Bio – Microelectromechanical Systems

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Module No. #01

Lecture No. # 20

Hello and welcome back to this lecture 20 on bio micro electro mechanical systems, we will just do a quick review of what happened in the last lecture.

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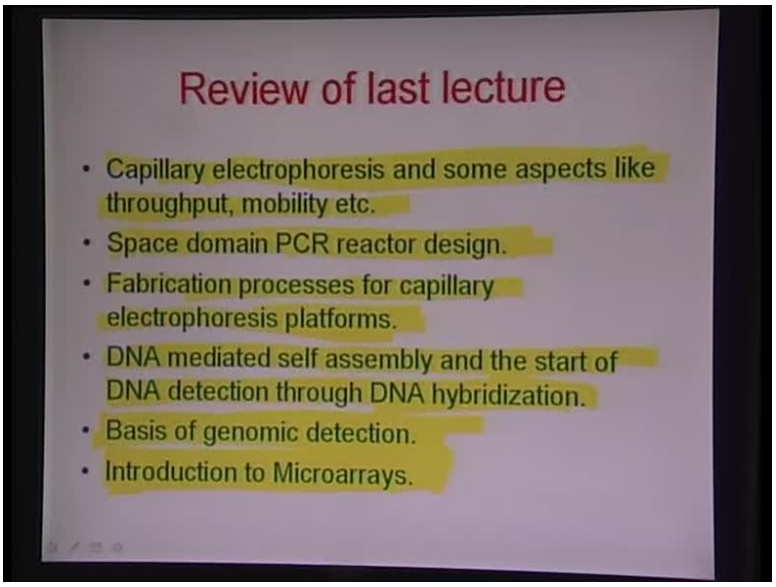

We discussed about capillary electrophoresis, which is essentially the fractionation of DNA molecules through thin capillaries loaded with gel materials Agarose, hydroxyl (()) cellulose, polychromide, so on and so forth.

The advantage here is that essentially, the detection throughput of these micro capillaries is much more than the conventional gels. One of the reasons being, that is because of the high surface area to volume ratio, the temperature of these gels or the amount of heat that these gels can absorb without getting broken or molten, is really very high and therefore, it can withstand higher amount of electric fields or higher amount of EMF - electromotive force.

So, voltage is as high as about 1000 to 3000 volts can be successfully applied into this micro capillary in order to move DNA fragments. Therefore, essentially there are two fold advantages. One is increased mobility, because of the higher electric field; another is ability, because this is a micro capillary, it is overall lesser in size. You can accommodate a dense network of such capillaries in a very small area and can do effectively array based detection. So, it is a high throughput system which you can generate using capillary electrophoresis.

We also talked about space domain PCR reactors and how to design them. I would just like to reiterate it again that all PCR microchip devices are categorized into time and space domain devices respectively. In the time domain device, there is a small chamber; essentially, this chamber is circulated through 3 or 4 zones of temperature - 3 or 4 different points of temperature as it is true for normal PCR process. There is a general confinement of the volume - the reaction volume inside that system. In the space domain devices, you have to oscillate physically this PCR fluid as a droplet or as a micro droplet over the three different heating zones, this lets you or helps you to avoid the ramp up, ramp down additional time as the time domain device would require.

Spatial designs, spatial domain space PCR reactors have been designed. Essentially, what happens is, in these cases you have to design it for the maximum velocity so that you can do the robustness of design. Even though the velocity the fluid is moving at its peak, the PCR fluid has enough interaction time with the temperature zones for it to get denatured, annealed and extended according to whatever process that is present. Essentially, space domain PCR comprises of a channel which is engraved over these three differential heat zones and then given multiple terms, so we designed such reactor device.

We also talked a little bit about fabrication processes for fabrication of these capillaries especially for electrophoresis platforms. Then we did understand DNA mediated self-assembly by referring to one of the very first works in this area from Alivastos group (()) state. Here it was one of the pioneering works to start this whole field of DNA detection through DNA hybridization. We also did some basics of genomic detection, how hybridization can be used as identification for detecting DNA molecules. Then finally, went through this very fascinating very new area of microarrays - gene microarrays.

We will start from the same area today and do some theoretical studies about the different commercially available microarray systems as on date. There are about two companies in the world which produce these arrays, who are from two different approaches, one is Affemetrix and another is Nanogen. Their approaches are totally different for making or realizing the capture probes on a surface to create the gene library. These cards which are immobilized with this library are either sold in one of the approaches directly to the customer. In another approach, it is basically the card platform which is sold and the customer is expected to mobilize himself the particular capture probe in the particular area that he intends to do.

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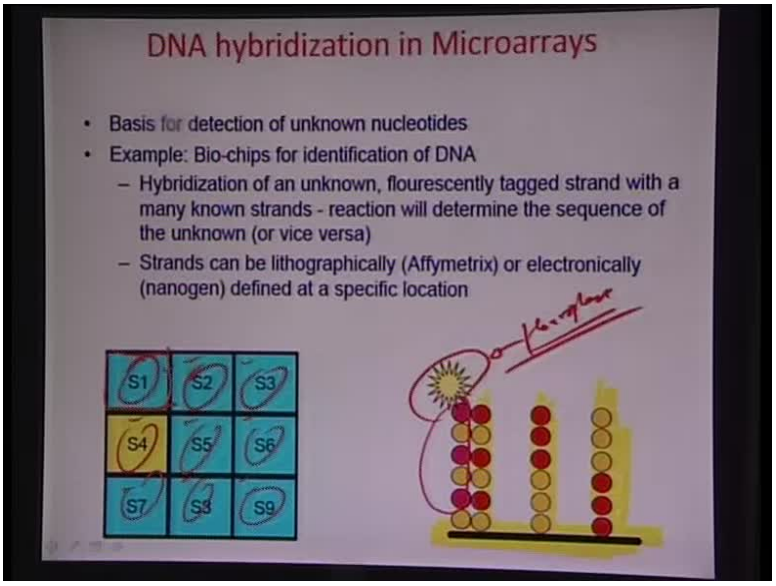

Let us look at what hybridization really is and how microarrays function. You have these captured probes as you can see here in this particular slide of multiple DNA molecules. These captured probes are immobilized on to the surface at the bottom. More so like this library, you have S 1 all the way to S 9, S 1 through S 9 is a different capture probe sequences. Somehow, if you can direct sequence S 1 to this square in the first row and first column S 2, in the first row second column and so on so forth, you could actually make or build the library of the sequences in this different boxes as you can see here.

The advantage is that when you actually have a target DNA and this; here is the target DNA. We are able to label it using a fluorophore molecule. The target DNA has the exact complimentary sequence of the capture probe that is there on the surface here.

Then this target DNA, kind of binds to the capture probe. Then when you wash the whole sample, the unbounded sample is removed except the target DNA which is bound, because of this binding and also because it has already been cultivated with the fluorophore you can see in one of such spots or one of such areas there is growth in fluorescence. If there is a growth in florescence that means, there is a perfect complementary probe which is present in this particular area corresponding to the target sequence.

If you already have information of the library as to what capture probe was there at that particular area, you could gauge the sequence on the target DNA molecule. So, essentially this is also the basis of detection of nucleotides and this hybridization principle. It is basically the hybridization of an unknown fluorescently tagged strand with many known strands and these are DNA strands, reaction will determine the sequence of the known and vice versa.

So the question is, how do you really make this particular strand here? As you can see with a certain sequence which is exactly complimentary to some of the targets that you would be planning to pick up eventually. How do you actually make these molecules in this different boxes or zones as it is illustrated by this big square?

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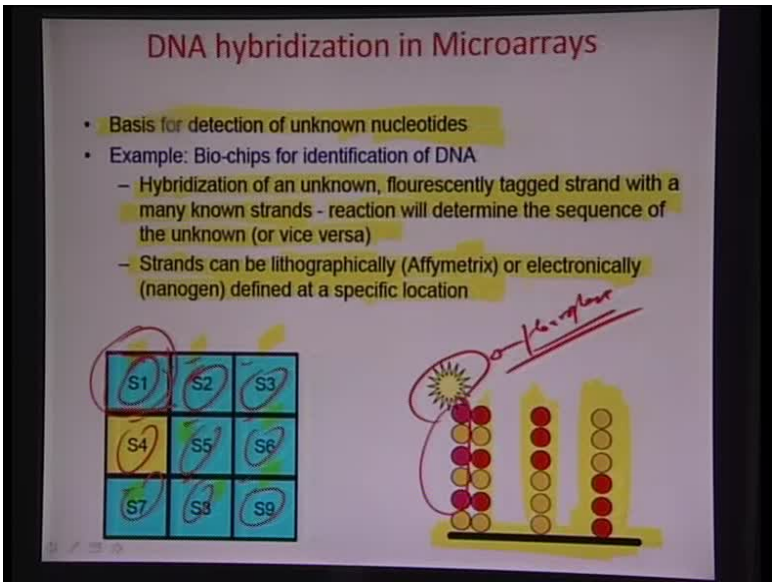

So there are two approaches which can be used for doing these kinds of studies, one is where the strand can be lithographically or placed on the surface that means, you can

actually put the DNA strand with light directed approach or light direct synthesis, that we are going to look in just about little bit. Alternately, you can actually direct the strand to the particular position by using an electronic addressing system as it is normally done by this company Nanogen defined at a specific location. So, that is how really the concept of hybridization works in biochips. Let us actually look at both these strategies independently.

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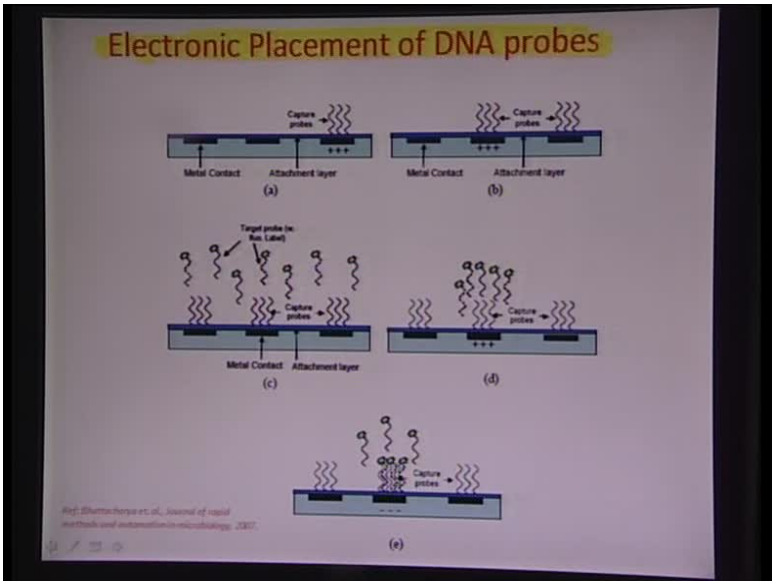

How do you electronically place the DNA probes? It is the approach used by Nanogen. Here, in this particular instance, the company really supplies the platform - the micro scale platform, which is actually having this electronic addressing system for directing a particular capture probe of interest to a particular area on its surface. The vendor or the other manufacturer supplies this platform without any mobilization to the customer. The customer is a biologist or bioengineer; he is expected to put probes in a certain sequence by using this platform and electronically addressing independent pixels.

As I will show you a little bit about how this addressing can be done. The customer actually takes care of building his own library according to his own requirement. So this is the basic difference in the Nanogen and Affemetrix approach. Nanogen is a company which would provide you the platform; it will provide you the protocol for immobilizing the capture probes. Your job as a customer to immobilize the capture probes that you are looking at and create your own library, so there is greater flexibility such that a product

would offer as opposed to the other approaches; they already build the library and give it to you at the very outset.

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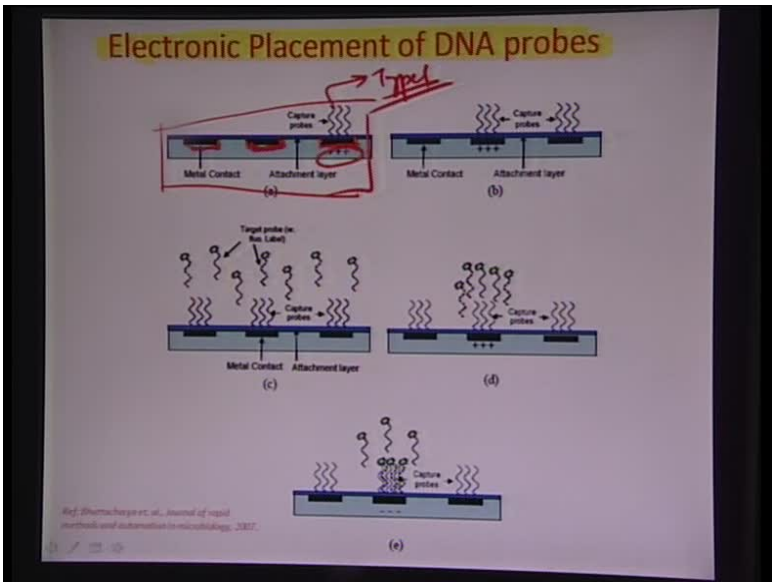

So here, if you see the Affemetrix the Nanogen approach you have this wafer, as you can see here we also have this metal contacts these are connected through conduits to a basic circuitry. The connectivity is in a manner that you could provide a charged surface, you could provide a charged surface onto the electrode, so that is how you have connected the particular electrode. Let us make this electrode connected to a positive DC voltage source terminal, so what do you expect will happen? There would be a tendency of these capture probes. Now, you place this particular assembly into a solution which has the capture probe for type 1, so it has type 1 capture probe solution above it.

When you are actually applying a positive voltage in this electrode it is very natural to assume that the DNA would move towards the capture probe using electrophoresis. The capture probe slowly comes and settles down on this particular electrode, because this is the only one which is a charged positively. So there is actually a lysine layer here in this area which can do this job of binding electrostatically the negative DNA onto the surface. So, you have a layer which would electrostatically bind negative DNA once it approaches this particular electrode onto the surface. If you want to put another library or another probe of type on electrode 2, this electrode is 1; in that case, if you apply a voltage - a positive voltage in electrode 2 here there would be a tendency of the capture

probes. Remove the voltage on the first one; you have to remove the voltage essentially on the first one, so the capture probes now would like to go towards this middle electrode here. The poly analyzing film here would again stick to the capture probe and immobilize it. So there is again a second set of capture probes which are put over this second electrode.

So, you have one capture probe in the previous step and one in the next, therefore you can repeat this process many times. All the different electrodes have a set of capture probes. Mind you, in this case, as we know what addressing is being done, if we change solution in between we will have very well idea of what is the sequence on the capture probe that we are putting on a particular electrode. So essentially, we have information for the whole library of the capture probes, which are this way immobilized over the whole silicon wafer with electrodes.

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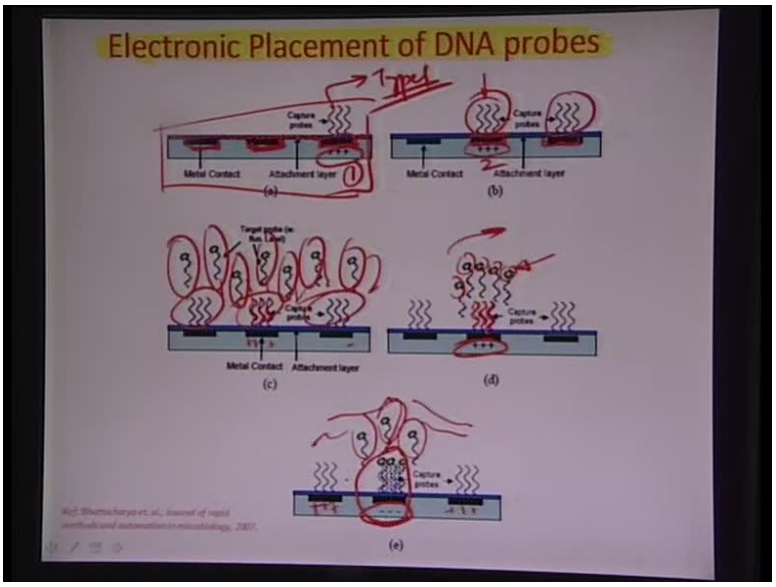

Now, we can actually pull the target DNA in pretty much similar way, but prior to that we also have to ascertain that they have some kind of an optical transduction mechanism, which can make them detectable. So basically, the targets that you put inside the solution, let us say A D I water sample; so the target that you put there - the target DNA should essentially be labeled and it should be single stranded structure that you would like to monitor on the sequence.

When you can actually put this green fluorophore labeled single stranded DNA molecule, as you are seeing here, applying a potential difference, you are starting to apply a positive potential in these electrodes. There is a tendency of these molecules to come close; these would now, based on whichever one has the potential. Let us say, in the first instance only the middle electrode has been given or provided a potential. So they would all emerge into this middle electrode and try to bind on the DNA which is there on the middle electrode. Effectively what it would mean? This is shown here. So essentially, you have electrophoretic mobility driving this negatively charged DNA onto the DNA immobilized on the middle electrode.

Mind you, these are all fluorophore level, if suppose, it is complimentary to the capture probe or we are going to test whether it is going to be complementary to a particular type of capture probe or not, if suppose it is positive in that sense there would be a binding action. So, when you wash this particular chip after the electrophoresis processes are over it does not let get rid of these particular fluorophore labels. Therefore, there is a pixel which is essentially turned on from which one can gauge what is the sequence of the target that you are looking at.

Now, you could also use the electrode for concept of repelling the negatively charged single strand. So suppose, now you have already registered a complementary binding over this particular electrode, this is now futile, you cannot use this electrode anymore. So what you do in order to prevent any further binding of the negative of the other single stranded DNA, so that the target concentration is not lost in the next cycle? You apply a small negative bias here so that nothing comes near this particular electrode. Once it has been already bound, the molecules stay back. This negative bias is used and the other two can then be converted into a positive bias, so that these molecules can drift essentially towards both the sides and then start binding into this immobilized DNA molecule.

This is how the Nanogen approach is essentially developed for making the capture probe; it is a very simple process in a laboratory. Also the experimentalist can actually go ahead and make a library of his own choice and then can use that for identification of certain target DNAs that he is looking into his samples.

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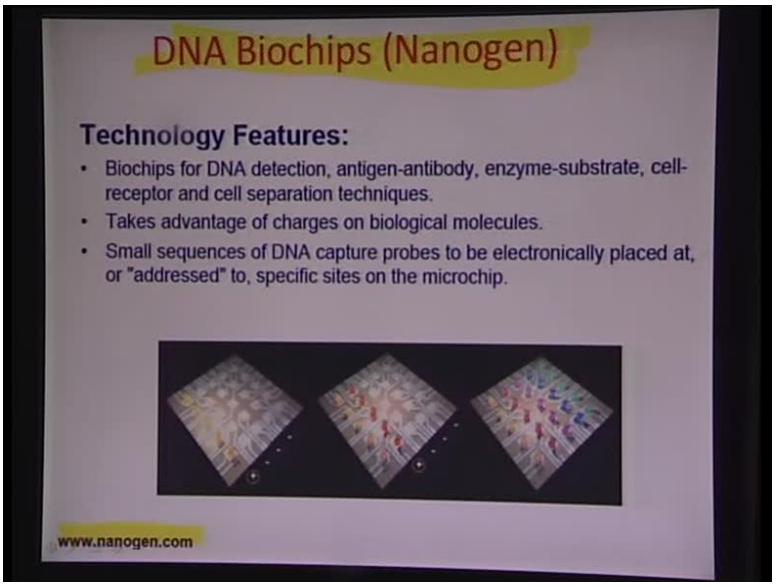

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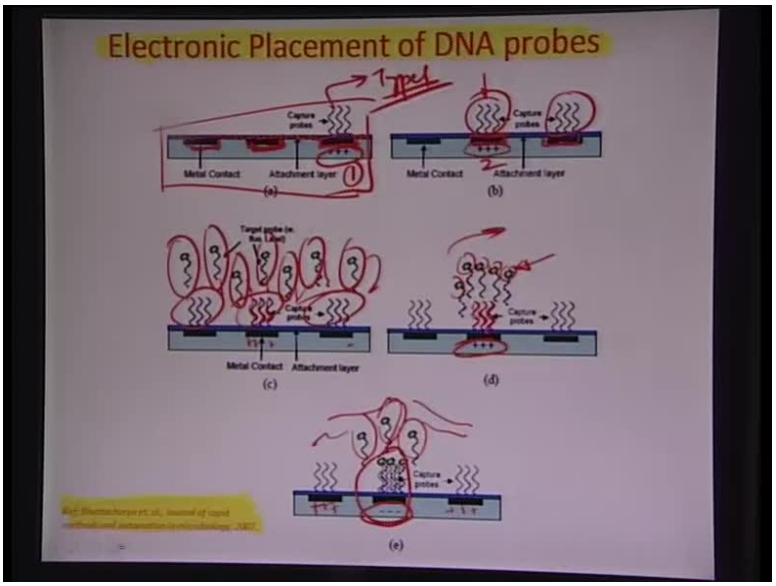

So summarily speaking, the DNA biochips from Nanogen has the following features. This picture actually has been borrowed from this website here WWW dot Nanogen dot com. Similarly, this particular example has been borrowed from this referred this cited reference here; it is one of my own earlier papers being reviewed for these kind of systems.

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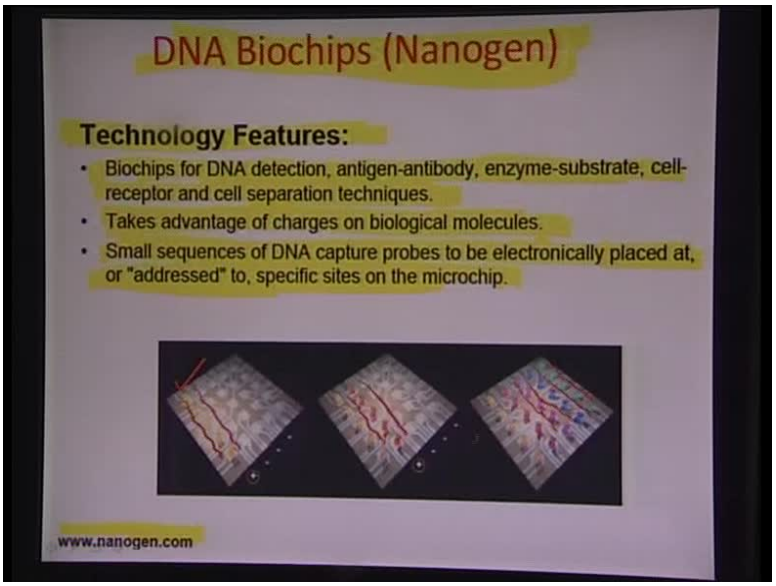

So essentially, in the Nanogen approach the chief technology features are the following: Biochips for DNA detection, antigen, antibody, enzyme substrate, cell receptor and cell separation techniques; all these kind of chips are available. It takes advantages of charges on the DNA or any other biological molecules if you are detecting proteins or antibody antigen etcetera. Every time it uses the advantage that some of these molecules are charged or it has a huge charge at least on its backbone. Then, small sequences of DNA capture probes that are electronically placed or addressed to specific sites on a microchip, which can be used for grabbing now different more and more different target DNAs.

So, this is how the chips really would look like; once the fluorophore labeled, target DNA is immobilized, so here is probably one corresponds to this yellow fluorescence, another red fluorescence has come up (()) fluorescent labeled DNA - single stranded DNA binds to the captured probe which is on the surface. Similarly, you have an array of these molecules; this is the pink fluorescence and this is probably the blue or green fluorescence. Depending on what is the particular captured probe or what is the sequence of the library of the capture probe it captures different targets, we can immobilize or we can actually know where it has been captured by using different fluorescent labels. On these multiple targets, you can have rows or columns of one color fluorescence which gives an idea of what really the sequence target DNA is. We very well know the

sequence of the capture probe, because we are the one as an experimentalist who are actually designing the library and making the library on the top of the chip.

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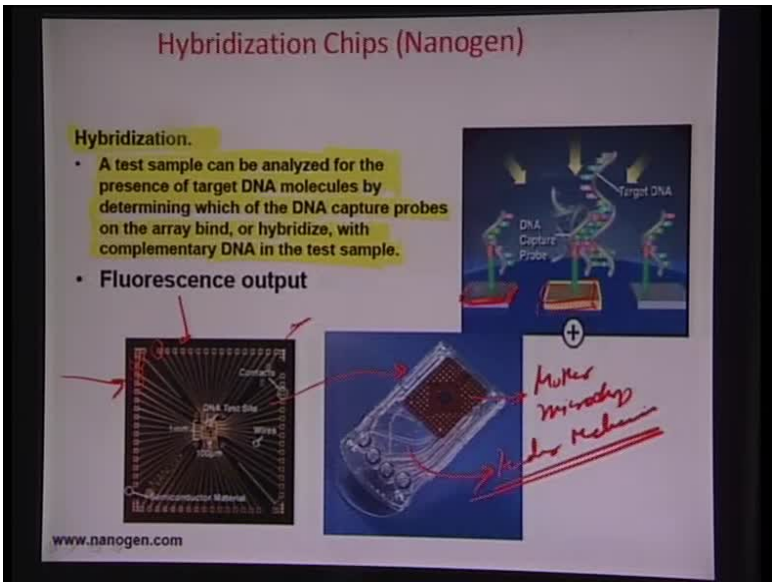

These are some of the main features of the Nanogen biochip. Essentially, what we can say is that in a nutshell the hybridization mechanism is essential the primary detection mechanism, it is used in these biochips, where a test sample can be analyzed for the presence of target DNA molecules by determining which of the DNA capture probes on the array bind or hybridize with the complementary DNA in the test sample. So, if there are some rows or columns which are left untouched, there is no fluorescence growth or no pixel turning on; that means, those capture probes are certainly not having the complimentary sequence of the target. But then, the moment there is a visible fluorescence after the wash cycle is over, it automatically means that corresponding to whatever was there in the library part or in the part of the library, if you are able to know that sequence, we can really predict what was there in the target as a DNA sequence.

These are some of the commercially available protocols. As you can see, this is essentially reader card mechanism. So essentially, this is the main mother microchip and the housing, surrounded surrounding is really the reader mechanism. So, these are available in a very compact manner. Nowadays, in order to do hybridization based array detection of DNA, if you look at the backs - the backside for the electrical connectivity of this particular chip you can find out very easily how it looks like. So essentially, these

are all the different contacts that you provide. In order to provide the electrical voltage to the different array of pillars or posts of principally gold, these pillar or posts as you can see here which are carved or manufactured on the top of the chip. So, this essentially is the river circuitry of this particular chip here, which gives you the connections between the various electrodes on the surface. You can actually keep biasing them positive or negative depending on your requirement.

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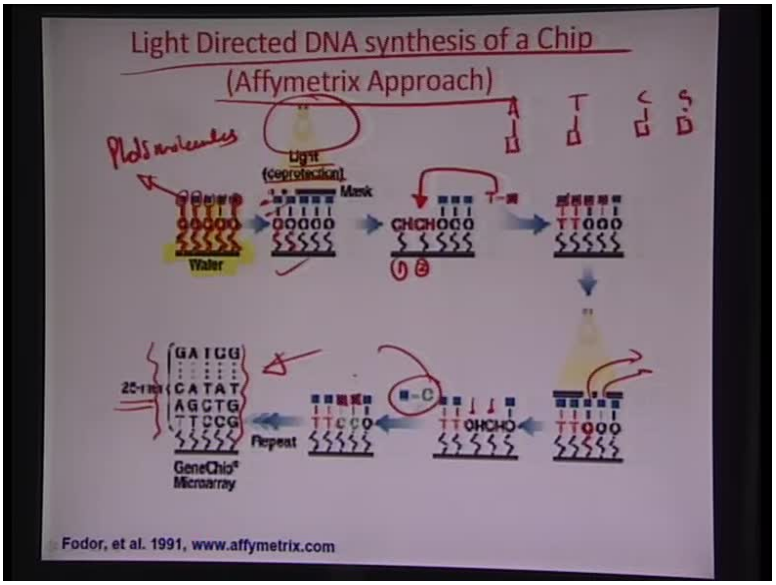

There is another approach which this company Affemetrix follows. In this approach, basically a beam of light is used to build capture probes molecule by molecule, the way you do it is that you take a wafer. By the by, actually this technique was reported by fodoratall back in 1991, which started this company Affemetrix, it is a very innovative approach to build molecules. So you take a wafer, let us suppose, these are different photo molecule capped linker layers.

There are two aspects in this particular layer; one is that these blue dots here as you see are photo molecules. They are capped over this linker layer, which essentially bonds the photo molecule to the substrate. In this particular approach, the first step is that some of these capping layers are first removed, they are uncapped. The way you do it is exposed to light, so you have this light source here. You do a light deprotection by using mask, we have been talking off and on about what a mask is. It is essentially kind of black and

white transparency or a chrome coated hard substrate made up of glass with certain features and designs, which would be transparent.

Wherever there is a transparent via or structure, light goes into and decaps or uncaps these photo molecule layers. So, you have deprotected this linker layer here by using a light signal, so you are deprotecting the linker layer in this particular illustration.

Once you do that you actually replace this linker - one of this top linker layer by CH groups. Use these particular groups to replace the thymine molecule, which has this capping layer at one of its ends. You have essentially these uncapped linker layers replaced with CH groups here, using a light deprotection technique and masking step. Then in the solution, you do have this different adenine, thymine, cytosine and guanine each with these photo molecules or a capping layer.

You have to grow the thymine in these two sites - site 1 and 2; that is the plan. So, what you do is, you actually use the thymine coded or thymine conjugated molecular or this thymine conjugated capping layer to this particular molecule such that it replaces the CH here by the thymine group. The arrangement actually does a capping back of the opened pixels in this particular step.

Once this is done, again you can use a different mask with an exposure in two other areas or two other sites to open the linker layer by decapping these two capping molecules or capped molecules or photo molecules. Then, you are again doing the same chemistry, were you have this OH bonded to this area. Then may be in this case, we are applying a cytosine coated with a photo molecule so that it comes and bonds to these two sites here, which have been opened up. After bonding, it again gets covered with this small capping molecule.

So essentially, you are doing a series of bonding and capping simultaneously. When you keep on opening and closing such capping molecular layers and keep on building the whole library, this slide here illustrates 25 mer library, you have the C A T so on up to G 25 in number. So, it is like a 25 mer library that you have built over each of these pixels in a different sense or in a different sequence. So, this essentially is again a series of masking and opening of the photo molecule layer, conjugation with a certain group which again brings in a photo molecule layer and then doing the masking process again

to open an another site. So, you are building a library essentially, planning your opening and closing of the photo molecules accordingly.

So that is what the light directed synthesis approach of Affemetrix is. In this particular case, though as in contrary to the Nanogen approach, the Affemetrix vendor actually supplies with these chips, which have been developed in the laboratory. They do have spec sheets, which they provide with the particular microchips. In which talks about the library and the capture probe information in different areas of the particular chip.

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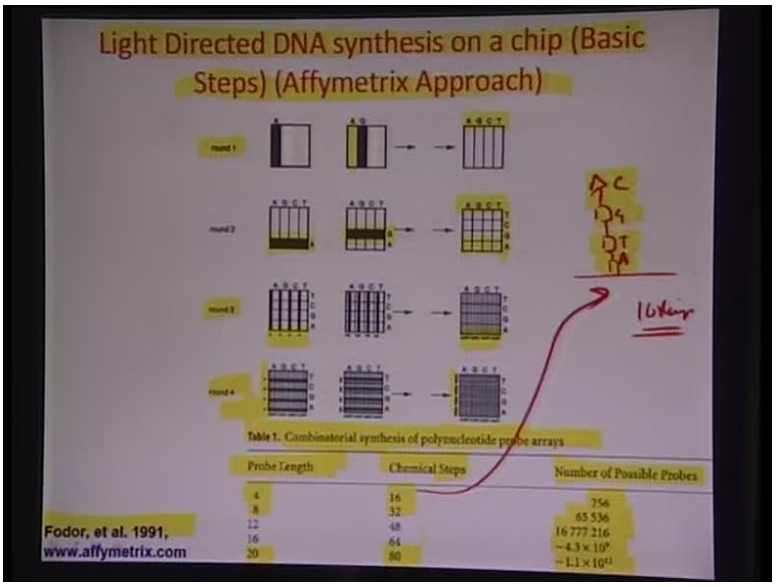

This is again a very different approach than the Nanogen approach; here the flexibility of using your own capture probe may be limited, because the vendor would give you this particular capture sequence or a capture probe, which is already synthesized at their end. In a sense, what you can do with this synthesis is that, you can actually try to build dense combinations of chemical steps or integrate a del dense combinations of chemical steps to build really long capture probe arrays.

So here, you are seeing round one, you are essentially opening and closing the different layers using masking process, so that first the A gets sutured, then the G gets sutured then C and T; so there are four steps essentially here. In round two, you may actually decide to go horizontal and then try to make a crisscross arrangement, so that you have now a combination of A with an A here, G with an A here, C with an A here or a T with

an A here. So, this is layer 1, this one is layer 2. In the third step or round three, you can do a layer 3 from this end, so on and so forth (Refer Slide Time: 29:10).

Then in round four, you would again do the layer from this particular end, which is dotted here. So, what I would like to do is just kind of assert here, is that the various layers of a certain capture probe could be just drawn by rotating the direction of using or the direction of placing the mask with respect to the wafer surface. That way you could have a dense integration of all different molecule layers one over the other (Refer Slide Time: 29:39).

Table 1 here really talks about this, this has been again borrowed from this paper – fodor et al and it is also listed in this affymetrix website essentially. So, the combinatorial synthesis of polynucleotide probe arrays would essentially mean that so many probe lengths; let us say length of 4 would essentially take about 16 chemical steps. The number of possible combinations that are available is 16 times of 16, so it is 256 (Refer Slide Time: 30:11).

Essentially, what it means is that when you are actually having a probe length of 4. So you are trying to build a sequence, let us say A T G and C, 4 probes. For these 4 probes you have to perform 16 steps. As you can see here, the first step can be from this end alright, just give me a minute here. (Refer Slide Time: 31:00).

The first step could be from this end, the second could be from this end, the third could be from this end and fourth could be from this particular end (Refer Slide Time: 31:30). You are essentially rotating the way that you can align the mask with respect to the wafer. You are essentially going through 16 steps in order to get the first layer, the second layer, the third layer and finally the fourth layer of the molecule.

If you want to build like an array with 4 different layers onto the surface, you need 16 chemical steps for this 4 layer. The number of combinations that you can have is, essentially if you assume 16 possibilities in 16 chemical steps you have about 256 numbers of possible probes.

Similarly, if the probe length is 8, the chemical steps is 32, the number of possible probes is 65536. So, you can see here that the way that this combination of different sequences

is increasing is tremendous, it can go up to almost 10 to the power 12 with a probe length of only 20, which would involve about essentially 80 chemical steps to get realized.

Therefore, it is really of vast utility, the library can be very diverse and also it can develop large number of possibilities just with a few probe lengths on the particular surface. Light directed synthesis in a very simplistic way of understanding, how you could capture different combinations altogether on a single surface different molecules.

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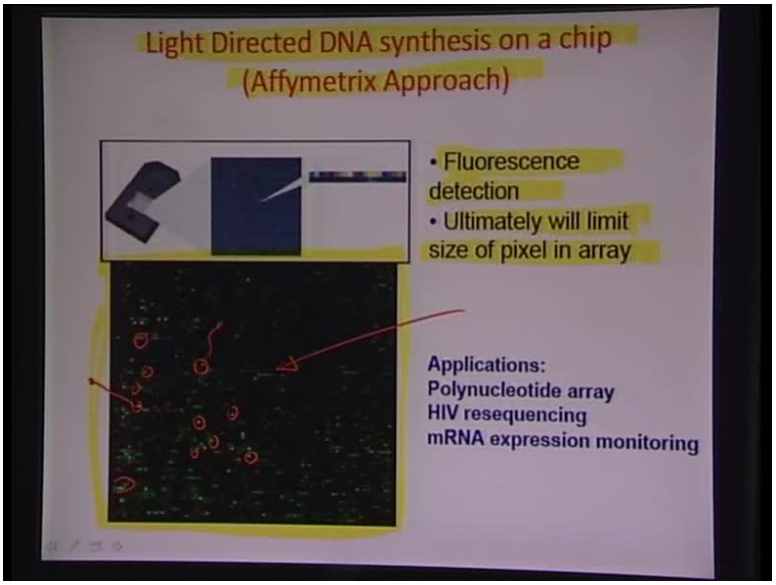

All these gene chips, whether it is Affemetrix or Nanogen, essentially would look in the product form something like this. This is essentially the readout of such a chip. If you can see here there are these fluorescent pixels, which have opened and closed at various regions of this particular chip, which essentially signifies the binding of the target DNA molecule.

Therefore, the capture probe, which may have been in this region, would have corresponded to the target. Similarly, in this particular region, would have corresponded to the target. You can get an idea of how what would be the sequence of the capture probe if you could already know what is the existing capture probe, which is here.

You could get an idea of the target molecule if you can know or if you can have prior information of what is there as a sequence of the capture probe. That is how real

Affemetrix readout would really look like. So ultimately, fluorescence detection is the basic means of finding out whether binding has happened or not.

Ultimately, this fluorescence detection would be able to limit the size of the pixel in the array, because you cannot really go on synthesizing smaller and smaller just because of the fact that you have enough fluorescence intensity for the detector. It should be able to tell you independently whether it is a pixel or which has opened up or which signifies the presence of the fluorophore.

If you go on reducing the size there may be a drastic loss in intensity. The detector may no longer be able to sense. So that is a limitation point for designing the pixel size - the final pixel size. Although, using fabrication you could have probably taken it much lesser almost orders of magnitude less than the existing size, so the detection imposes the limit on the final size of the array.

These are immensely utilized nowadays, these different so called gene chips using the various approaches in various laboratory related experiments, polynucleotide array, HIV resequencing, mRNA expression monitoring. Some of these protocols are very often in great need of such gene chips to tell you accurately, rapidly, quantifiably how much amount of what can be a sequence on the target? Or what can be the concentration of the target?

So one more information that you get out of this fluorescence readout is the fluorescence intensity. The fluorescence intensity can signify what is really the binding level in a particular capture probe. If the intensity crosses a certain threshold, you take the data in that case, also beyond that threshold, whatever change in intensity is recorded is also a change in concentration of the target molecule. Therefore, the florescence intensity provides in addition to the yes no type answer of the sequence an idea of what could be the concentration of the particular target sequence, so that is exactly what this gene chips essentially do for gene analysis.

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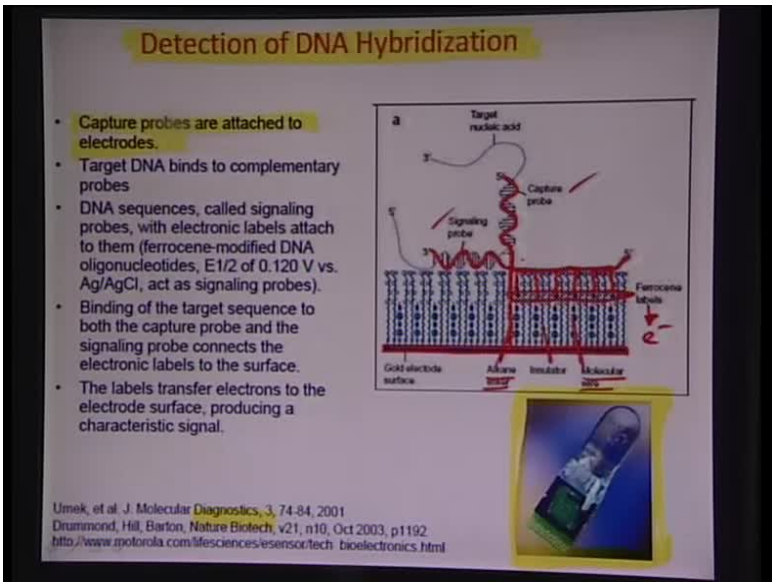

There are other protocols and means for detection of certain sequences of DNA. This one approach here, we would discuss next, it is essentially a chip which has been developed by Motorola. This was reported also in this nature biotechnology journal back in 2003. Here, this is how the chip really looks like, if you look at the whole platform this is what the gene chip really is. Here, the detection again is based on a label which is more like an electro chemical label.

The capture probes are attached to the electrodes in the first step. Here, if you look at, this is the capture probe, which has been bound this blue stain; here is the capture probe, which has been bound all the way down to this alkane linker. The substrate preparation in this particular process is very critical. The substrate is prepared in a manner so that you have molecular wires. In this particular illustration, here as you can see, there are these surfaces are not really a plane surface, it is certainly complicated. There is a gold electrode surface, which is shown by the blue here; this is essentially the gold electrode surface.

There is alkane linkers, which is in this particular area, is an insulator. There is a molecular wire, which is their inside these alkane linkers, so the molecular wires essentially are the conductors. There is an alkane linker, which is an insulator and in parallel to that there is a molecular wire, which is a conductor. So, it conducts it is like a

nanoscale conductor, which would be able to detect a single electron transfer event. So, the way that this works is based on electro chemical sensing.

If you can see here in the signaling probe; this essentially is the signaling probe let us just rub all this off to make this thing a little more clearer so this essentially is the signaling probe. This is other end, this is complimentary end of the DNA and it has a signaling. Why it is called the signaling probe, because it varies from 3 prime to 5 prime side of the DNA and it contains an electro chemical label called the ferrocene group. So, what a ferrocene label typically would do? It essentially is an electron transfer agent; it transfers electrons from the ferrocene label when it is in close proximity to an electrode.

In this particular case, the label if you can see is bonded molecularly to the signaling molecule like this. You have a capture probe here bonded to one of these alkane linkers, which is an insulator. You have a signaling probe here, which has a ferrocene group and which is lying somewhere around in the surface; this is the situation.

Now, let us suppose, we have a target nucleic acid which we want to detect. Very intelligently, the system can pick this up by aligning the signaling probe flat to the bed of the molecular nano wires, getting the ferrocene very close to the molecular nano wires; let us see how? You have a certain sequence in the capture probe and a certain other sequence in the signaling probe. Now, these sequences can be altered in a manner that a large DNA molecule - a large single stranded DNA molecule can bind to both the capture probe as well as the signaling probe.

Let us suppose this 3 prime to 5 prime molecules is the target nucleic acid, it is a single stranded DNA molecule. It has groups complementary to a portion on the capture probe and also another portion on the signaling probe. So essentially, it is making the capture probe and the signaling probe kind of one integral by binding halfway through the capture probe and halfway through the signaling probe.

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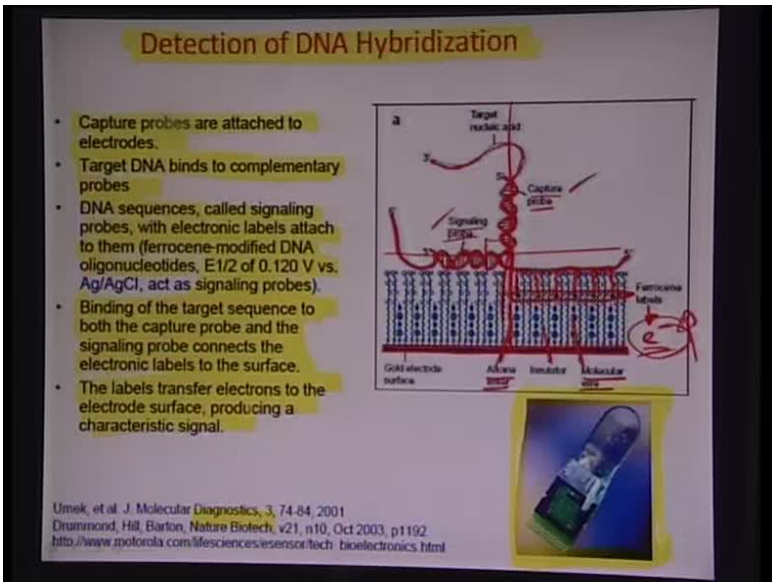

But, there is another effect, which it leads to or generates. When this kind of a binding happens, there is almost always a perpendicular angle between the signaling probe direction and the captured probe direction. So, when the binding event happens, the binding event ensures that these things are just perpendicular like this. This is the capture probe standing upright, this is the signaling probe and they are just perpendicular to each other (Refer Slide Time: 42:42).

It gives some pressure to the ferrocene molecules which are just simply lying on this wire of brushes, which has an alkane linker, which is essentially an insulator in nature. As the pressure is given, because of this perpendicular alignment suddenly by the capture and signaling probe, the ferrocene label gets to a little bit depth crosses the alkane linker layer and comes very near to these molecular nano wires, which are little shorter than alkane linker. Therefore, the single electron transfer events are rapidly recorded.

As they are recorded, it gives an idea of whether the DNA has bound both to the capture and signaling probe. This is a little complicated process and essentially there are a few reasons why this kind of technique or technology works very well. One is the specificity of detection. If you think about the case, when there is a DNA molecule, which has a sequence, which is aligning not only to a capture probe but also to a signaling probe. To consider this electron transfer effect or event to happen, it is a much better selectivity

over just a normal hybridization essay, which has only one capture probe binding to the target DNA molecules. So that is one aspect of this.

Another is that electronic labels, especially electro chemical labels. This has always been the buzzword in the industry because of the fact that it can align them very well with microprocessor so that they can do automatic measurements, data analysis and the signal acquisitions so on so forth. Therefore, microchip architectures almost always have been associated with some kind of an electro chemical or electrical detection. This thought process of this signaling probe also falls in line with same idea, because of these two reasons this is a very well-known architecture which is currently available for DNA detection.

Motorola is a company which actually sells some of these chips for laboratory usage. Essentially in a summary, there is a target DNA which binds to complimentary probes, capture probes are attached to the electrodes. DNA sequence is called signaling probes with electronic labels attached to them. So, ferrocene modified DNA oligonucleotides E 1 by 2 or E half the EMF generated in this particular example is about 0.120 volts.

Ferrocene modified DNA essentially acts as a signaling molecule by emitting an electron, binding of the target sequence to both the captured probe and the signaling probe connects the electronic label to the molecular nano wire which is there on the surface; this can do the electron conduction. The labels transfer electrons to the electrode surface producing characteristic signal, which would give an indication whether the DNA that you are looking at the target DNA has base pair complimentary to the capture as well as the signaling probes.

So in a nutshell, these are some of the DNA hybridization arrays, which are as on date available commercially in the market. I have been probably able to take you through at least some of the few integrated gene chip arrangements that are commercially available.

Now, let us look into a kind of totally different aspect of how to sequence a DNA. This reaction is also popularly known as the Sanger’s reaction developed by a scientist called William Sanger in 1975, for which he subsequently won the Nobel Prize. It is one of the first steps in identifying the sequence of information that is there on a DNA molecule. This is also the very foundation for the field of molecular biology or diagnostics to have developed; it has kind of emerged from this sequencing activity.

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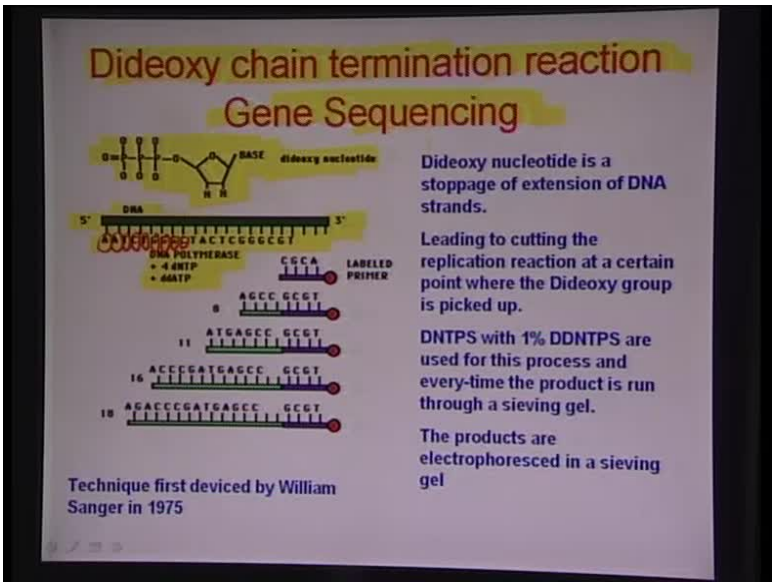

Let us look at how this reaction has been designed in a step-by-step manner. To do this thing first what we need to ascertain is to find out what all are the constituents of this reaction. Let us say, we are trying to sequence this particular DNA product which is from 5 prime to 3 prime, a single strand DNA structure. You want to find out what follows what in terms of these molecules. You are seeing here this molecule A A T C T G G G C and so on so forth is the sequence of information that is really not visible at the outset when you have this DNA molecule with you.

You want to find out what is there in this sequence by looking at this molecule as a whole. This reaction is also popularly known as the dideoxy chain termination reaction used for gene sequencing. Let us suppose, as we were talking before we want to detect the sequence on this particular molecule - single stranded DNA molecule which you are seeing here from 5 prime to 3 prime.

You need the following for detection, you need a DNA polymerase. You already know how the DNA polymerase works, it is an enzyme, which is kind of zips the DNA open, then tries to suture the two daughter strands to make complete strands by fixing oligonucleotides from the solution one by one on the 5 to 3 prime direction. From the 3 to 5 prime direction, it does so by producing fragments the Okazaki fragments, which we discussed about in the last lecture. So that is what is the DNA polymerase.

So you need DNA polymerase for this, as well you need the nucleotides A T C and G independently is called DNTPs; you have four different DNTPs. Then you have a dideoxy chain terminated, let us suppose A T P in this particular case. It is a dideoxy chain terminated nucleotide, also represented as this here. This is the chemical structure of this nucleotide. The advantage of the dideoxy chain terminated nucleotide in such a reaction is that as and when this particular nucleotide binds to a certain region of the chain the reaction terminates; that is why it is called chain terminating nucleotide.

So, the dideoxy group essentially prevents the first further development or suturement of the sugar molecule to the next phosphodiester linkage. It gives an energetically least favorable configuration. Therefore, whenever there is a dideoxy group which comes and sets towards the end of the chain. The chain does not replicate any more it kind of terminates there. We use this as an idea for stopping the reaction in a controlled manner as and when we require it to stop.

Now also important to mention here is that this dideoxy group has some kind of so it What is important for me to tell you is that, for dideoxy group in this particular case it has a certain concentration with respect to the DNTP. So, the dideoxy chain or the dideoxy nucleotide is of a certain ratio in comparison to the actual the DNTPs which are present without the dideoxy group.

So normally in all Sangers reaction processors the DD NTPs as we popularly know them the dideoxy NTTPs is about 1 percent by volume of the DNTPs. This is a standard which is followed in the reaction design. Now interestingly with this kind of reactions proceeds and let us suppose we have a DDATP as in this particular case the DDATP.

There would be fragments produced from this DNA, wherever there is a binder molecule T we know that the thymine binds to the adenine from earlier knowledge about the DNA. Whenever there is a thymine molecule somewhere placed in the chain that may be a point of cleavage or cleaving the molecule as and when the dideoxy group binds with thymine on it on the particular A.

Suppose, there are 50 centers with the nucleotide A on the parent strand of the DNA molecule, this dideoxy chain terminating thymine group which is present as 1 percent of the normal DNTPs that kind of binds; whenever it binds to any of those 50 sites for a it

terminates the chain. So the end result of this process of this reaction is a set of fragments of chains which are terminated each at the adenine.

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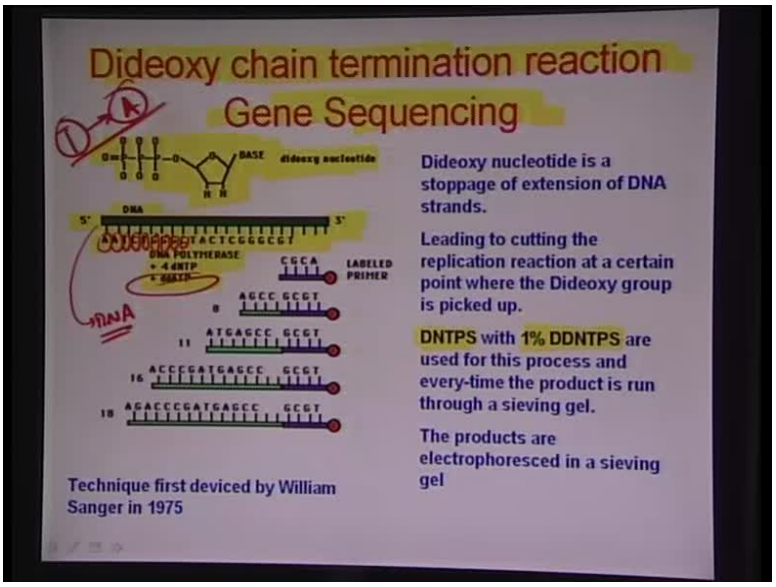

It gives you the location to us of the adenine on the particular base pair, on the particular parent strand of the DNA molecule. Now, let us suppose we have 4 different such reactions with D dideoxy ATP, dideoxy TTP, dideoxy CTP and dideoxy GTP each of them are 1 percent of the normal DNTPs in the reaction and these 4 reactions are all independent in 4 different vials.

What is going to happen is that wherever there is a presence of a gene molecule it would be terminated and fragments would be developed in the reaction vessel which contains the dideoxy G molecule. Similarly, when there is a fragment present in adenine sorry, when there is an end group or a group that we are trying to decipher is guanine in that case we can produce the fragments of wherever the guanine was present in the parental chain by putting it with 1 percent DCTP or dideoxy CTP, cytosine, nucleotide.

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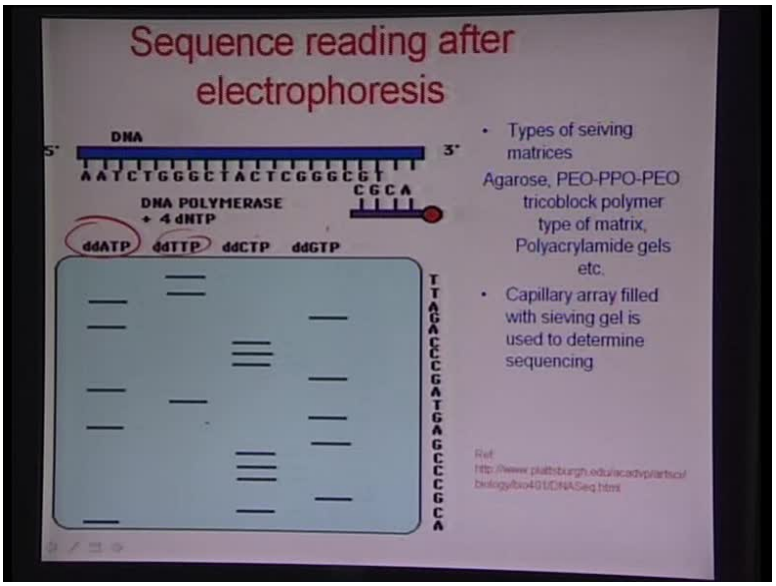

So, if we take all these four products and run it on a gel something like this what happens is that wherever there is a chain termination on the ATP would develop, based on sizes of these fragments which we can later on glow up by using fluorescence. For the DTTP case there can be fragments in this manner. Similarly, for the CTP and GTP if you do an overall readout we will be able to get the position of what follows what in this particular chain.

I am going to take this ahead a little bit later; we have almost come towards the end of this particular lecture. So, the next lecture we will talk about the Sanger's process a little bit more in details and try to see how micro scale architecture can be synergistically developed with respect to Sanger's reaction to do rapid sequencing, thank you.