BioMEMS and Microfluidics
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Lecture – 14 to 16
Review of Lectures

Here, we would like to review from lectures 14 onwards. So, 14, 15 and 16 where in the first half we would like to describe more about electroosmotic flows and silicon micro channels, where we would take a surface to the point of zero charge and then just above it or below it to have a dual layer of charge created. And then would study the aspect the flow or mobility associated with such a dual layer which has been created in the influence of the potential, which has been provided.

So, here we would typically design a crisscross kind of electro kinetic network, where through Thevenin's network theorem we would like to as a function of no current being generate at a particular node would like to see what is the charge movement, what is the mobility associated with a longest channel. So, we complete this particular design and then go on to very interesting area which is the real important aspect of BioMEMS which is the introductory concepts into molecular biology or the biology of DNA or other molecules as we know it better from a stand point of an perspective of diagnostics.

So, here we first reviewed the basic cell and we try to analyze the cell in terms of the various different organals which are present in such a cell and various different small mites and this with did from a size aspect, particularly how we would arrive at the region which is central to the nucleus of a cell which is also known as the chromatic region, where there would be the presence of the chromosomes which are long and interrupted pact super coiled linear polimer strands of deoxyribose nucleic acids, we discussed about the detended sizes of human chromosomes to be about close to 50 to 400 into 10 to the power of 6 units long.

This typically would be a few centimeters and we also describe that mother nature is such a good pacts such a 5 or 6 centimeter long object into a very small volume which is close to only a micron cube, which is the nucleic. So, one of the greatest integration density is that can ever be achieved, because of this folding nature provided in the very simple unit of cell that is a nucleic of the cell. We started unfolding the chromosomes and through TM as well as physical tools arrived at the single, the double standard DNA structure, which is also one

particular helix with in this chromosome and such many folds of such double strands would actually make what you call the chromosome.

Then we looked at the DNA structure to be two different strands with one strand composed of sugar phosphate backbone comprising of a five carbon sugar and ((Refer Time: 03:31)) linked between two such sugar molecule and also bases which would be four different bases in numbers, adenine, thymine, cytosine, guanine we looked at the different bases in terms of it is chemistry a bonding to each other, normally this would be hydrogen bonded to each other.

What we also observe was that two is like cytosine and guanine would be bonded with three hydrogen bonds and the other two adenine and thymine would be bonded with two hydrogen bonds and there would be something associated with the stringency of the binding process and the binding process we would also known as hybridization. So, basically it was a phenomenon associated with the opening of the unzipping of this double scanted DNA, because of the exposure to the certain high temperature followed by a reversible association or linkage of this DNA again under the certain critical temperature, where you could bring provide sufficient kinetic energy to bring them close and of to start bond, rebonding again.

So, in one case as a function of temperature you are completely debonding the DNA another case again as a function of temperature or kinetic energy you are rebonding them and this process are phenomenon would also be known as hybridization. And again what we discuss this that hybridization could be a function of course, the temperature, the salt and buffer concentrations which are present the GC content the Guanine Cytosine content of a particular DNA and so on so forth.

They should typically happen between DNA and DNA, DNA or a RNA and then again RNA and RNA. So, all this hybridization phenomena would be very important from a diagnostic perspective has been see later and one of the other lectures. We talked about two different cases, one is the reduced stringency a case of hybridization, where there would be many mispairs a misparing between the different you know groups on such a double scanted DNA.

For example, if you were to bond a cytosine to a cytosine or even a cytosine to an adenine there would always be a misfit, because in one case that would be an additional hydrogen dangling bond which should be unsatisfied and other case there would be a complete disparity, because of size issues etcetera. And therefore, in such cases the bonding would typically not happen or when it happens it happens very mildly and on a statistical in more

significant bases, if such instances of reduce tendencies are available within a molecule, the molecule would spirit apart very easily and it would not obey the particular melting temperature, phenomena that would be associated with and otherwise normally available DNA.

So, we talked about the various theories of association related to this base pairs as related to the finally, the composition of the whole DNA molecule. So, we covered very interesting phenomena call the polymerase change the action, which is invented the bibulous sign a 19 to towards beginning of 1980's. And we also described about how an enzyme ((Refer Time: 06:57)) that is the DNA polymerase could be used for suturing the various base pairs which are commonly available tries within the PCR reaction.

So, does making copies of template DNA which would initiate or which would start initially from the solution. We also investigated what is the use of such a polymerase chain reaction towards diagnosis, forensics, so on and so forth. And then briefly talked about how the three step PCR process could be obtained on a laboratories scale by preparing different temperatures either through a heater cooler assembly or even through as basic components like a differently heated solutions into which you would amerce this reactant for many times.

So, that it could have exact copies of the molecular DNA, the template DNA the more number of times you circle thermally across this three different temperatures which are 95 degrees for the denaturation, the around 54 degrees for the nailing and again 72 degrees for the extension you would have many number of copies of same DNA strand. And also we talked about the various ways of calculating the rates of such PCR reactions by looking at the speed at which the enzyme would suture the DNA to extend the DNA particularly in the extension step, the last step that is 72 degrees Celsius and which is incidentally also the rate limiting step of the whole process and we found out that enzymes with all set of speeds varying from 30 to 100 base pair per second suturement are possible or possibly available.

In a way you know if you look at real time what is going on the reaction rate limits itself because of unavailability of the various other participating species in the solution of interest etcetera. So, in that sense we derived the amplification efficiency of such a reaction, where we talk about factor gamma we define by 1 plus efficiency PCR for a encycle process to the power of n, typically the efficiency PCR would be one for the first 20 cycles are so, but beyond that there would be a rapid deterioration of the efficiency of the PCR process going to the fall down of concentration as the various participating specious.

For example, the DNTP of the dinucleotide triphosphates are even the let say the solutions which are containing magnesium, iron, etcetera they would deteriorate rapidly and also the availability of the primers would also reduce quite a bit, because a primers all shower finitely available within the solution and therefore, there is a self-plateauing action which would formulate. So, the first seven cycles or probably first ten cycles of a PCR process would actually result in the growth of PCR at a linear bases and then it would suddenly have a 2 to the power n type when exponential increase followed by this plateauing.

The first seven or eight steps cycles would typically not result in complete yield because of the fact that still there is lot of diffusion limited react and spread around as conglomerates and not properly makes or uniformly mixed was the mixing is complete then every PCR would result in exact two copies of whatever molecular concentrations are present till and until the concentrations again fall down. So, we described the efficiency of the PCR process in this particular manner and then we also looked at the various determining steps of transaction, where this PCR process could be gazed.

Some of the steps would include things like or die likes Syber green or PCR or even molecular beacon assembly, where what is happening within a reaction can be on a real rime manner gazed in terms of the concentration at the falling down specious. So, would start with something like a simple syber green reaction or where there would be a die which would inarticulate to the double stranded helical structure of DNA and they would be a lease of sudden increase in the quantum yield of the particular die.

So, the idea is that where ever there are such a case like structure with the cyber there would be a interculsion and there would be a increase in the florescence intensity, because of the increase quantum yield of the reference to that gage like structures, there would be a obvious fall out of the process that any double stranded DNA available in the solution would yield light signal, which would talk highly about it is non specificity of recognize a process.

But, that is very important way, because ultimately the statistically significant numbers would reflect all of the copied strands and not the other strands, if you talking about 20 cycle process, so on and so forth, where there is the involvement of millions of molecules and trace concentrations of other specious which have represents may not be significantly contributing to the overall growth of the light signal, so on so forth.

In this slide we also described another Taqman base PCR, where it would be involving between a flourophore of one kind with a certain excitation and emission frequency, which

would then be associated with the die of the second kind with it is emission with it is excitation in the same range as emission of the previous die and it is emission in a completely different range.

So, in this slide we described the FRET process, the fluorescence resonance energy transfer process, where whenever in a primer these two specious are these two molecules are put together and they are separated by a distance equal to a fortress distant which is very small number, there would always be a coupling of energy between the first die of the pair to the second resulting in the emission of the first die going down, then the emission of the second die going up.

Typically, if you could identify such a pair of die associated with each primer, the each binding event of the primer specifically to a DNA molecule would cleave of one of the deist going to the solution, where it would result in exactly the opposite kind of signal, were the first of the prior would have a growth in the emission frequency and the second of the pair, which is already onto the DNA would have a reduction in the emission frequency.

So, such a turnover of the intensity at different frequencies of this die would definitely comment about the way the PCR amplification process is happening and this would be a very good real time gaze or process for determining how the DNA is being replicated within a solution. A similar arrangement was described in terms of molecular beacon assembly, where there would be again present in a primer as a hairpin loop DNA and the idea as that the primer would allying itself flat and bind to a end of suddenly in a strand it would result in the separation between the observer and the die and the die is the signal would though, because of this binding.

However, such molecular beacon assembly and only present for very miniscule section of primers and they cannot be use this a generic technology for the whole PCR process. So, in a nutshell this is what we described about how to read the PCR, we talked about how thermal cycle associated with the PCR process could result in increasing light signal in real time PCR reaction, where we described a strategy real time PCR device with multiple wells about 96 wells high through put assembly.

There be also further described that how the read out would happen typically across the 72 degrees step at the end of the 72 degree step which is also the extensional step in the whole PCR reaction and conglomeration ((Refer Time: 15:36)) of such steps as a function of cycle could be plotted which would yield in that linear exponential of the PCR reaction, which we

described earlier for the PCR process as such.

Following this we also described how to design a PCR reactor on a micro scale and for this the very important, the most important part would be that how the temperature would vary with let say a surface which heated already to a certain temperature and a very small film of the PCR reactant contained within a small chamber sitting over this surface and in that light what we try to look at is a very important electrical analogy associated with such a temperature system, where there would be a heat capacitance and there would be a heat resistance associated with such a circuit.

Then where what we try to determine is that how the you know the whole reactor can be made into the heat resistive and heat capacitive control finally, resulting in a RC circuit equivalent of the heat flow here the way that the heat is flowing dq by dt would be treated as the sort of current flow and the way that temperature would be vary as a function of distance or the temperature gradient would be treated more as a potential associated with that heat flow or current flow.

So, by doing that we finally arrived that time constant for the RC circuit which would typically mean it is a point were three times the time constant would be the time needed for the temperature to go to the set point. For example, in one case if the temperature needs to be of the particular PCR mass needs to be going to 95 degree Celsius that is the set value of the temperature and we need to weight for at least three or C times which is again a function of the geometry of the geometrical parameters related to the chamber in the micro scale.

This way we could be predictably able to determine what could be the time needed for the temperature to go up to that particular set value. In order to design the control system using a proportional integro differential controller, which would ultimately we needed for designing a micro PCR you need to go through this process steps of the RC circuit analogy or method to hit upon the right times which would be needed for the micro scale PCR process to go on .

In that light we design some in using some problems or some you know design parameters some micro PCR chambers where we could estimate the overall PCR cycle time to be much smaller in comparison to the time that it would otherwise state for a bulk PCR process. So, this in a nutshell completes are gives a complete review of the lectures 14, 15 and 16 and also probably extends some extends of 17 what I am go to do in the next review is to start of complete the remaining lectures associated with this subject of BioMEMS and microfluidics.

Thank you.