BioMEMS and Microfluidics
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Lecture - 30
Review Lecture of 17, 18 and 19

Hello and welcome to this review lecture on lecture seventeen, eighteen and nineteen. In this lecture we had done basic constitutional details about the deoxyribonucleic acids, DNAs and then, we studied about different aspects of how to amplify the DNA in vitro, what are the proof reading mechanisms, which are there for the enzymes for extending the DNA.

As you know, PCR is the process where there are three different steps of, different at, carried out at different temperatures, 95 degrees Celsius corresponding to denaturation of the DNA, 54 degrees corresponding to the annealing or the primer binding step of the DNA and finally, 72 degrees step, which corresponds to the extension of the DNA molecule.

And in, in respect of that we also studied how to take a real time snapshot of the reaction through some fluorescence based or optical based method where there would be a single transduction mechanism. As the molecule grows, number of copies grow. Within the solution there is a growth in the fluorescent signal and that gives you an illustration of how the PCR process can be carried out in various stages along its amplification cycle.

We know, that there is a stage, which corresponds to the first five or six cycles where there would be a linear growth in the number of copy because of several diffusion limitations within the solution. There is again another stage where there would be an exponential growth. And then, finally, a plateauing stage, which would indicate, that the reactants, the primers, the enzyme, the dNTPs and the other constituents, which are there apart from the template in the in the PCR reaction, they, they fall down. The concentrations of these fall down because of which there is plateauing. So, that is an indicative of a real snapshot of the PCR process and this can be very well gauged by looking at the florescence response.

There are several essays which we discussed. One is the Syber green based detection where there is a molecule Syber green, which can interpolate with the double stranded DNA and result in a higher quantum yield. There is also another essay where we use Taqman probe of two different dipairs and carrying out mechanism called Fred, fluorescence resonance energy

transfer, where the two probes are bound to a primer before the primer binds to the template DNA.

And the moment there is a binding of the primer to the template DNA and an enyzimity activity, one of the probes or one of the molecular probe or a molecular dye, it comes out of the primer. And the two probes separate through a distance more than ((Refer Time: 03:06)) distance and there is no resonance between the two molecules and therefore, there spectrofluorometric response changes, which gives you with certainty information about the right binding event of the right template DNA being amplified.

We also further studied about molecular beacon technology where there would be a hair pin loop DNA acting as a primer and it would be immobilized with the reporter and quencher die, which would separate, thus leading to enhancement of the fluorescence as the primer binding event would happen. So, these are some of the real time mechanisms we studied for the PCR process.

We tried to take the PCR reaction to a microchip scale and used some basic thermal capacitance, thermal resistance based modelling. We would take a mass of the PCR fluid and we would study what is the heat capacity and heat capacitance or thermal capacitance and would also study what is the thermal resistance. So, this would be on an equivalent model, just like an electrical circuit where the cause really is the voltage and the effect is the flow of charge.

In a similar manner, in a thermal circuit, the cause is the temperature difference and the effect is the heat flow. So, we considered, that the equivalence and tried to derive the thermal resistance and thermal capacitance and finally, put them, embedded them together into RC circuit and tried to find out the response of the RC circuit. Just in a way an RC circuit behaves, that the capacitor charges to its full value in about close to 3 times the time constant, the time equivalent equal to 3 times the time constant, which is also the product of the resistance capacitance product. So, in the same manner, we have the thermal resistance thermal capacitance product and we multiply that with 3 to find out what is the ramp up time for the temperature to go to a certain pre-set value. In case of a PCR, as you know, these values are 95, 54 and 72 degrees Celsius.

So, therefore, whenever there is a small confined volume, which were heating up and there is a small thermal mass, which were heating up the way that it would go is, that in one cycle of

the PCR, there would be at least three such ramp ups and ramp downs. Ramp down, the first ramp down takes place from 95 to 54 degrees. The second ramp up from 54 to 72 degrees and the third, again from 72 to 95 degrees. So, in one particular cycle of the PCR, we would have 9 times RC as the total ramping temperature of that small thermal volume and this is purely dependent on the thickness.

So, as we noted down, that if you can take the thickness of the volume by making a thin film rather than a volume, ok, so over, over a spread, over a huge surface area, obviously the amount of time needed to ramp up would be very, very small, is proportional, in general, to the square of the depth of the film or the thickness of the film. So, therefore, film PCRs, which would be easily carried out in a micro-fabricated scale or the microchip scale would be much better in terms of its thermal, its, its, thermal ramp up, ramp down nature. So, it would have extreme rapidity because of very less such times.

So, we also looked at various mechanisms to transport the DNA. DNA being negatively charged, electrophoreses is one of the driving mechanisms for flooding the DNA through gelatinous medium there. As you may recall, we talked about model where we would have the DNA charge being shielded by the equivalent positive charge of the medium and this shielded charge would again transport across the gel and, and then, there would be spherical capacitance ((Refer Time: 07:17)) electrophoresing mobility. And that mobility would again be a function of the field and therefore, the velocity of flow of the DNA species would be proportional to the mobility, the electrophoretic mobility, which is very well dependent on the dielectric constant of the medium and also the total amount of electric filed, which is present.

So, we modelled electrophoresis and then, we tried to look at some of the different aspects of genomic detection where we would simply put a PCR reaction and a chip scale and then pack gel samples within small capillaries and be able to translate the DNA fragments of different lengths as amplified by the PCR reaction through these capillaries. So, it becomes, essentially, a sieving problem and we could be able to detect the various length strands at a very high throughput by making all sort of radial disc like arrangements.

There was some review from the literature, which was carried out throughout these three lectures for indicating that. So, that in a way, brings us to the end of the review of lectures 17 to 19.

Thank you.