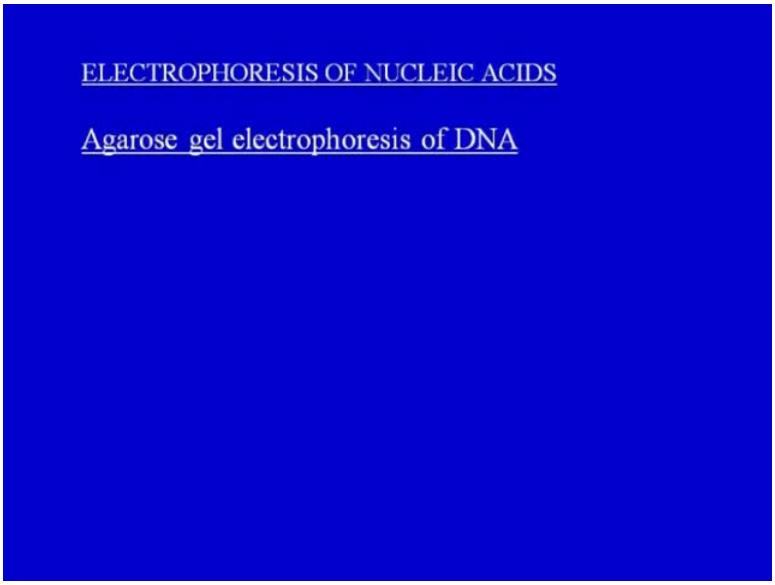
Analytical Technologies in Biotechnology
Prof. Dr. Ashwani. K. Sharma
Department of Biotechnology
Indian institute of Technology, Roorkee

Module - 4
Electrophoresis
Lecture - 5
Electrophoresis of nucleic acids

In previous lecture, we have discussed about the electrophoresis of proteins. We have discussed about various techniques used in the analysis of protein molecules or polypeptides, which may be smaller. If you could recall, we have talked about STS page electrophoresis, which is a form of discontinuous cell and developed by lamellae. We have talked about iso electric focusing, where pi value is taken for separation and proteins in the electrophoretic gel. They focus on a particular ph, which equals to the pi of a protein or amphoteric molecule. Also we have discussed about 2d gel electrophoresis, the most widely used one combines isoelectric focusing and STS page. There the whole protrusion could be analyzed like 2 particular systems, which could be native and deceased. If you are looking for expressed protein, it could be analyzed.

(Refer Slide Time: 01:45)



Now, in today's lecture, in this lecture we are going to discuss about electrophoresis of nucleic acids. Here we are going to discuss various techniques, which are used for in the analyses of nucleic acids. Now, nucleic acids mean DNA or RNA; mostly it is the DNA,

which is utilized. Now, nucleic acids could be separated by both polyacrylamide gel electrophoresis and agarose gel electrophoresis.

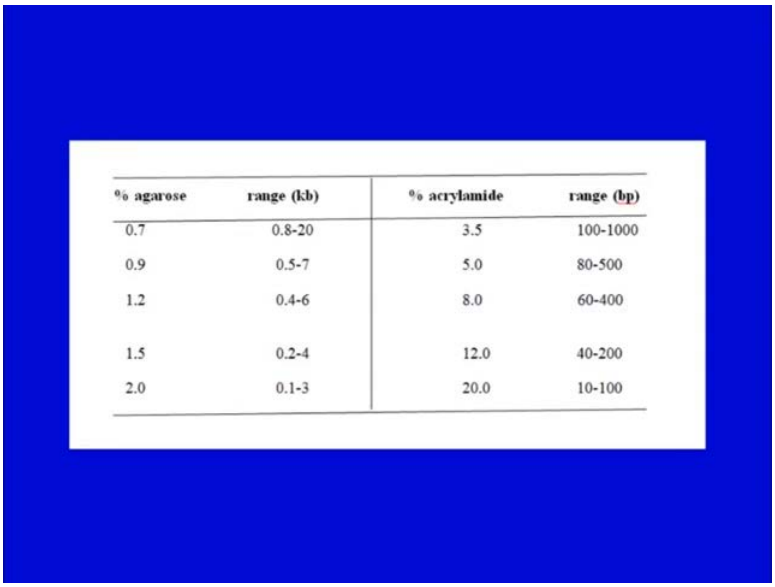
Now, agarose gel electrophoresis is commonly used method to separate nucleic acids and polyacrylamide gel. As we have discussed in previous lectures that polyacrylamide gel is utilized for smaller fragments of DNA or small sized DNA analyzed on polyacrylamide gels, so the agarose gel electrophoresis is most common method used. In agarose gel electrophoresis, nucleic acids that are DNA or RNA both could be analyzed. This we have also discussed in the horizontal gel electrophoresis.

So, the negatively charged DNA molecule or the phosphate backbone of the nucleic acids gives them a uniform charge to mass ratio. Therefore, migration of DNA or RNA in gel is inversely proportional to the nucleic acid. The structure of the nucleic acid, for example, it could be linear, it could be circular, double stranded, single stranded. So, all these structures of the nucleic acids will also affect the migration in the gels.

Now, it is possible to distinguish various DNA fragments that differ by as little as one percent and in some applications. For example, DNA fragments where it is mostly done on polyacrylamide gels, fragments that differ in size by single nucleotide can be resolved. So, the wide range of sizes could be analyzed on this gel electrophoresis.

It could range from less than 10 base spheres to 20 over the 20 kilo base spheres. Agarose is the most common matrix. As we discussed earlier for the electrophoresis of nucleic acids, this is mostly done in horizontal electrophoresis set up. So, nucleic acids ranging in size from say 100 base sphere size to 20 kilo base spheres can be resolved depending on the those concentration. If you could recall when we were discussing horizontal gel electrophoresis, you can go from 0.7 percent to say 3 percent, agarose can be taken. Now, acrylamide, which we will discuss in little bit in DNA sequencing gel is used for only smaller size nucleic acids and oligo nucleotides.

(Refer Slide Time: 05:08)



The percentage of agarose or acrylamide and the fragments could be separated. If you could pay attention here, 0.7 agarose can, 7 percent agarose gel can fractionate or separate say from 0.8 to 20 kb. Likewise, up to 2 percent agarose can give you 0.1 kb to 3 kb separation.

So, depending on like as you increase the concentration of pore, side will be decreased. The size of the DNA, which is separated, will also be smaller as compared to one with 0.7 percent where pore size will be much larger. Now, in acrylamide, the pore size will be much smaller as compared to agarose gel. You can see 3.5 percent acrylamide could be used for only say very small like 100 to 1000 base spheres only. When you increase the percentage of acrylamide say to 5 percent or and up to say 20 percent, you can see the size of the oligo nucleotides, which can be separated also becomes smaller. At 5 percent, it is 80 to 500 base spheres. At 12 percent, it is around 40 to 200 base spheres. Likewise as per the pore size, these fragments will be separated.

So, the larger the pore size, larger fragments will be separated. Smaller the pore size, smaller the pore size that is higher the concentration of the matrix, then smaller size fragments can be separated. So, the agarose gel are usually pored and electrophoresised in an apparatus. This horizontal apparatus provides more support for the gel. It is easier to prepare if you call because in vertical fashion, these agarose gels most of the time,

they break or they are not able to be in vertical positions. So, best way to run them is horizontal position.

Now, shape as we said also affects the migration of nucleic acids in the gel. So, like structure as we have seen like, it could be linear, it could be relaxed circular. All those things will affect as well as shape will also affect. For example, RNA and single stranded DNA can form secondary structures due to internal base sphering and this can certainly affect.

So, these secondary structures will lead to a lower mobility. So, different factors will affect the mobility of the DNA and the gel. Now, the secondary structures or this base sphering could be eliminated by running the gels under the denaturing conditions. For example, you can use 7 or 8 molar urea even. You can have elevated temperatures for running these gels, it will prevent the formation of base spheres in RNA or single stranded DNA.

(Refer Slide Time: 08:19)

This is typical electrophoresis apparatus for horizontal, a typical horizontal gel electrophoresis apparatus for running the agarose gels. As you can see that here tank buffer is put and there will be two electrodes, which will be one positive and negative. The STS shape gel is placed and agarose gel is placed here. Then sample will be loaded and this we have discussed. So, the wells are created and the sample is loaded. The DNA will run towards the positive electrode.

Now, lots of different agents, denaturing agents are utilized such as urea form amide or formaldehyde is included in the gel buffers for the electrophoresis of single stranded DNA or RNA. If size is needed to be accurately determined, one has to make sure that there is no internal base sphering from secondary structures or double stranded sort of a structures, which can hamper determination of their sizes accurately. Now, samples are prepared by mixing DNA in a buffer solution that contains sucrose or glycerol or it is phicol. Now, this makes the solution dense.

As we have discussed in protein gels also many times, dense solution in form of glycerol or other things is put in so that it will sink or settle down at the bottom of the well without diffusing. You do not have to really remove the buffers. You can directly load the samples into the well where the buffer is overlaid on to the gel. It could be tae or tbe buffer. Then certain dyes such as bromophenol blue are also included in the sample.

So, it makes easier to see the sample that is being loaded. It also can be marker of the electrophoresis front and same if you could remember was used in polyacrylamide gel. Now, once the system has been run, the DNA and the gel need to be stained and visualized. So, there could be different fluorescent dyes could be utilized for visualizing DNA. The most widely used fluorescent dye is dye is aecidium bromide.

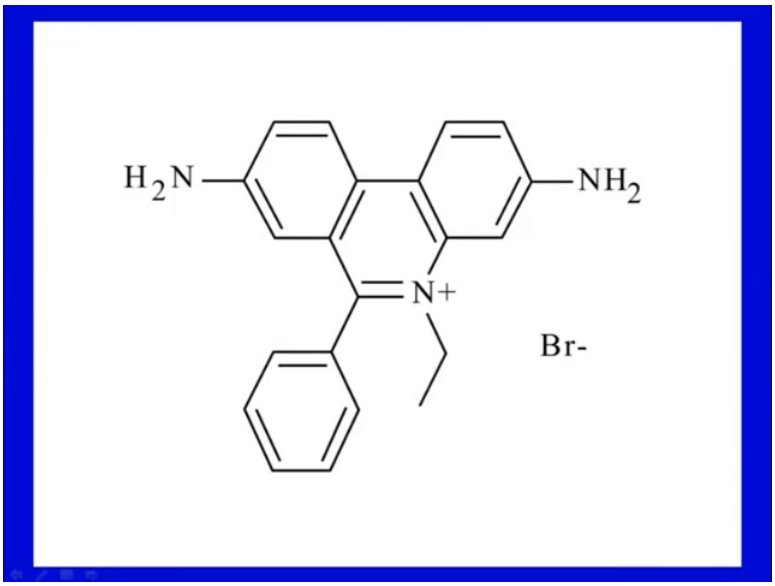
Now, aecidium bromide is the cyclic plainer molecule that binds or intercollegiate between the place of DNA and aecidium bromide concentration. Therefore, it builds up at the site of the DNA bands. Under UV light, the DNA bands fluorescents as orange red. So, you can see them very clearly. Now, there are 2 ways you can stain or you can do the visualizing of DNA. One is that aecidium bromide can be copolymerized with the agarose gel. That is when you are preparing the agarose solution; you can put the aecidium bromide in it. It is in the gel when you have polymerized it.

Other method could be that you can run the agarose gel first. Then you can put the gel into aecidium bromide solution. So, it will be stained after certain period of time. Then the extra aecidium bromide can be washed off with water. Then the gel could be visualized under UV light. So, both ways it could be done. One important thing is that when you run, when you put copolymerized aecidium bromide aecidium, bromide runs in opposite direction.

So, many times for the bands, which run faster on other side of the gel towards the anode, they will be hard to see. So many times, we have to add little bit aecidium bromide tank buffer on the other electrode. So, it should be noted that aecidium bromide can be harmful. If you are doing extensive viewing of the DNA with UV light that can result in the damage of the DNA by nicking and base sphere damagisation. Aecidium bromide could be harmful. It is a cartogen. So, it should be handled with care.

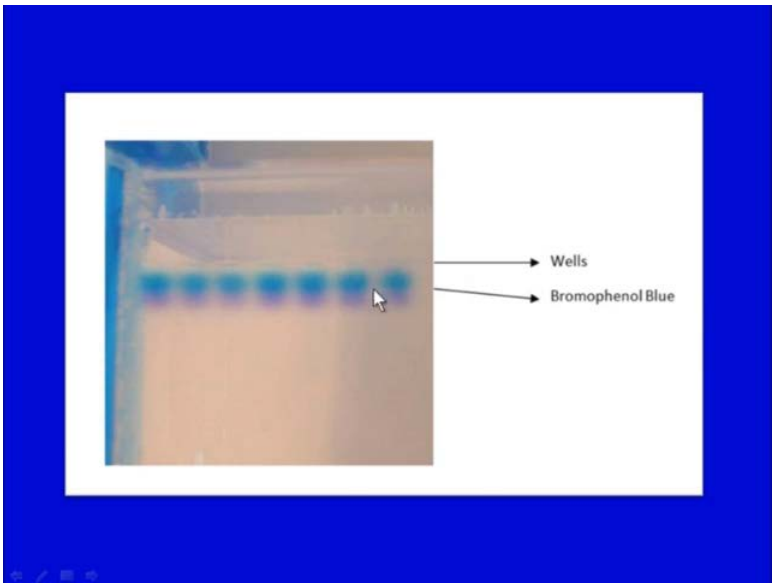
When you have this aecidium bromide stained gels or aecidium bromide solution, it should be it should be properly disposed off after treatment. It should not be directly thrown into the sink. That is very important that in doing these experiments that you take proper care and you do not harm yourself or others.

(Refer Slide Time: 13:18)



So, this is a typical structure of aecidium bromide where you have cyclic cleaner structure with bromine.

(Refer Slide Time: 13:29)



When you load your samples, these are wells where you have loaded samples. Bromophenol blue can be seen and can run these samples. When they run towards positively charged electrodes, they have a negative charge with phosphate backbone. Now, DNA can also adjust. There could be different structures. It could adjust as a circular molecule as in plastids. It could be further more circular. DNA can adjust extend multiple confirmations that is it could be relaxed, it could be super coiled.

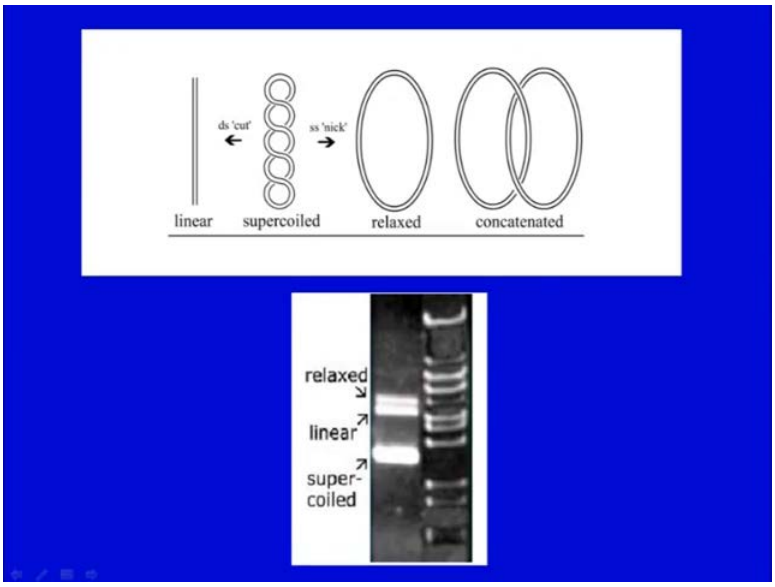
Now, if it is super coiled, it is having super helical twists. When you bake a single bone in the backbone of one strand, it results in relaxed circular DNA molecule. If you bake both strands, it will result in linear like you do it through restriction digestion. So, super coiled circular DNA, if you compare these three that is super coiled circular relaxed circular and linear DNA and there are of the same molecular weight, they will migrate at different rates through the gel.

So, if you compare them, typical order of the migration will be from fastest to slowest. That super coiled DNA will run faster than linear DNA and the relaxed circular DNA will run slowest. So, what you can see is that the shape, the size, the structure, so what you can see is this the structure of the DNA molecule will certainly affect its migration. How about the exact migration is influenced by the agarose concentrations? Also, the applied current, the ionic strength and number of super helical twists, so lot of factors do

affect. In general, it is super coiled DNA will run faster than linear DNA and circular DNA.

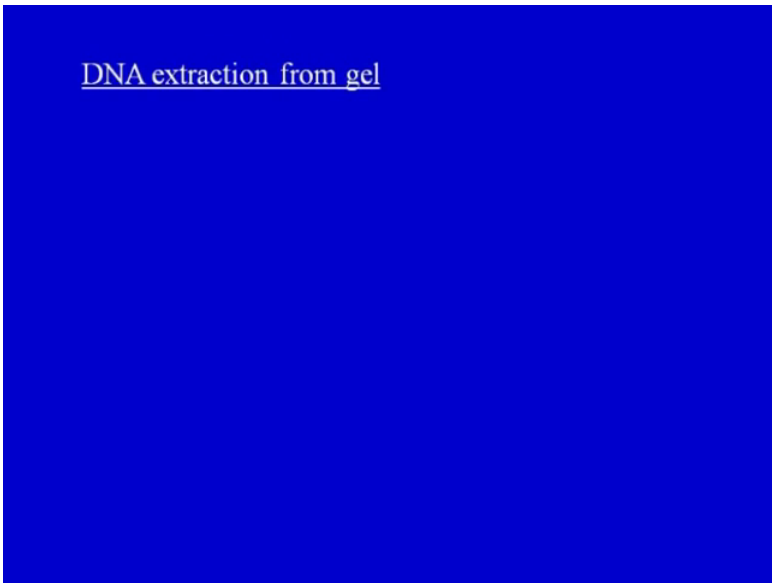
Furthermore, it could happen that circular DNA can form dimmers or trimmers or concatenated form. Multiple bands are seen where you have highly purified preparation, but you are seeing those because of these diammers and triammers. Now, a complete digestion with restriction and endonucleus, which only cuts once within the plasmid, will result in the formation of single band corresponding to the linear DNA molecule.

(Refer Slide Time: 16:10)



Now, if you see here in this figure, if these are linear DNA, this is an example of super coiled DNA. This is example of circular relaxed DNA. These are what you call concatenated or dimmers formation. Here, if you see the pattern of running, you can see that super coiled runs faster linear than linear and the slowest is the relaxed one. So, this is the pattern how they will run on agarose gel. As we have seen, this is how DNA molecule could run and separate on the agarose gel.

(Refer Slide Time: 16:55)



Now, DNA could be extracted also from the gel. If you could recall, we were talking about preparatory gel electrophoresis in the last lecture and recovery of proteins as we have seen recovery of proteins. Likewise, DNA could also be extracted or recovered from the gel. Now, the methods are same here. What you can do is you can have passive diffusion or electro illusion could be done. One way is that dialysis bag can be placed into an electrophoresis chamber and the dialysis bag contains this cut out from agarose.

Now, when we apply the current, the DNA fragment will migrate out of the agarose, but will not go out of the dialysis bag. So, then the DNA is recovered from the dialysis bag by precipitation or other methods. So, that way, you can drive the DNA fragment out of the agarose gel. Another method involves dissolving the exercised agarose gel pieces with say sodium iodide or other chemicals or silica resins, which binds. DNA is added to the dissolved gel solution and following several washes, the DNA is eluted from the resin from low ionic strength.

Then, one can use low melting temperature agarose gels. What happens is when DNA fragment is exercised and gel is melted, the temperature below 60 degree Celsius will really have an adverse effect on the DNA. So, low melting gels are very good way to recover the fragments of DNA material and fragment is then recovered from the solution by ethanol precipitation. So, that could be done.

So, you can melt the gel first. Then you can recover the DNA. So, DNA is the very commonly or routinely used method to recover DNA, pure DNA from the agarose gels, particularly from low melting agarose gels. So, this was about the agarose gels or DNA separation on agarose gels. Now, there are lots of other techniques, which are also employed for DNA analyses. One is DNA sequencing gels.

Now, DNA sequencing gel have been widely used earlier and like the final, so when you do DNA sequencing, there was lots of processing lot of steps to be performed like one has to restriction digest a DNA molecule to generate fragments. Then those fragments are sequenced. Smaller fragments are sequenced by labeling them and by cutting them at specific bases, so that you generate array of partial array of fragments. They are kind of separated by one nucleotide. They differ in one nucleotide where array of fragments could be run on the electrophoresis gel and analyzed.

So, in DNA sequencing method, the final analysis usually involves separating single stranded DNA molecules, which are shorter than about 1000 nucleotides. They differ in size by only one nucleotide. Now, remember nowadays, these methods may be less used because automated sequencing method and new generation methods have come up. They are much more automated and much more efficient and fluorescent based where directly data could be collected. Till DNA sequencing gels are been widely used, it is one method where electrophoresis is used.

So, in chemical sequencing method or in enzymatic method, starting material is the fragment of DNA. What is done is the DNA fragment, which is obtained after restriction digest is labeled at one end. This end labeled fragment particular in chemical subjected to four chemical treatments wherein each one of these fragments backbone is preferentially broken adjacent to one particular base, so four reactions where four types of fragments will be generated. They are like preferentially breaking the fragments at a particular base. You have lot of fragments generated at a particular base.

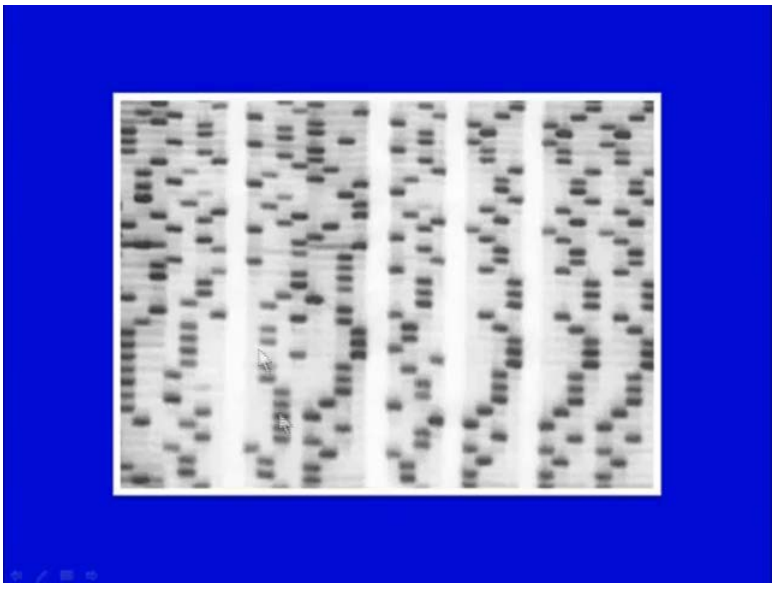
Now, enzymatic methods like dioxi methods utilize polymer chain reactions to generate different fragments. Again, four reactions are there here where you have dioxy counterpart for each of the base. Lot of fragments is generated when this particular modified base is incorporated in the sequence during polymer chain reaction. Now, both sizes generate different chain reactions different mostly single nucleotide.

So, to analyze these small fragments, it is necessary to have small pore gel as compared to agarose gel where the pore size is larger. So, here polyacrymalide gels are used here. Mostly 3.5 percent to 5 percent polyacrylamides gels are used to separate DNA in the range of 82,000 are nucleotides and 12 percent gels. As I have shown you, the table could resolve the fragments from 20 to 100 nucleotides.

Now, since it is necessary to separate DNA molecules that are very similar in size, DNA sequencing gel stands to be very long like say from 50 to 100 centimeters to maximize the separation achieved. These gels are much thinner. Also, they may be almost 4 millimeter or less thinner. They are run in tbe buffer containing 7 molar urea may be other denaturing agents could also be added.

This is because the single stranded DNA should not form base sphere or form secondary structures. For that reason, they are also run at limited temperatures. So, these combinations, the base sphering are avoided. They are run at 50 to 70 degree Celsius. Now, the gels are run. You will find a pattern like this auto radio graph.

(Refer Slide Time: 24:07)

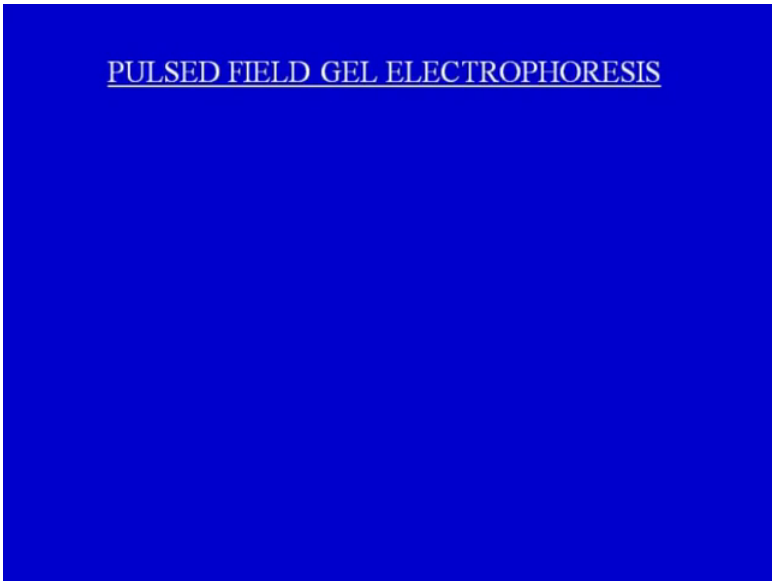


What is done is once the gel is run, then the gel will be wrapped in a particular wrapping paper. Then once it is wrapped, then it is exposed. The x ray film will be exposed because it is labeled. These are labeled fragments. These will expose the fragments. We have discussed in auto radiography. Then auto radiograph will be generated.

So, what you will see is that you will see lot of fragments here. These are different. There are different experiments for each base or combination of bases. They will be read and analyzed. The whole sequence will be generated like different fragments are being sequenced. Overlapping sequence can give complete sequence of a larger DNA molecule. Now, after getting that, you see the pattern here or auto radiograph.

This is a very tedious job that one has to set in dark room. Then analyze these gel patterns or bands and even many times, artifacts have to be eliminated to get right sequence. So, DNA sequencing was the widely used method earlier. It was very tedious method. It has been replaced by automated methods, which we are going to discuss in another section. So, if you could recall, we were discussing about agarose gel electrophoresis.

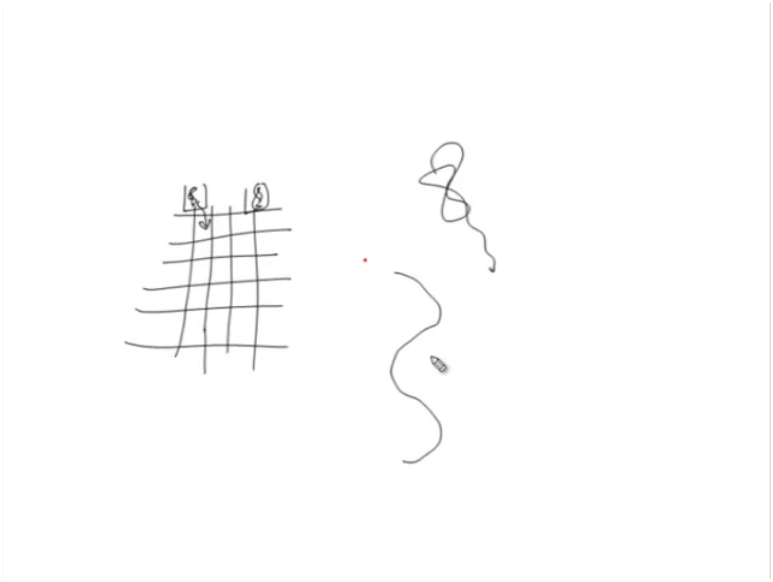
(Refer Slide Time: 25:41)



We said that up to 20 kb fragments or 20 size of DNA of 20 kb can be easily resolved. But, these conventional gel electrophoresis systems have an upper limit of 20 kb size that can be resolved and DNA fragments larger than that are not resolved actually in simple agarose gel electrophoresis. Now, what is the reason? What could be the reason is like under best condition, if you could go to very low agarose concentration, low ionic strengths may be up to 50 kb could be resolved, but still 20 kb is norm. What is the reason? To understand this, there is a process called rapidation or snake like movement through the gel. Now, why 20 kb fragments or the DNA above 20 kb cannot be resolved?

To understand that, we have to little bit understand how the DNA runs in the gel. So, let me show you that on your screen a little bit. So, what happens is like if you could recall when we were talking about the gel, gel is a very random structure. It is not uniform structure. The pores are also not uniform sized pores. It is a mesh work, which is very random structure.

(Refer Slide Time: 27:26)



If I say this is your gel, if it is a mesh work, if I am making it a random structure, now what you do? You load like in here. You load your sample actually. So, one thing has to remember that the DNA in the gel is in the relaxed form. Once it is put in the agarose gel, it is kind of constrained because of the gel matrix up to 20 kb the agarose; the gel interactions are such that the 20 kb fragments could be resolved, but when they reach above 20 kb, then a common phenomenon occurs that we were talking about.

It impedes the motion of the DNA and does not allow the resolving of fragments or DNA molecules above 20 kb. So, what is happening here is when you are apply electric field, then the DNA molecule that is constrained tries to resist the movement actually like it is to enter to the pores. So, what happens is that if you have DNA molecule, suppose this is your DNA molecule. Then one side which may be any side becomes a leading side. Actually, it leads here and it will enter. So, one side will enter into the pore like it will kind of lead and it will enter into the pore.

So, once it enters into the pore, rest of the fragment that is the lagging side, you can say lagging area of DNA will follow. As the electricity is applied, it will start moving. You can compare this movement to snake moving field, which is having bamboo trees arranged in a particular fashion. It has to move in a manner, I think everybody knows about snake moving. It is a rapidation movement. It moves through each of these. It is a sort of this kind of movement is occurred.

So, likewise same movement occurs in DNA here. When you load DNA and when you apply electric field, there is one side of DNA leads and thrust the pore, the rest of the DNA follows. Now, the molecules about above 20 kb, when they move in this agarose gel after very short while, as their leading front goes, rest of the DNA follows. They are entangled into these. You can say mesh work here. They are entangled so much that their movement is impaired. They are not able to move further.

So, even when electricity is or electric field is applied, there is no resolution of the fragments. This is for DNA molecules above 20 kb. So, you have to switch off the electric field. Again, it will relax and reorient. Then it has to move again. So, this problem is the reason why this whole thing that you cannot resolve or normal gel DNA of the size above 20 kb. Now, what is the solution to resolve these large size DNA molecules? One solution is to disrupt the rapidation movement.

Now, in pulsed field electrophoresis, this problem is kind of solved. So, this technique overcomes the problem of resolution of large DNA fragments. What it does is you have to disrupt the rapidation movement. So, it utilizes two sets of electrodes and that are fixed at angle to each other. So what happens? You switch on the electric field in one direction, and then you switch off. Then you switch on in another direction.

So, what will happen? The DNA will reorient first in one direction and then move. As you switch off that and switch on in another direction, it has to move in another direction. So, this particular one exploits the length dependent reorientation time of DNA in electrophoresis. The current is alternating between these 2 sets of electrodes at defined interval. So, here as separation is based on the ability of the DNA molecules to re reorient and then move in the electric applied electric field.

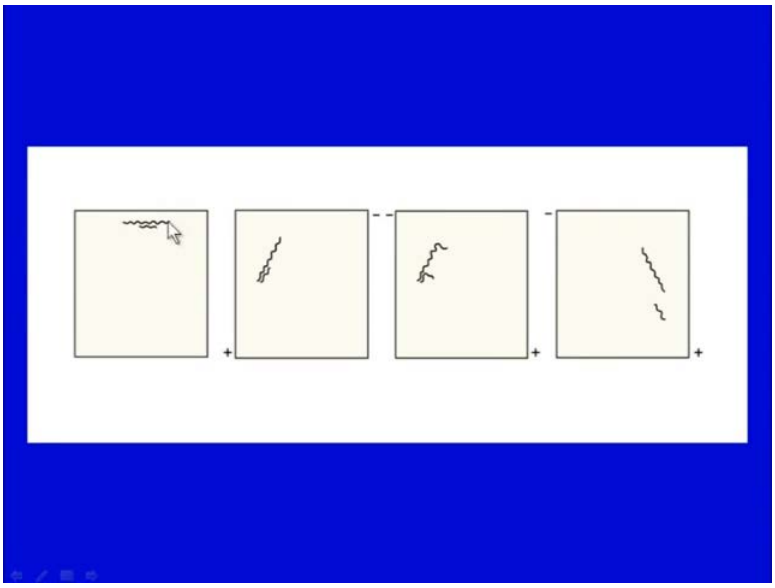
So, what will happen is that you have three kinds. We can divide these large DNA fragments. We are talking about DNA fragments, which is bigger than 20 kb here.

So, if I say small, it does not mean smaller DNA, it is above 20 kb, but smaller in terms that it is smaller than 100 kb or 1000 kb. So, there are smaller, medium sized and large sized DNA molecules and that particular range. So, what will happen is when you switch on and switch off this electric field in different directions, then smaller DNA molecules will reorient faster. They will move also in the applied electric field direction. As you switch off and again switch on another direction, they will move in another direction. They will reorient faster, and then again move in another direction.

So, they will be migrating a little faster. Then there will be medium sized DNA molecules, which will reorient, but they might not move too much as compared to the smaller size. So, they will reorient and move somewhat, but not equal to the smaller sized DNA. That is above certain size. Then there are large DNA molecules, which will spend most of their time in reorientation only. They will not move at all. Now, because of this disruption of this rapidation movement and exploitation of length depended reorientation, different sized DNA molecules above 20 kb could be resolved in these gels.

Now, here almost like million base sphere DNA fragments, DNA molecules could be resolved by pulsed field gel electrophoresis. For example, many genomes of small organisms like yeast have been resolved in this technique. So, many lower eukaryotes, which have chromosomes, say 0.5 to 3 mb sizes could be resolved here. Pulsed field gel electrophoresis provides a means to characterize map genes and chromosomes of these organisms. In higher eukaryotes, this pulse field electrophoresis is used for long range restriction mapping with restriction times that cut frequently. So, lots of applications are there for pulsed field gel electrophoresis.

(Refer Slide Time: 35:12)



If I see this figure here, this kind of shows you that there are two DNA molecules. One is large, another is smaller. As one side in one direction, current is applied. The smaller one moves little faster. Then as you switch on the electric field, then you can see the reorientation is taking place. Then smaller one has migrated ahead of the larger molecules. So, this is how as you shift the field from one direction to other, smaller ones will reorient as well as they will move and migrate faster. This is the basis of separation in pulsed field gel electrophoresis.

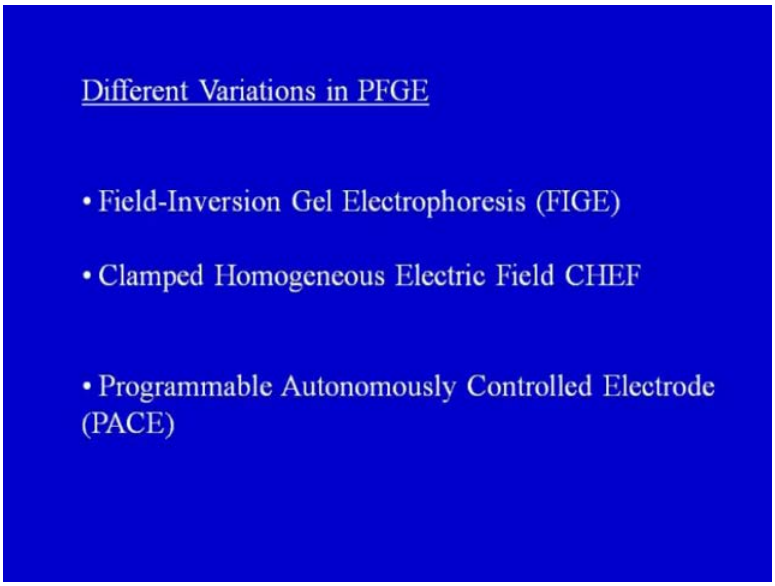
(Refer Slide Time: 35:58)

Now, there are lots of variations of pulse field gel electrophoresis. We will see some of them here. Variations in this technique are based on changing the direction of migration. The differences between the methods are primarily in the position of the electrodes. Remember, the switching time, which we were talking about? The time for which different direction applied electric field is called switching time.

So, you switch on one, then to other. Mostly, when you are switching in 2 directions, you make sure that resulting factor is straight. The whole time and amount of electric field applied is such that they are moving in one direction only. Now, there are lots of different kinds of pulsed field gel electrophoresis, which is like they could be moving back and forth in 180 degree direction. They could be like moving in 120 degrees. They could be lot of other positions of electrodes in different directions.

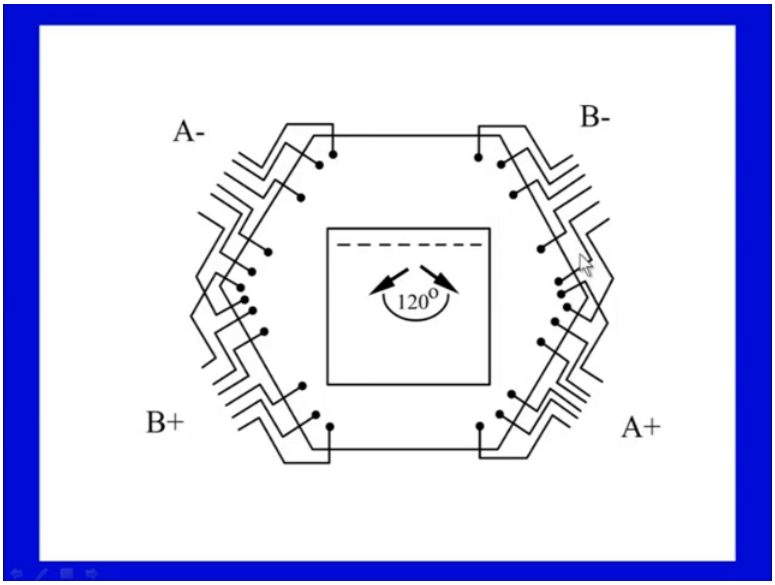
The clamp homogenous electric field is probably the most popular method. Here, in this particular technique, the apparatus is arranged in a hexagonal array. There is a generation of homogenous electric field. I will show you that how do it look like the result in the lanes being straight. It allows for an easy comparison of different samples, though there are directions, two directions, you can load many samples at a time. There is lot of variations, which could be tried.

(Refer Slide Time: 37:47)



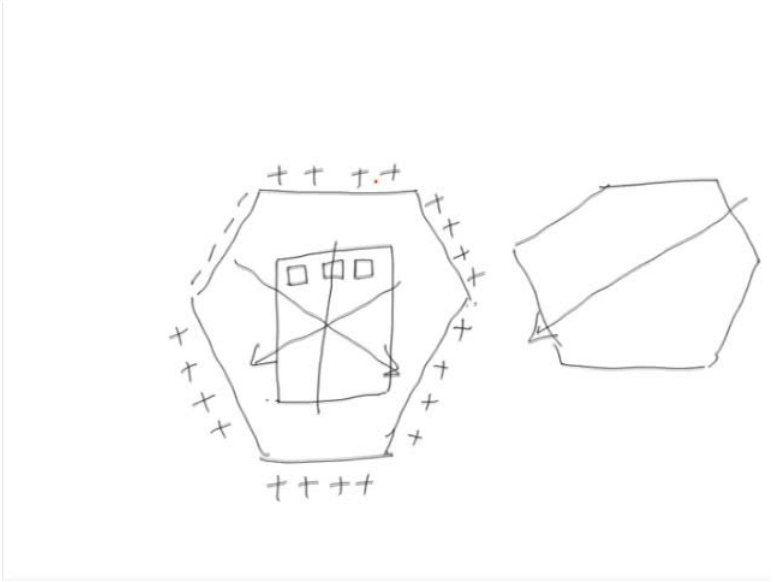
Now, there are different variations in pulsed field gel electrophoresis. There are lots of variations. I have just listed three here that is field inversion gel electrophoresis where the field is applied. The field is switched on and off at 180 degrees. Then it is clamped homogenous electric field where it is at 120 degrees. There is a very advanced system called programmable autonomously controlled electrodes. This could be that you could control each of the electrodes separately and can generate lot of different kinds of variations.

(Refer Slide Time: 38:28)



This is a very common way. This is a hexagonal array of electrode. You can see. Now, here each of this is shown, two directions are here shown. This is a gel placed in electrode pattern. The applied electric field is 120 degrees here, which is like plus 60 minus 60 directions. The resulting movement is in straight line here. Now, let me show you these three variations, which we were talking about on screen. Here, pulsed field gel electrophoresis like I was telling you are a hexagonal array of electrodes. It looks something like this.

(Refer Slide Time: 39:16)

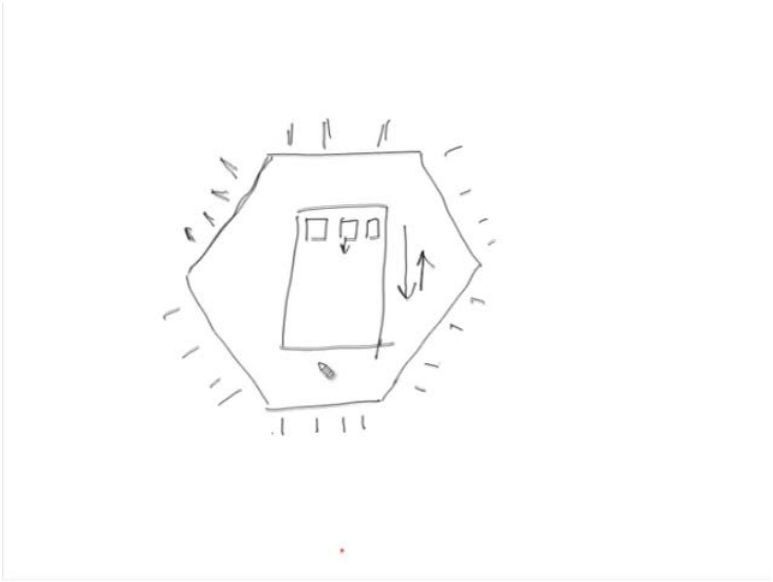


It is not perfect, but I think you can understand that. Now, you have like each leg contains this hexagonal array of electrode arrangement. Here, each leg contains 4 electrodes. I will just make one. These are 4 electrodes. These electrodes are made of platinum wires. So, what you can do is for now, the direction force on the DNA say there is a DNA molecule here.

If there is a gel placed in the centre of this electrode array and the samples are placed in here, then they will be in this direction. Another electrode when you switch on in second direction that is this, if I say from the centre, this is plus 60 degree. There could be another arrangement where you can have this in this direction. That is I can make it here.

So, these will be the negative electrodes. This could be switched on to negative. This will be positive here. So, this is the clamped homogenous gel electrophoresis. You can call chef. So, clamped homogenous pattern here is given where you can change to 120 degrees. There could be other patterns. So, these four electrodes, which are here in say field inversion electrophoresis pattern will be different.

(Refer Slide Time: 41:17)



You have six legs in here. In field inversion pattern, what you have is gel loaded in here and you apply the electric field in two directions. One direction is in this direction and the other direction is in this direction that is at 180 degrees. So, accordingly these four electrodes, which are placed in here, will change. Either they will be negative or they will be positive. So, they will be either negative electrodes or positive electrodes accordingly.

Now, these are four. These are platinum wires. Remember in field inversion gel electrophoresis, what you want is the molecule should move in forward direction. So, always the time and the amount of electric field of the forward direction will be higher than the reverse direction, so that the resultant movement is towards forward movement. That is done in program controllable pattern. Each of these electrodes could be separately controlled. As we see in clamped homogenous or field inversion, what was there is that each leg is controlled separately.

In case of field inversion in case of programmable autonomous control system over, you can control each of these electrodes separately. You can generate various types of variations or directions, in which applied electric field could be applied. So, it is much better. Now, if we compare them, all these techniques field inversion gel electrophoresis, you can separate up to 100 kb fragments or 100 kb DNA molecule.

If you go to clamped homogenous system, then you apply plus 60 minus 60 directions or 120 degrees to each other. Electric field is applied there. You can go up to say 2000 kb. The best results could be obtained up to 7000. It could be extended up to 7000 kb. In programmable autonomous, you can have lot of different variations and that way you can have much better results.

So, these are three variations. There could be many more variations at different angles that could be found where you can vary and you can get best results. You can load many samples. I have shown you since the resultant movement are forward in one direction, so there is no problem. There will not be any mixing or so you can run lot of samples at a time. The factors which affect resolution are one is the uniformity of two electric fields that is very important and you have to remember. For example, in field inversion, the forward electric field has to be higher. Time also has to be higher.

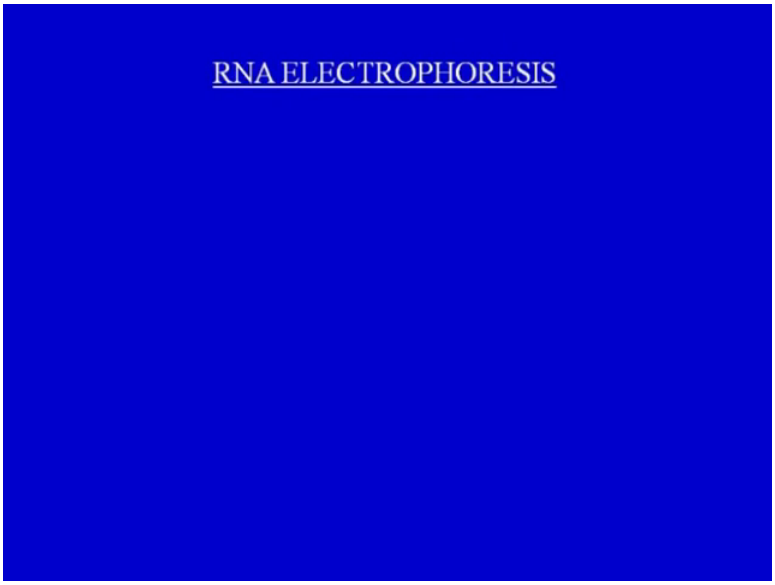
So, all these things have to be taken into account. So, homogenous electric field has to be applied for best results. The lengths of the electric pulses are also important. So, that has to be seen and that has to be standardized. Then the ratio of the lengths of the pulses is important. In effect, the resolution the angles of the two electric fields are also important. The strengths of the electric fields are important. So, all these factors have to be taken into account while running pulsed field gel electrophoresis.

Now, apart from electronics or the automation that has gone into pulsed field gel electrophoresis technique where you can have a lot of different directions, you can pulse the field as such. There is another important thing when we are dealing with such big DNA molecules. These DNA molecules like say genomes and others can easily break. They are very fragile materials and in solutions, there are assure forces which can break these to pieces. Then you will get sphere. You will not get really good bands or good separation. Also, your DNA material is broken here or it is destructed.

So, when you are dealing with very large DNA molecules, then DNA is not allowed to come into the solution, rather what is done is the DNA material is or the organism or the source from where the DNA is being separated or isolated is directly, the whole thing is put into a plug, which is low melting agarose. So, DNA is not allowed to come into the solution. All the processing for example, deproteinization or breaking the cell wall, all those things will be done in that plug only.

The whole plug is then loaded on to pulse field in the slot pulsed field gel electrophoresis in the gel. So, the DNA is not coming out in the solution. The whole thing could be done very nicely. So, that is another important part of running pulsed field gel electrophoresis.

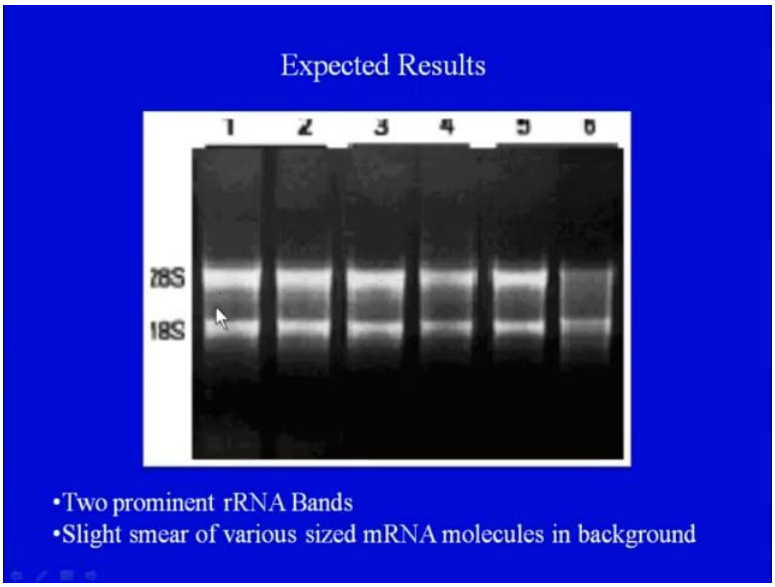
(Refer Slide Time: 47:14)



We come to the last part of this that is RNA electrophoresis. This is also very important, but not as prevalent as DNA electrophoresis. So, like that of DNA, electrophoresis of RNA is also usually carried out in agarose gels. The principle of the separation is based on the size; the same. Often, one requires a rapid method for checking the integrity of RNA, immediately following extraction.

So, RNA is highly susceptible to intra strand x bonding. Secondary structures could be formed, which will affect the migration through the agarose gel. So, what is done is you use denaturing agents or denaturing agarose gel electrophoresis is run; certainly, denaturing agents are put in loading buffer. So, RNA samples could be heated up to 65 degree celsius for 5 minutes prior to electrophoresis and it could be run.

(Refer Slide Time: 48:15)



You could update the results in this form where mostly about what you see is two prominent RNA, r RNA bands are seen which are 28 and 18 s RNA. Then there is a slight smear of various sized mRNA molecules could be seen in here in the background. So, this is how the RNA electrophoresis can be done.

So, this completes this lecture on electrophoresis of nucleic acids. Just to summarize, we have seen how a DNA up to 20 kb could be run on agarose, simple agarose gel electrophoresis fragments above 20 kb cannot be separated or resolved on a simple agarose gel. Pulsed field gel electrophoresis is the technique to be used for analyses where the field is given in pulses in different directions and where process of rapidation is disrupted.

As we have discussed, length dependent orientation of DNA molecule in electrophoresis is exploited, which can give you separation of very large DNA molecules. Even the genomes of small organisms like yeast can be performed there. Then we have discussed about a little bit of about RNA electrophoresis. So, this completes our chapter on nucleic acids separation on electrophoresis. In the next lecture, we are going to discuss about the immune electrophoresis and capillary electrophoresis.

Thank you.