**BioMEMS and Microfluidics**
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**Lecture – 17**

Let us just to could preview before the last lecture of before preceding here.

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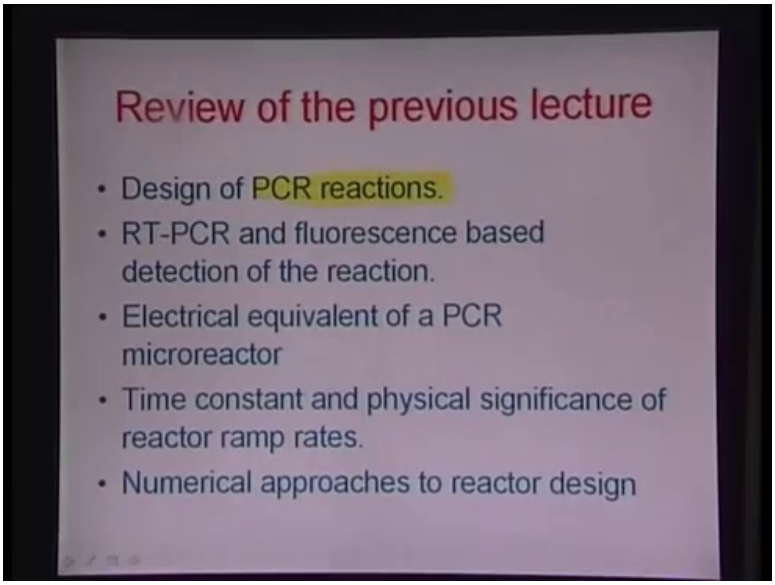


In the last topics covered in the last lecture, we discussed about the very famous PCR reaction, PCR is Polymerase Chain Reactions. So, essentially DNA amplification technique which is done invitro by the use of enzyme and some of these other different reactant like oligonucleotides you know different primers then essentially this reaction takes place on the certain PH condition.

We also talked about real time PCR which is essentially a snapshot by snapshot view of how the amplification reaction proceeds it is essentially done by using fluorescence readout methods, where in some sources are used to either intercalate are used or appeared with some other di-molecule or some quencher molecule in a manner that when the reaction proceeds the distance between the primary fluorophore and the secondary fluorophore of quenchers the changes as a result of which there is an increasing fluorescence you can detect whether successfully the reaction is being carried out or not.

So, real time PCR really gives you an idea of what is the current amplification rate of a certain strand of DNA molecules as the reaction proceeds. So, we also try to design PCR

micro reactors, PCR again because of the rapid thermal cycling issue associated with the process and be very easily translated on to a micro chip level. And the lot of benefits to do such a react one of them being rapidity.

Because, essentially you are using or you are trying to heat up very little mass of a fluid in such a micro chip architecture by going into a thin surface film. So, you are spreading out a volume or a bulk into a thin surface film and essentially are using the surface area for promoting higher heat transfer rates and does quicken the ramp up and ramp down times. So, that the overall cycle time can get reduced, because of that and the PCR reaction happens much more quickly in the micro reactor mode.

So, we talked about developing an RC modeling, where we took thermal capacitance, thermal resistance and try to see by derive in an analogy between the electrical parameters like voltage with physical parameters like temperature, which drives the process. Now, we also looked at how the charge in an electrical circuit can be kind of equivalent to the heat flow in a thermal circle and then try to develop the time constant from circle point of view.

So, essentially at the end of the day the take home message that we had is that really the ramp up ramp down time is independent of the reactor volume, especially at these scales and there is a certain finite time constant which has to be used or spent in order for the reactor to get to a certain level of the temperature that is equals to three times of RC or three times of the time constant value.

So, we tried this numerical approaches on one PCR reactor design example, we will do some more today and then move on to our next topic when we will study a little bit more about how this DNA polymerase within a cell tries to replicate DNA molecules.

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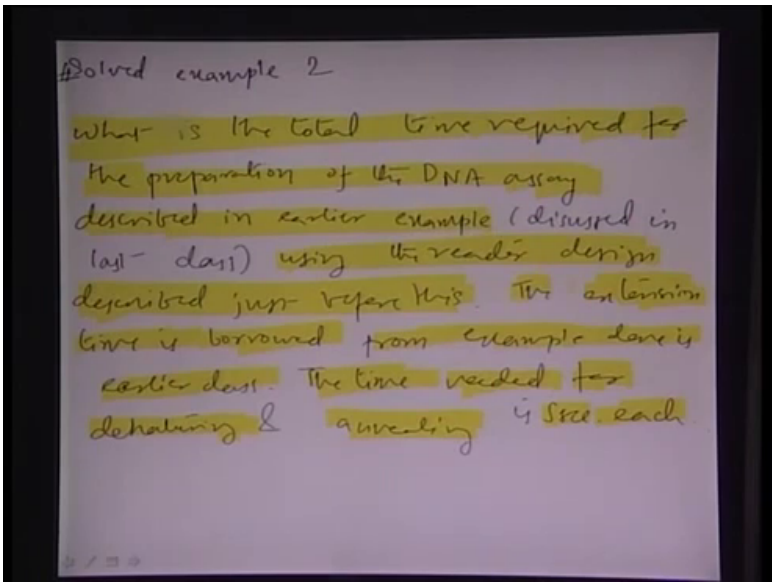


So, let us to another example on designing a whole PCR process by using this time constant. So, in this particular example we want to find out the total time require for sample preparation of this in this DNA assay, not sample preparation really for the whole assay to get completed. So, we assume the constants from the earlier example, where in we already found out that the time constant for the process whose about 0.28 seconds.

Essentially using the reactor design fundamentals we found out that the total time there it would take for the temperature to hit the 90 percent mark would be about three times such time constant, which is a 0.84 seconds. Here, we assume the extension time again from the earlier examples, where we assume that 1000 base pair DNA was really the template DNA, the extension rate of the enzyme was about 50 base pair per second which makes the extension time longest step times that about 20 seconds. We further assume the denaturing and the annealing times to be 5 seconds each and in this case we want to find out the total time needed for the PCR assay.

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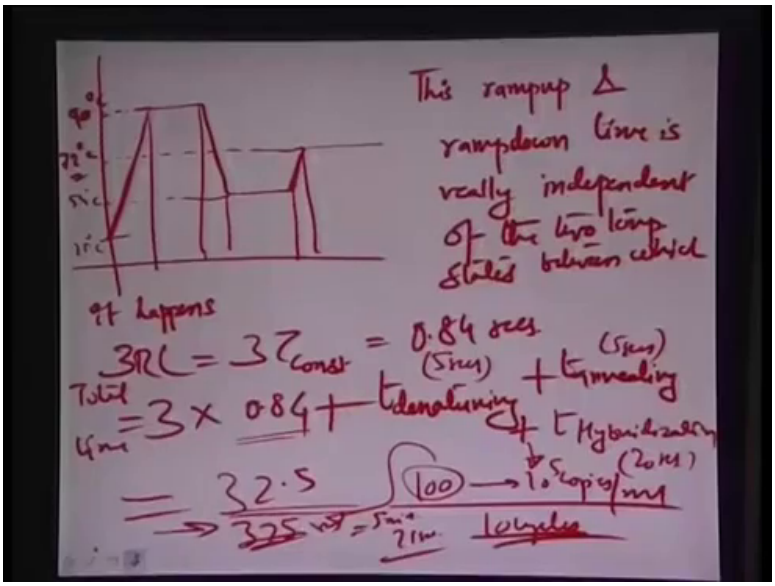


So, let us try solving this problem by assuming a certain time constant of 0.28 has had been indicated before, in this particular case again the total time you know which the total cycle of the PCR could be represented by 3 temperatures step here. Here we go from let say about 25 degree Celsius all the way to about 90 degree Celsius stay here for little bit go down to about 54 degree Celsius stay here for little bit and then again go up all the way to about 72 degree Celsius.

So, in one cycle really you are doing this heating cooling mechanism thrice, this is the first time you are doing in the ramp up, essentially you are ramping down and then again you are ramping up to on the way to about 72 degree Celsius. So, as we know from our last illustration that this ramp up and ramp down time is really independent of the two temperature states between which it should take place between which it happens. So, it is essentially dependent on the reactor volume.

This particular case on the 3 RC or the 3 time constant was found out to be 0.84 seconds and in a whole PCR cycle you are seeing that this time constant has to be executed 3 times, because there is essentially two heating and one cooling steps. So, there is one heating here, one cooling and then there is another heating up here. So, it gets repeated three times this time constant plus you have the independent the extension in healing and denaturing step.

So, you have t denaturing t healing plus t extension. So, this essentially is a very important concept to understand I would just like to point here attention to this fantastic problem, where in it is really independent of the temperature you know in between which this body of the

reactor is oscillating, irrespective of whatever the two temperature points are. So, in this case for instance you see that you know you have to go in one instance from 25 degrees to about 90 degree Celsius and then you have to come down from 90 to 54 and from 52 back to 72.

So, really the delta t is varying in this case, but we assume that the time constant the amount of time there it would take for the fluid or the particular volume of fluid inside the chamber to reach the temperature of the walls of the chamber, would essentially be constant, it will dependent just on the properties of the thermal mass. Now, if you are to assume that there is a surface, where we can rapidly change the temperatures without really having to for the temperature of the surface on which this reactor is place to reach the particular temperature point.

So, in that case the only time that is needed assuming the temperature of 90's just met just about met or the temperature of 72 is just about met, the only time needed is to need or is to heat up the bulk which again is constant. It is not a function of the two temperature states for just the volume to area ratio, which determines in fact, the time constant in that the particular case.

So, really if you can have a control on the ramp up ramp down of the material that is involved in the heating process like for example, the remaining portion of the ((Refer Time: 09:41)) which probably the heaters are placed. Then controlling from the surface which is enclose approximately to the solution to the solution is really independent of the two temperature states, this is the very important concept to be understood at this stage.

So, in micro chip architectures the advantage which such architectures provide is really the fact that such a surface which is essentially the surface wall holding the bulk or holding the fluid that that surfaces is essentially very small it has it is own thermal mass which is effectively very, very small. And so therefore, it can rapidly reach certain temperature point from another point whether it is cooling or heating also, because the surface area is more prominent it happens rapidly.

So, this issue is of extreme importance then once this temperature is reached we do not need to bother about what is going on it is in terms of quick transfer within the bulk of the particular solution. So, you have this the total time of the reaction in this case as 3 times of 0.84 seconds which is the time constant for essentially for the PCR volume or for the micro reactor volume from the last question and plus denaturing time plus a meaning time plus hybridization or extension time.

So, we already know that the healing and the denaturing times are all 5 seconds as illustrated in the question itself from the earlier example, where we calculated a 1000 base pair DNA and extension rate of about 50 base pair per second we found out you know the total time hybridization is about 20 seconds. So, this is 20 seconds and therefore, the total time that one cycle would need really is about 32 seconds and in this case.

If you work to assume that you have to produce $10^5$ copies per ml from about 100 copies per ml of the particular solution then... So, assuming that about 10 cycles would be needed to do this you will left with about 325 watts seconds for by the by this is actually 32.5 here. So, it is about 325 seconds that you would take for cycling this for about 10 times. So, there it could produce about 10 to the power 5 copies of DNA from 100 assuming the efficiency is that we have disused in the last lecture in the last example.

So, you are total time for the whole reaction to have 10 to the power 5 copies per ml starting with about 100 copies per ml is about 325 seconds or about 5 minutes and 25 seconds in a macro scale reactor thermo cycler will there is a huge element a blog, this would go as high as about close to 2 to 3 hours. So, there is definitely an advantage that micro scale PCR has to offer.

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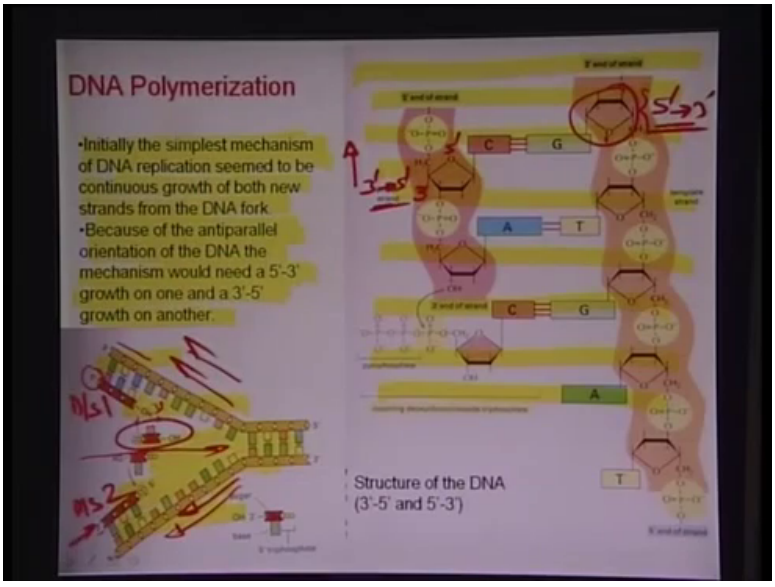



So, after this designing problems are over let us try to understand this process of DNA polymerization which is extremely important process for you know for understanding of the basic kinetics or kinematics of the way that DNA is reconstructed between cells as this split and get reconstructed. So, essentially if you look at the DNA structure again I am closely the

evaluated the way that the sugar phosphate backbone is being laid out is really opposite to each other.

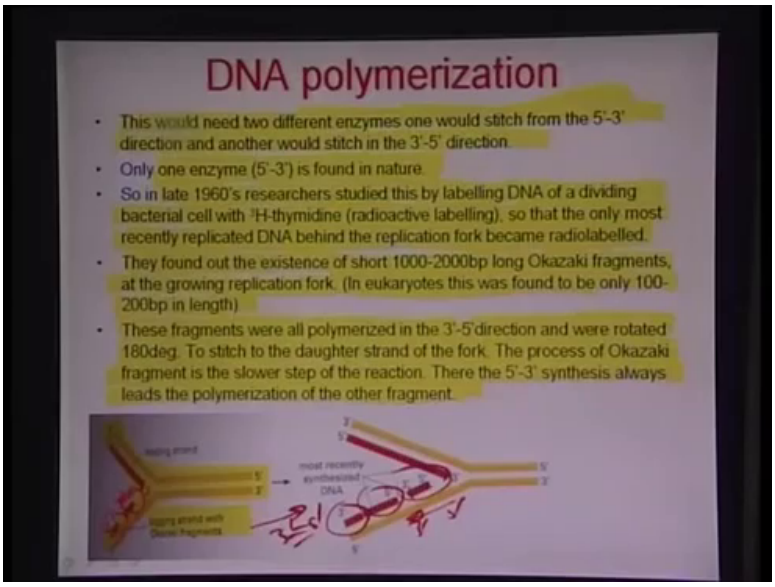
Here, in this particular illustration as you can see shows how if you look at this particular side, let say this side the sugar phosphate or the sugar bone or the sugar molecule is really pointing from the 3 prime to 5 prime side. If you may remember from our earlier discussion for DNA molecule, this is the third carbon it is called 3 prime, this is the 5'th carbon of this sugar called 5 prime. So, this direction is from 3 prime to 5 prime very amazingly the other strand of the DNA has an opposite direction of the sugar molecule. So, it is starting from the 5 prime end and going up to let say the 3 prime end of the earlier strand.

So, as you see here this sugar molecule the 5 prime is pointing downwards as suppose to this where the 5 prime is pointing upwards. So, the two stands of DNA really or just opposite to each other in terms of the way that sugar molecule is aligned or oriented. So, it is very interesting phenomena, because that kind of defines the way also the DNA polymerase able to replicate these moieties independent moieties specially when within cells.

So, initially of the simplest mechanism of DNA replication seem to be continuous growth of both stands and daughter strand from the DNA four. So, this right here is the DNA molecule as you can see here and if you see this closely there is a zip, there is an unzipping action here, and there is an opening of these two stands in both directions like this. And therefore, there after there is a continuous growth of both the new stands from the DNA for.

So, essentially people use to think that when these two daughter strands have been oscillated, one in this direction, one in this other direction there would automatically replicate from the moieties which are present in the solution that is the tri phosphates and the kind of dNTP's are tri phosphates and the enzymes which do the suturement. So, people used to think that as for the fork is been separated and as the new daughter strands generate of the suturement kind of happens parallely and therefore, you get this red think here or this red portion of the molecule is sutured portion, this is the artificially sutured portion.

So, just because the DNA molecule has the base which is anti parallel to each other and just you saw just you know in the last point discussed. So, just because the orientation of the DNA has one sugar pointing from 5 prime to 3 prime on one flank another sugar molecule pointing from 3 prime to 5 prime an another flank they can be said that they are anti parallel. So, therefore, they are really needs to be a growth for these daughters strands which are emanating out here.




So, in the anti parallel direction, so this is let say daughters strand 1, this is daughters strand 2, the emanating and getting unzipped and getting reconstructed, the red portion is the reconstruction on the particular strand and both ends. But, in that case just for you know the sake of maintaining the anti parallel nature one of the suturements should be starting from 5 prime to 3 prime which is opposite to this 3 prime to 5 prime, this is anti parallel to the other 3 prime to 5 prime another should be from 3 prime to 5 prime.

Now, which is anti parallel to the flank 5 prime to 3, so this concepts needs to be little more clear. So, just because the two stands on the DNA and both sides or two flanks on both sides or anti parallel nature it automatically means that should be from 5 prime to 3 prime on one side from 3 prime to 5 prime on other side.

Now, effectively this should need two different enzymes in nature if you look at what really does the job there is only a single type of enzyme, which does this jobs. So, then effectively there should not be any stitching in one of the directions. So, here the need would be really of two different enzymes one would stitch from the 5 prime to 3 prime another would start stitching from the 3 prime to 5 prime direction, but only one enzyme has been oscillated so for in nature which can only do this suturement from the 5 prime and the DNA molecule to the 3 prime and them molecule.

So, therefore, there has to be a mechanism which can accommodate this very fact that the presence of only a single enzyme would also let this stands grow in a manner that is a well suited and still develops this anti parallel nature or characteristics. So, in the late 1960's researchers for the first time labeling DNA of a dividing bacterial cell with this radioactive die H-thymidine and this labeling was essentially used to a certain what is going on, especially in along with 3 prime to 5 prime direction.

We already know that the enzyme there exists in a nature does it suture vent only from the 5 prime to 3 prime direction. So, this labeling was done in a way that only the most recently labeled DNA or replicated DNA behind the replication for became suddenly radio labeled. So, you have a fork and let say it is opening up like this and if I somehow I am able to label this particular, you know DNA behind this replication fork; that means, if this is the fork which is opening just behind it; that means, this parent DNA which is able to split up into two, if I can suddenly labeled radio labeled I have an idea of what is going on, how this label changes from one parent DNA to the daughter DNA.

So, from all this we do have information or we have some very good idea about what is going on particularly in the 3 prime to 5 prime direction. So, sign is find out that in the 3 prime to 5 prime direction it is really small fragments of about 1000 to 2000 base pairs which get first separately made in the 5 prime to 3 prime direction and then the suturement really happens in the 5 prime to the 3 prime direction of these groups or fragments, not single base pair by single base pair as normally happens in the 5 to 3 prime direction.

Let me just retreat again what I said that by labeling techniques by labeling DNA which is just try to open into replication fork people have try to find out that particularly in the 3 to 5 prime direction as there is no existence of an enzyme. They have found that there are this growth of short fragments abut 1000 to 2000 base per long which are essentially sutured from the 5 to 3 prime direction and they are replicated also in the 5 to 3 prime direction.

But, then instead of doing the single base pair at a time what happens in the 5 to 3 prime direction normally in the 3 prime to 5 prime plane or the 3 prime 5 prime portion of the four it is the growth by group procurement of the fragment by fragment procurement, which happens and which can cause the 5 prime to 3 prime enzyme to replicate on a 3 prime to 5 prime fragment or strand or flank of the DNA. So, these fragments are known as essentially Okazaki fragments on the name of the guy who really found these fragments find out first.

So, therefore, they found out the existence of short 1000 to 2000 base pair long Okazaki fragments or the growing replication for. So, in this we found to be only about 100 to 200 base pairs, where is in eukaryotes this would be as high as about 1000 to 2000 base pairs and these fragments would then attach in bulk as fragment by fragment rather than individual base pair by base pair, these also illustrated in this figure here.

So, if you see this particular example this is the fork which is radio leveled and this is the DNA which is radio leveled and it is opening up as a fork and slowly one portion is getting

replicated from the 5 to 3 prime direction which is perfectly for the other portion getting replicated from the 3 to 5 prime the replication. Actually happens in the reverse direction, it happens in the 5 to 3 prime directions; however, it happens through the short segments which get generated and the segments get connected to each other rather than individual base pairs, you can see it lot more bigger here may be you know.

So, you can see these segments can getting created and this segments which are actually able to get secured also in the same direction in the 5 to 3 prime direction even though they are on a flank which needs to go 3 to 5. So, that is what Okazaki fragments are, so in nutshell these fragments are polymerase in the 3 to 5 prime over rotated 180 degrees into stitch the daughter strand of the fork and the process of Okazaki fragments is slower step of the reaction. So, it defines the overall reaction time is well.

So, there in the 5 prime to 3 prime synthesis always leads the polymerization of the other fragments. So, irrespective of whatever be the case then enzyme used to only one which is 5 to 3 prime and in order to make this process a little quicker nature mother nature has given this wonderful ways and means of forming a fragment and the stitching fragment by fragment, rather than going into individual base pair by base pair. So, this is essentially a very important aspect about the DNA polymerization.

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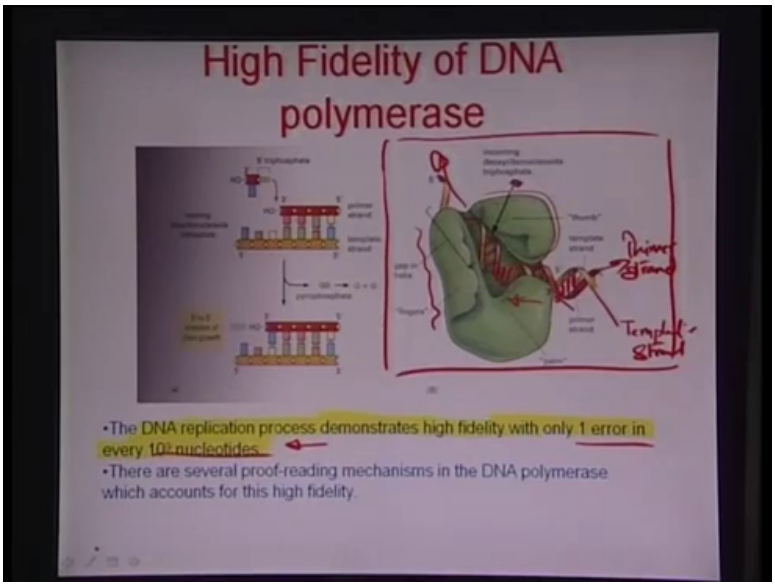


Now, this is essentially this is an engineering course I am not go into the full details of the way that this enzyme would work, but then essentially we can look at enzymes really as a procuring hand you can look as if there is this kind of palm of mine which I fold like this and

then the molecules kind of past through from one side of this palm and goes out. In the other side and this palm actually presses two stands to gather and there is bonding because of that this is the nice analogy also thermo dynamically this is what happens.

So, this is can be a kind of thermo dynamic map of what is going on the reaction by looking it as if the molecules are passing like a chain through this gap of a palm of a hand and we are pressing this palm from time to times. So, that we are suturing different molecules together. So, using this model again you can see the very fact, so this essentially is what the hand really looks like, you know this particular illustration here you can consume as if the templates strand which is given by this yellow portion and the primer strand which is given by the red portion.

The kind of move together and one end of this palm from this end of this palm and they are moving out from this other end of the palm and you can think of as if this these are really the fingers which are kind of pressing and this is the thumb which are kind of pressing on the molecules and developing these bonds between the base pairs.

So, essentially it is also a hand drawn by thermodynamic considerations, this is the way the energy would kind of, in order to suture in the molecules to each other. So, there is a very good aspect of this DNA polymerase mechanism or this mechanism of suturement by the DNA polymerase that it is a very, very high fidelity process there is about one error which it demonstrates only about 10 to the power 9 nucleotides it is fantastic.

Because, if this error rate for any different or any bigger than this by there would be sever notations and then we would have a continuous process of changing outwardly physiological, responds, behavior, shape, size, everything else with time, but; however, because of this high fidelity process there is almost one error in 10 to the power 9 such suturement which this DNA polymerase can ever make and that makes the process of suturement a very, very high fidelity less error process.

Then there is hardly any mutation or very slow of nitration which happens over considerable amount of time one subject to certain stimuli or conditions related to the environment. So, therefore, of the DNA replication nutshell is very high fidelity process, what about one error in about every 10 to the power 9 nucleotides, there are several proof reading mechanism of the DNA polymerase, which accounts for this high fidelity.

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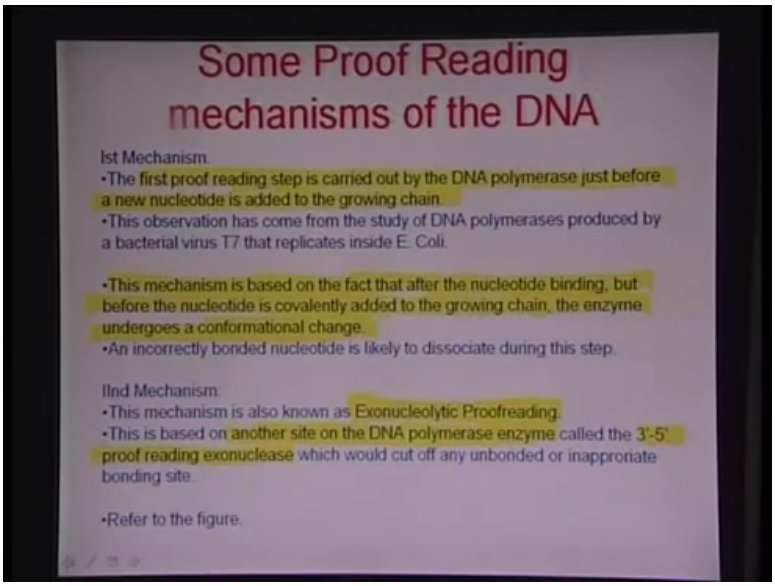


Let us look at some of the mechanisms here. So, the first mechanism really is where the first proof reading step is carried by the DNA polymerase is just before and new nucleotides is added to the growing chain.

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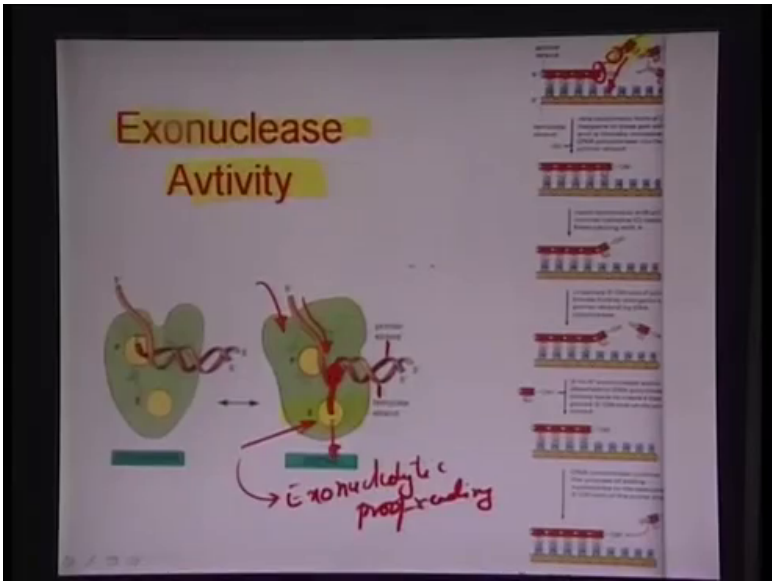


So, essentially something wherein think I have illustration here, when there is a new nucleotide which is added to the grow in chain, there is a proof reading step which is carried out. So, this mechanisms is based on the fact that after the nucleotide binding, but before the nucleotide is covalently added to the growing chain, the enzyme undergoes a conformational change. So, let say you have a moiety here which is coming and it is about a bind to the complimentary strand which is there on the template.

Once it binds on to this can complimentary scan it has to get attached on to this other portion of the sugar phosphate chain, before this happen the enzyme if it feels there is a wrong binding by any chance gives a jerk or a conformation change it kind of leads to cleaning this molecule away. And so in the next step enzyme trays to typically the different molecules see that is going to work or not. So, before adding this portion of the input molecule to the chain after this is base pair has been bonded to the base pair here on the template, the enzyme tries to proof read and see if there is an out of shape you know situation.

So, if there existent out of the situation in it suddenly changes it is conformation and tries to cleave the molecule off from the growing DNA chain. So, therefore, that is no question of suturement of the phosphate part on this dNTP or the nucleotide to bonded to the remaining part of the DNA chain. The other mechanism which is of interest is also known as the Exonucleolytic proofreading mechanism and this happens, because another site because of the presence of the another site on the DNA polymerase enzyme.

So, the site is also called the 3 prime to 5 prime proof reading Exonuclease and it would cut off any unbonded or inappropriate bonding sites. So, this is very fine the illustration here, this is the site as you can see is color does not worked ((Refer Time: 30:06)) very well. So, this is the site as you can see are the exonucleotide proof reading mechanism. So, this essentially is the primer strands, this is the template strand and they are all going in to this hand like future and going out.

So, therefore, the moment here the DNA molecule of this hand senses or this enzyme senses that there is a miss fitment of two base pairs wrong each other, it redirects the primer strand towards the exonuclease site on the enzyme and it does not let and this is the different direction all together. So, it does not let the primer portion which is inappropriate bond to you know the other template portion. So, the template portion in this case is coming from this end and the primer portion in this case is kind of getting sutured here.

So, the moment there is a difference in the base pair and it does not match of very well, the molecule kind of changes direction and goes to this exonuclease site, which causes the particular base pair complimentary base pair on the template strand to go and bonded. So, these are the two mechanisms on DNA polymerase enzyme, which leads a two kind of accurately and with high fidelity produce repeatably whole DNA chain.

So, basically that is a totally all about how the DNA polymerase you would try to you know do the proof reading and suturing of the different stands to complete the whole DNA

molecule. This is of course, in view process and this is a where done inside the cell and that is the path way to for the daughter stands to split equally and going to the two daughter cells from a parent cell which splits up.

So, after doing this another interesting concept which is relevant for the diagnostics industry and that is concern with the detection of the DNA is electrophoresis. As we have already discussed before in the whole classification of an electro kinetic flows there are two different kind of flows, where in one case an external EMF is apply to cause of a flow in another case there is a pressure driven flow which causes in EMF to happen.

So, in the first category in the two different processes that we discuss electrophoresis and the osmosis, electro osmosis we have discussed in much details, electrophoresis which is left with the basic definition which is that of the motion of a charge molecule in site medium and then electric field.

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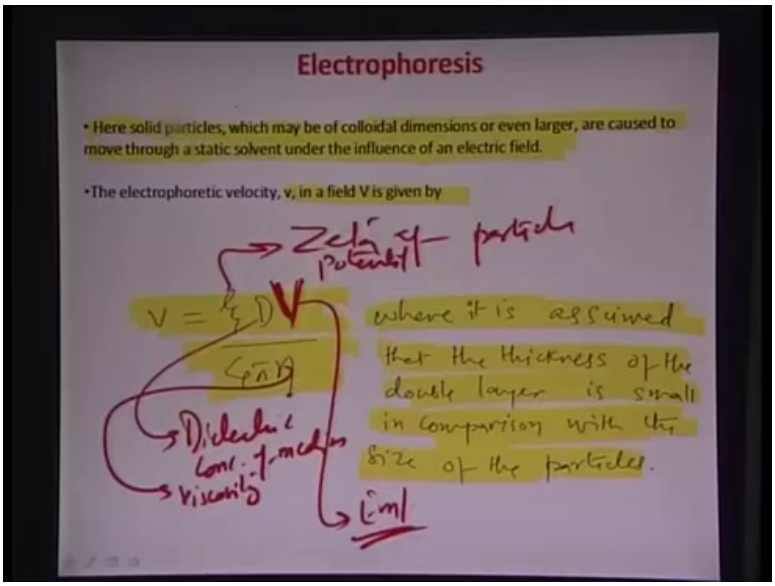


So, here solid particles which maybe of colloidal dimensions or even larger are caused to move through a static solvent under the influence of an external electric field, the velocity of the field is given by $\frac{\xi DV}{4\pi\eta}$ and where it is assume to the thickness of the double layer is a small in comparison with the size of the particles. So, we assume that there are no effects little the double layer close to the surface or at least they are not tangible effects of this surface the double layer.

The only effect that can happen is in term of reduce charge an increase via by the counter

ionic cloud for a nine that is moving into this electron. So, here in this equation V is the electrophoretic velocity of course, zeta here is the zeta potential of the in the particle with respect to the medium, D is the dielectric constant of the medium it has a viscosity and this essentially also that apply field and this is really applied the EMF external EMF.

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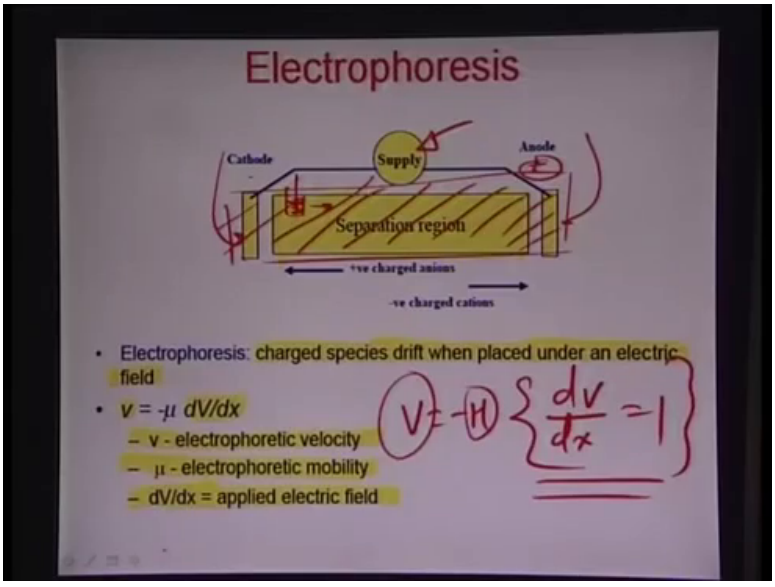


So, let me ((Refer Time: 35:01)) is done particularly from molecules like DNA and it gives a lot of utility to find out of the overall base pair numbers or size of the molecule. The way it can be done it is through porous networks comparing with the size of the DNA, if you 100 nanometers of pore size is probably. So, if you push the DNA molecule by virtual of dragging electric field with in such a sequence of ports, on a network of pores it is going to straight enough the molecule.

If this pores are randomly oriented this molecules would tend to move a surface with in to those course towards the positive electrode and in the process they would face a couple of different forces which a balance between which you need them to move in a particular reaction of course, there is a force of a capture that the positive electrode would provide on the negatively charge DNA and then there is a force which is associated with the friction that the DNA would undergo while moving through such a set of pores or sieves ((Refer Time: 36:10)).

The balance between another two forces that is the frictional forces probably in the backward direction is drag force we tries to stop the DNA or end of the DNA from moving through this pore network and the electric field force is a forward force it is a driving force moving the

DNA. So, the balance between these two really determines at what rate the DNA would move about would be the velocity of the DNA.

So, assuming that we have a bunch of different learns that you want to separate and you jumble them all to gather in a solution and try to use in electric field and inject the through this serious of pores or you know sees in the gel, it is almost always true that they started the strand there is the lesser is the fiction level effect in the start moving faster in comparison to the longer sizes or length of a DNA.

So, therefore, that is always fractionation effect which comes into a picture with the longer once moving slower and the short revenge moving faster and after while you can see this dependently, suppose assuming that you have intercalated some of florescence molecules to make them visually distinct to could see them as the different bands or ladders across a DNA gel, if you can illuminate a such as a gel with you know the UV excitation frequency from the particular die molecule.

So, here the following things are required you have a separation region as you can see in this figure here of this separation region essentially is the matrix on the sieve ((Refer Time: 37:48)). So, if put the DNA somewhere inside in this matrix by making a pocket and the simple poring. So, you put the DNA here somewhere and then you apply a field wherein you make one side the cathode, another the anode which is positive electrode and then dip this whole configuration in some kind of a buffer solution and ionic buffer solution.

So; obviously, as you apply a voltage on to this electrodes through this externals supply, there is a tendency of the negatively charge DNA to move through this gel network through towards the anode the positive charge, essentially what it does it is gets inject into the gel material. So, definition is the electrophoresis is really the drift of charged species when placed under the electric an external electric field and that a velocity are related to electrophoresis of the electrophoretic velocity, essentially proportional to the field gradient.

So, the applied electric field of the voltage gradient, so d V by d x mu here is a constant of proportionality which is essentially and estimation of the velocity per unit electric field applied and it is a constant of proportional between the electric velocity and the electric field. So, mu equals v as d V by d x equals to 1, so it is the velocity per unit electric field that you are talking about in terms of the mobility.

So, let us try to a really see at a single particle level what happens identical to what we did before in kinds of in terms of electro osmosis overall particular electrodes, here we try to

estimate the double layer the diffuse layer and then how the dynamics between you know the upper layer double layer, one side it is being pull it through the externally applied perpendicular electric field and another side is being help back for the shear forces or the forces how the dynamics works their we did all these things in details.

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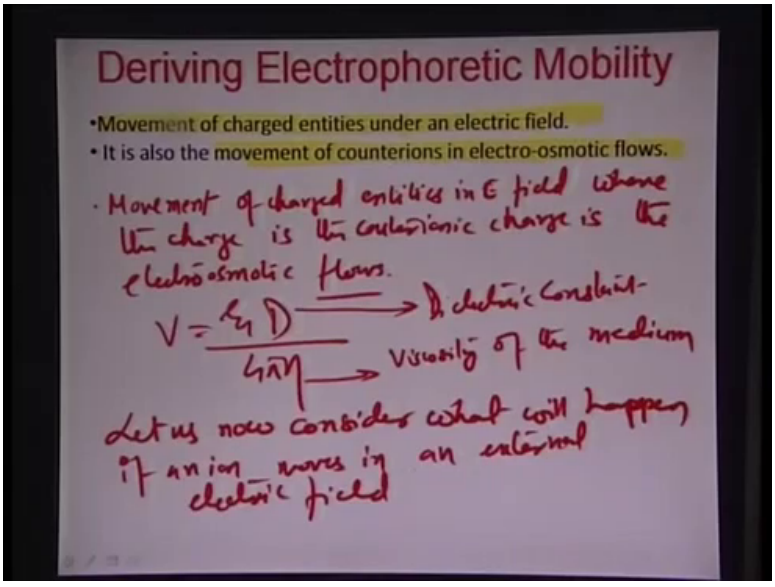


Here, we would try to build an identical model for charge particle by deriving what the electrophoretic mobility of that particle will be once you take a through medium or through you know some kind of ionic fluid. So, electrophoresis is again a movement of charge the entities under an electric field and also in the movement of counterions and that is electro-osmotic flows. So, couple of important things we like to illustrate we will quick here one is that the movement of charge entities in E field, where the charge is the counterionic charge is the electro-osmotic level.

If you remember there is a surface which is a negative charge it is a counterions which would comprise the bulk flow in case of a electrosmosis electro osmotic flow, here it is just the opposite all though the velocity V would still be defined as $\frac{\xi D}{4\pi\eta}$ essentially is the dialectic constant of the medium and I am borrowing this equation from the electro osmosis case, because whether it is counterions or primary ion, if theory does not make much of the difference as long as for as the equations of motions for the charge type where as in this particular case, so $\eta$ is the viscosity of the medium. So, let us now consider what will happen if an ion moves in an externally applied electric field.

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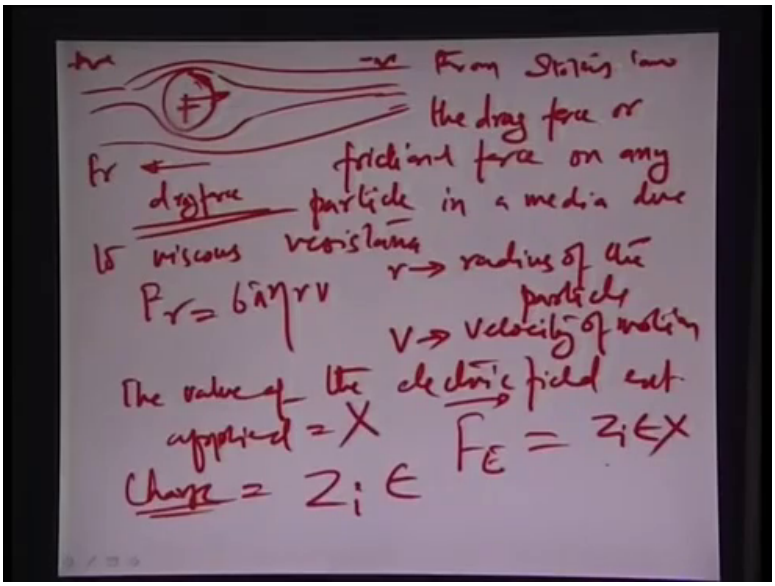


So, the first instance that is come to the mind is Stokes law. So, you have a particle which is moving inside of fluid. So, that is always going to be a drag force, so see on the particle removes and direction from the left to right. So, the drag force what essentially being the opposite direction and this that force can also be found out from Stokes law, this is the kind of frictional force that are particle phrases once it flows through medium.

So, frictional force on any particle in a media due to viscous fiction, viscous resistance. So, the drag force F r is also represented by $F_r = 6\pi\eta rV$ , r is the radius of the particle, v is velocity of motion. Now, let us suppose that the value of let say this is a positive charge it goes towards the negative electrode here. So, we suppose that the value of the electric field the externally applied in this particular instance this given by term x.

So, if our charge here has a $Z_i$ E being the electronic charge, epsilon in the electronic charge. So, the total charge on the particle here which moves is about $Z_i$ E and the total force that the particle would face in the electric field is also given by $Z_i \epsilon X$ . So, the particle will only be moving in the solution, if these two forces equal essentially cosing you know the particle to have a uniform electric linear motion without any acceleration of forces of course, there are going to be in driven forces in sedimentation forces, gravity driven forces which happened to the particles.

But, assuming that the particle size is small let us we talking about by bunch of different ions we hardly get any of these we can we say is for as the gravity effects of the particles scope.

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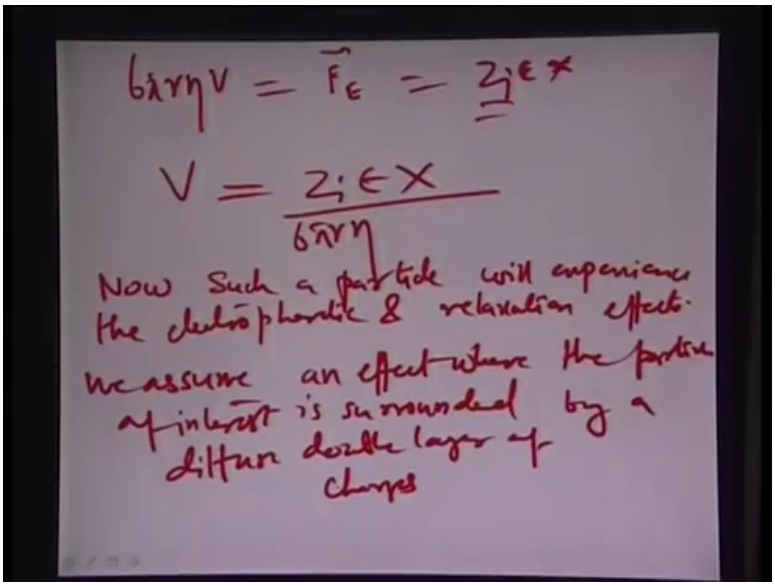


But, then the very facts that the drag force only if a equalized to 6 pi r eta v if equalize to this other electrical force f e would create a situation, where the particle would move through the solution on a plain the electric field. So, $6\pi\eta rV$ the drag forces also equal to the electric force which is $Z_i \epsilon X$ , x is the external of the field, epsilon is the charge on one electron and $z_i$ essentially the valency ((Refer Time: 46:58)) of the ion that we are considering in this particular example.

So, v here for the velocity of the particle in a medium would be given by the term $\frac{Z_i \epsilon X}{6\pi\eta r}$ .

So, this is just a plain case with the particle does not phase any other restrictions from the counterionic cloud that it is in; however, it is a at most important to mention here in the particle is not alone, there is a tendency of this particle to drag bunch of different can counterions in the solution arounded and the effective charge of the particle is shielded because of the counterion cloud or the ion density which is around.

Number two, another very important point is that the overall diameter of the particle also kind of increases notionally, because of the availability of this counter ionic charge. So, if you really involve the differential the charge differential and also the diameter differential of this particle core with an iron clouded, you know surrounding going through or being drag the through the solution by the external field, the equations of motion we will slightly change.

So, let us look at some of the aspects if we consider this particular relaxation effect. So, we call this particular effect of counterionic cloud coming and surrounding the particle of

interest, the electrophoretic relaxation or the relaxation effect. So, such a particle will experience the electrophoretic and relaxation effects, we assume an effect where the particle of interest is surrounded by a diffuse double layer of charges.

Now, this diffuse a layer thickness is really a function of the concentration of the ions in the solution will go to the concentration is more. So, higher concentration we typically have a more number of charges in the counterion cloud. So, we will kind of a stop here and try to look into the whole derivational aspect of this relaxation effect on electrophoretic in our next lecture.

Thank you.