have an absorption maximum at this wavelength, for this experiment we need DNA for standard cup preparation, distilled water, a DNA sample of unknown concentration, micropipette tips, a cuvette, and a spectrophotometer. According to this table, we will prepare different concentrations of DNA solutions for the standard curve. After preparing different concentrations of DNA solution, we will measure the absorbance at 260 nanometers using a spectrophotometer.

We will take the absorbance of the blank sample. Now we will take the absorbance for the 5 microgram per ml concentration. These are the absorbance values. From the absorbance values, we have plotted the graph and obtained the regression equation.

Our absorbance for the unknown sample was 0.478, and this value is the value of y. Substituting the y value into the regression equation and solving it will give the x value, which is our unknown concentration of 22.319 micrograms per ml. Now the second method is that you can actually do the DNA estimation with the help of the chlorometric method, and that method is called the estimation of DNA by the diphenylamine reaction. So diphenamine is a chlorometric reagent; when it reacts with diphenamine, it actually gives you the blue-colored complex, and that blue-colored complex gives you the absorbance, which can be used to determine the total DNA content present. Now, the question arises: why are we doing this instead of measuring the absorbance at 260 nanometers? The answer to this question is that the absorbance at 260 nanometers is a quick method.

And it actually gives you quite reliable results. But it is not very quantitative. It will not give you the absolute correct answer. And that's why there is a colorimetric method in case you want to verify, because if you are measuring absorbance at 260 nanometers, there are other molecules that can also contribute to the reactions. So, what is the principle of beta-flamin reactions? So, the deoxyribose, the sugar part in DNA, is the presence of acid that forms the beta-hydroxylinolaldehyde, which reacts with the

diphenylamine to give a blue-colored complex with a shock of absorbance at 595 nanometers. In DNA, only the deoxyribose of the purine nucleotides reacts.

So the value that you're going to obtain represents half of the total deoxyribose. So what you have is a DNA. DNA has pyrimidines, right? And the purines, right? So, when you are going to put them into the acidic reactions, right, it is actually going to have the purines, and then you are also going to have the sugar part. This sugar, in the presence of sulfuric acid, is actually going to react with diethylamine to form the beta-hydroxyliuminoaldehyde, right? And then the beta-hydroxyliuminoaldehyde is actually going to react with the diethylamine, and the dipylamine is actually going to give you the blue-colored complex, and this blue-colored complex is going to absorb very strongly at 595 nanometers. What is metallurgy, right? The material requires that you actually use the spectrophotometer and a water bath.

So you require a boiling-water bath. Remember that you actually require a water bath that can maintain 100 degrees Celsius. Then you require the chemical. You require the standard DNA solutions, diatomine reagents, and DNA sample.

You require the citric buffer. You require acetic acid, concentrated sulfuric acid, and ethanol. The glassware you require includes a test tube, pipettes, and a graduated cylinder. Then the procedures: you are actually going to prepare the diethylamine reagents, and while you're preparing them, remember that these are the reagents where you will actually have the glacial acetic acid and the concentrated sulfuric acid, so you should be very careful. 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 one 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 diaphragm in the actions. You always have to be very cautious because you are actually going to deal with concentrated sulfuric acid and concentrated glycolic acid. So, you always wear the eyewear protection and use the fume cupboard for these reagents. The diphylamine reagent is also very harmful. So, if ingested or inhaled, it may irritate the skin or eyes, and it comes into contact with them.

Now we are going to set up the assay. So you are going to prepare a series of dilutions of a standard DNA starting from the 0.25 mg per ml stock in a saline citrate buffer to give a concentration of 50 to 500 micrograms per ml. You prepare all the reaged samples in triplicate. Then, to 2 ml of each of these blanks, standards, and unknowns, you add 4 ml of digramine reagent and mix. Tube 1 is used as a blank, and tubes 2 through 7 are used as a consideration of a standard calibration curve, whereas tubes 8 to 11 are for the unknown samples.

This is anyway you are going to see in the table, right? Then you incubate all the tubes in a boiling water bath for 10 minutes, cool the temperature, and read the absorbance at 595 nanometers. Then you can actually make a calibration curve of the absorbance at 595 nanometers versus the concentration of the DNA. And this is the table; this is the recipe table that you are going to use. So the first reaction is actually the blank reaction in which you have not added the DNA.

So this is the minus DNA reaction. So this is actually going to be blank. So whatever the reaction you are going to have, whatever the values you are going to get, that has to be subtracted from this value, right? So this is the value that you are going to get. Right. That is the average of this, and this value has to be subtracted. And when you do that, you are actually going to get the corrected absorbance value at five hundred twenty-five nanometers.

And these are the standard reactions that you're going to use. Right. And these are the unknown samples. So DNA, which is present in unknown samples, and then using these reactions and values, you can make a calibration curve. And using this calibration curve, you can determine the DNA concentrations in the unknown samples. So this is the standard curve that you're going to prepare, where you are going to have the corrected absorbance value on the y-axis. This is the y-axis and the concentration of the DNA or amount of DNA is on the x-axis, and then you are going to get the calibration curve. You can actually have two options: either you use the equations to determine the concentration of the unknown samples, or you can use this calibration curve to determine the concentration of the unknown samples.

So, to show you all this, we have prepared a small demo, and with this demo, you will be able to understand how to prepare these reactions and what the different places are where you are supposed to take precautions because you are dealing with 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 diphenylamine method. The basic principle in this method is that the deoxyribose in A of the purine nucleotide, in the presence of sulfuric acid, is going to form beta hydroxy levonol dihyde, which in turn is going to react with diphenyl amine, forming a blue-colored complex with an absorbance at 595 nanometers. So, the materials that will be required are the standard DNA solution prepared in citrate buffer of 250 micrograms per ml, the diphenylamine reagent, and the saline citrate buffer. The test tubes for the preparation of the standard curve for the three unknowns, as well as the one for the blend, and the spectrophotometer for the absorbance, have also been needed.

So, coming to the procedure to prepare the standard curve, we need to add the known concentration of DNA to each of the standard tubes. So in standard I, we will be adding 50 micrograms of DNA; in standard II, 100 micrograms of DNA; in standard III, 200 micrograms of DNA; in standard IV, 300 micrograms of DNA; in standard V, 400 micrograms of DNA; and in standard VI, 500 micrograms of DNA. So, to add this particular concentration of DNA in each of the test tubes, we need to use the standard DNA solution of 250 micrograms per ml that we already have. So for 50 microgram of DNA to be added in standard I 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 100 200 300 like that we will be adding the DNA known DNA concentration along with the distilled water to make up the volume 2 ml in each of the standard test tube now we will be adding the known concentration of DNA into each of the standard test tubes. 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 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 cubes with the known concentration of DNA, we will be making up to 2 ml in each of the standard cubes. 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 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 color 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 color was also increasing now to record the absorbance we need to check at 595 nanometer in the spectrophotometer so this is the spectrophotometer device where we are having two cuvette holders one is for the blank other is for the test samples so first we need to set the absorbance at 595 nanometer and i'm going to take the blank in one of the cuvette and place it in the holder next we are going to take the standard one in another cuvette and place it in the another cuvette holder this would be constant and for the standards we would be changing from second third to till the unknown samples now i will be 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 standards 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 colored 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. 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.