BioMEMS and Microfluidics
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Lecture – 20

Hello and welcome back to this lecture 20 on Bio Micro electro mechanical systems.

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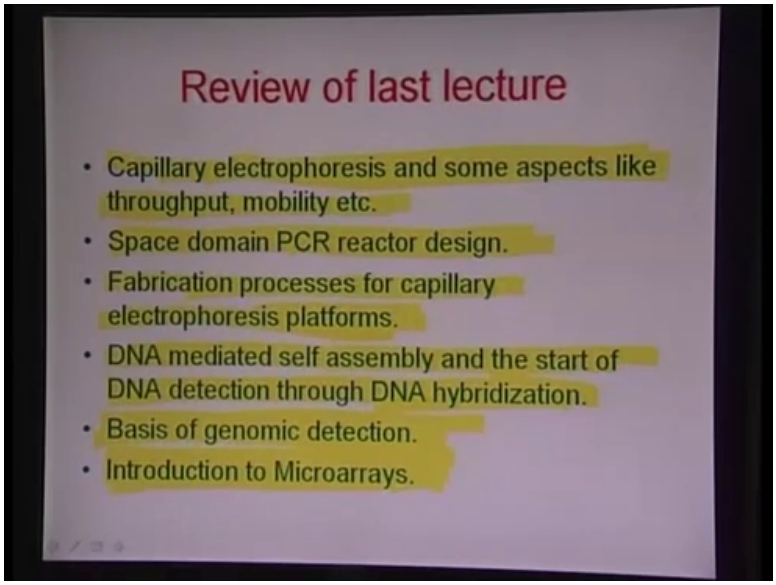

We will just do a quick review of what happened in the last lecture. So, we discussed about capillary electrophoresis, which is essentially the fraction nation of DNA molecules through thin capillaries loaded with gel materials agarose hydroxyl little cellulose so on, so forth. So, the advantage here is that essentially the deduction through put of this micro capillaries is much, much more than the conventional gels. One the reason being that, because of the high surface area to volume ratio the temperature of these gels the amount of heat that the gels can absorb without getting broken or molten is really very high.

Therefore, you can it can which stand higher amount of electric fields or higher amount of EMF electromotive force. So, voltage is as high as about 1000 to 3000 volts I can be successfully applied in to this micro capillaries in order to move DNA fragments. And therefore, essentially there are two fold advantages one is increase mobility, because of the higher electric field and other is you know the ability, because this is micro capillary it is

overall size you can accommodate dense network of such capillaries in a very small area and can do effectively and array based detections.

So, it is a high through put system, which you can generate using capillary electrophoresis. We also talked about space domain PCR reactors and how to design them just like to retreat again that all PCR microchip devices have been categorized into time and space domain devices respectively. A time domain device there is a small chamber and essentially this chamber is circulated through three are four zones of temperatures three or four different points of temperature as is true for normal PCR process and there is a general consignment of the volume the reaction volume inside that system. And in this space domain devices you have to kind of oscillate physically this PCR fluid as it droplet as a micro droplet over the three different heating zones and this let us you helps you to avoid the ramp up ramp down additional time as the time domain device would require. So, special deigns special domains PCR reactive happen design and essentially, what happens these cases is, is here to design it for the maximum velocity.

So, that you can do the design even though the fluid is moving at its peak the PCR fluid as enough interaction time with the temperature zones for it to get denatured and yield and extended according to whatever process. And essentially space domain PCR comprises of channel which is in great over these three differential heat zones and then, given multiple turns, so we designed such reactor device.

We also talked about little bit about fabrications processes for fabrications of these capillaries especially for electrophoresis platforms, then we did understand DNA mediator itself assembly by referring to one of the very first works in this area from here it was one of the works to start this whole field of DNA detection through DNA hybridization. We also did some basic genomic detection, how hybridization can be used as an, identification for detecting DNA molecules.

Then, finally, went to this very fascinating very new area of micro arrays gene micro arrays. So, will kind of start from this area today and do some theoretical studies about the different commercially available micro array systems as on date there about to companies in the world, which produce a these arrays from two different approaches one is alpha metrics and another nano gen.

Their approaches are totally different for making or utilizing the capture probes on surface to create the gene library and these cards, which are immobilized with this library or either sold

in one of the approaches directly to the customer. And another approach it is basically the card platform, which is sold and the customer is expected to mobilizing self the particular capture probe in the particular area that intense to do.

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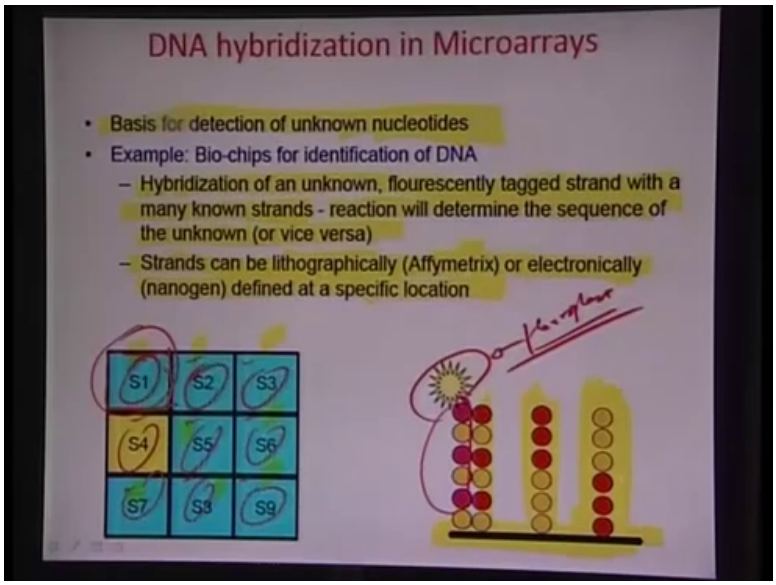

Let us look at what hybridization really is and how microarrays function. So, you have this capture probe as you can see here in this particular slide of multiple DNA molecule and is this capture probes and mobilized on to the surface the bottom, so more like this library. So, you have S1 all the way to S9, S1 to S9 is different capture probe sequences and somehow if you can direct sequence one to this square in the first row in the first column S2 in the second column and so and so forth.

You could actually make or build a library of this sequence in this different boxes as you can see here. The advantages that when you actually have a target DNA on this right here is the target DNA and are able to label at using a fluorophore molecule and target DNA as exact complimentary sequence of the capture probe; that is there on the surface here. Then, the target DNA binds to the capture probe and then, when you wash the sample the unbounded sample is removed except the target DNA, which is bound.

Because of this binding and also because it was already being conjugated with the fluorophore you can see in one of such spots or one of such areas there is a growth in fluorescence. So, if there is a growth in fluorescence; that means, there is in perfect complimentary probe, which is present in this particular area corresponding to the target

sequence and if you already have in information library is one at capture is there in that particular area if it gaze the sequence on the target molecule.

So, essentially this is also the bases of detection of nucleotides this hybridization principle. And it is basically the hybridization often unknown fluorescence, many node strands, then there is a DNA strands reaction in determine sequence of the known of vice versa. So, the question is how do you really make this particular strands here as you can see with the certain sequence, which is exactly complimentary to some of the targets, that would be planning to pick up eventually and how to we actually make these molecules in this different boxes all zones as is illustrated by this big square.

So, there are two approaches really which can be used for doing this kind of studies one is, where the stand can be lithographically placed on the surface; that means, you can actually a put the DNA strand with light directed approach a light direct synthesis, that we are going to look just about little bit. Alternately you can actually direct the strand to the particular positions by using an electronic adjacent system as a normally done by this company nanogen defined that is specific location. So, that is how really the concept of hybridization works in bio chips in, let us as actually at both these strategy independently.

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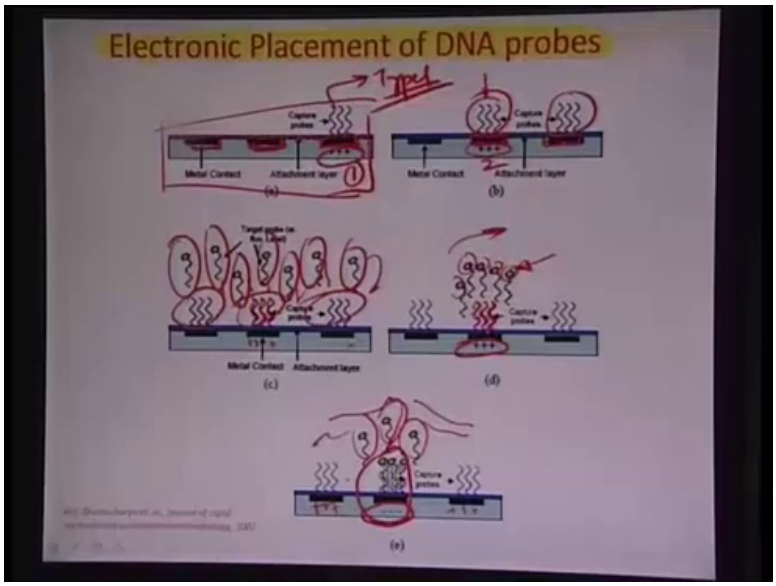

How do we electronically place the DNA probes as is the approach used by Nanogen. So, here in this particular instance the company really supplies the platform the micro scale platform, which is actually have in this electronic addressing system for directing a particular capture probe of interest to particular area on it is surface. And the vendor the manufacture really supplies this platform without any mobilization to the customer. And the customer is expected are a bio engineer is expected to kind of put probes in a certain sequence by using this platform an electronically addressing independent pixel as I show just about little bit, how this addressing can be done. And the customer actually takes care of building is own library according to his own requirement.

So, this is the basic difference in the nanogen and the affymetrix approach that the nanogen is a company, which would provide you the platform and it would provide you the protocol for immobilizing the capture probes and it is essentially your job is a customer demobilize and the capture probes you are looking at and create your own libraries, so there is great of flexibility that the product often as suppose to other approach like already build in the library in the and give it to you at the very outside.

So, here if you see the affymetrix the nanogen approach really you have this wafer, so you can see here and you also have this metal contacts. So, these are connected through to basic circuitry and the connectivity is in a manner that you could provide a charge the surface you

could provide a charged surface on to the electrode. So, that is how you have connected the particular electrode.

So, let us make this electrode connect to positive DC voltage source terminal. So, what you expect will happen. So, there would be a tendency of these capture probes and now, you place this particular summary in to a solution, which has the capture probes type one, so it as type one capture probes solution above it. So, when you are actually applying positive voltage in this electrode it is very natural to assume that the DNA would move towards the capture probe using electrophoresis. And the capture probes slowly comes and settle down on this particular electrode, because this is the only one, which is charge to positively. So, there is actually a layer here in this area, which can do this job of binding electro statically the negative DNA on to the surface. So, you have a layer which would electro statically bind negative DNA once it is a prose this particular electrode on to the surface. If you want to put another library or another probe type on say let say electrode two this is electrode one.

So, in that case if you apply a voltage a positive voltage in electrode two here there would be a tendency of the capture probes and remove the voltage on the first one you have to remove the voltage essentially in the first one. So, the capture probes now, would like to go towards this middle electrode here on the **poly-L-lysine** film here would again kind of stick to the capture probe and the mobilizer. So, there is again a second set of capture probes which are put now, in this over second electrode.

So, you have one capture probe in the previous step and one in the next step and so therefore, you can repeat this process many times. So, all the different electrode has set of capture probes and mind you in this case as we know what the addressing, what addressing is being done and solution in between we have a very well idea of what is the sequence on the capture probe that we are putting on a particular electrode.

So, essentially we have the information for the whole library of the capture probes, which are this way in mobilize with the whole silicon wave for with the electrodes. Now, we can actually pull the target DNA in pretty much similar way but prior to that we also have to certain that they have some kind optical construction mechanism, which can make them deductible. So, basically the target that you put inside the solution now, clean with let say D I water sample may be.

So, the target that you put there the target DNA should essentially by labeled and it should be single stranded structure that would like to monitor on the sequence. So, now when you can

actually put this green fluorophore label single stranded DNA molecule as you seen here and start applying a potential difference. So, you are starting to apply a positive potential, now this electrode.

So, there is tendency of these molecules will kind of come close and these would now based on whichever one has the potential, let say in the first instance on the middle electron as been given are provided potential. So, they would all kind of emerge in to this middle electrode and try to bind on the DNA, which is there in the middle electrode effectively what it would mean this is shown here. So, essentially we have electrophoretic mobility again driving this negatively charge DNA on to the DNA immobilize on the middle electrode. And, so mind you these are all fluorophore level and if suppose it is complimentary to the capture probe or we are going to test whether it is going to be compliment to a particular type of capture probe a not. And if suppose it is positive in that sense they would be a binding action and, so when you wash this particular chip after the electrophoresis process is over it does not let get rid of these particular fluorophore labels.

So, therefore, there is a pixel which is essentially turned on from which one can gaze, what is the sequence of the target that you are looking at. Now, you could also use the electrode for a concept of repelling the negative charge single strand. So, suppose now you have already resister a complimentary binding over this particular electrode and this is now few tile you cannot use this electrode anymore. So, what we do is in order to prevent any further binding of the negative other single standard DNA.

So, that you know the target concentration is not last in the any cycle you apply a small negative bias here. So, that nothing comes near this particular electrode once it is already bound and the molecules stay back. So, this negative bias is used on the other two can, then be converted to a positive bias, so that these molecules can draft essentially toward both the sides and then, stop binding in to this mobilized DNA molecule.

So, this is how the nano gen approach is essentially develop 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 DNA is that is looking in to examples.

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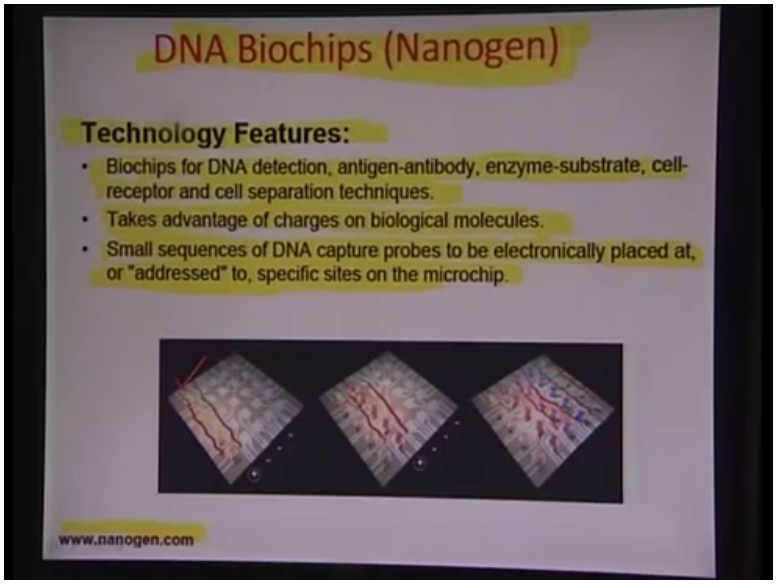

So, the summarily speaking the DNA bio chip Nanogene has the flowing features and this actually have we borrowed from this website here the picture www dot nanogen dot com. Similarly, this particular example has been borrowed from you know this referred this sited reference here shown of my own earlier papers doing review for a these kind of systems. And, so essentially the nanogen approach the chip technology features are the following biochips for DNA detection antigen antibody enzymes substrate cell receptor cell separation technique all these kind of chips are available it takes advantages of charges on the DNA are in other biological molecule if you detecting proteins and antibody antigen excreta. Every time it uses the advantage that the some of these molecule a charge it as a huge charge at least in back bone.

Then, small sequence of DNA capture probes are electronically placed are addressed to specific sights on a micro chips, which can be used for grabbing, now different one more different one more different target DNA. So, this is how this chips really would look like once the fluorophore labeled target DNA is immobilized. So, here is probably one corresponding to this yellow fluorescence another red fluorescence come up and the eared fluorescence label DNA single strands DNA binds to the capture probe, which is on the surface.

Similarly, you have an array of this molecule this is the pink fluorescence this is probably the blue or the green fluorescence. And, so depending on what is the particular capture probe that is the sequence of library of the capture probes it captures probes it captures different targets become mobilize on we can actually nowhere it has been capture by using different fluorescent label on these multiple targets that you can have tows or columns of and you

know one colored fluorescence, which givens and idea of what really you know the sequence on the target DNA is we very well know again the sequence on the capture probe. Because, we are the one is experimentalist is to one designing the library by making the library on the top of the chip. So, these are some of main features of the nanogen biochip.

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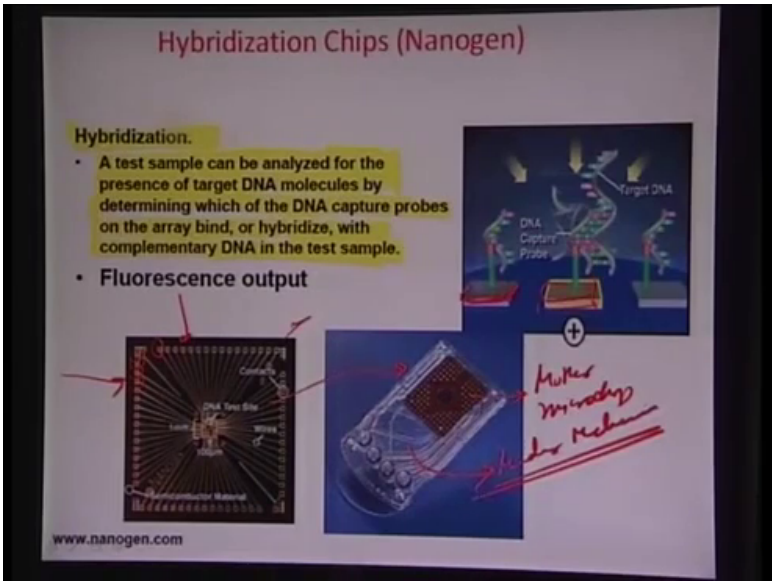

So, essentially what we can say is that in nutshell the hybridization mechanism is the primary detection mechanism used in the biochips, where in at a sample in analyze for the percents of the target DNA molecules by determining, which of the DNA capture probes on the array bind are hybridization with the complimentary DNA in the test sample. So, if there are some rows of column which a left and touch there is no fluorescence growth no pixel turning on; that means, those capture probes certainly not have in the complimentary sequence of the target.

But, when the moment there is a residual fluorescence of to the wash cycle is over it automatically means that you know corresponding to whatever was there in the library part and the part in the library if you are able to know that sequence you know really predict what was there in the target as in a DNA sequence. So, these are some of the commercially available protocols as you can see this is essentially a reader card mechanism.

So, essentially this is the main mother microchip and the housing surrounded around guess against really the reader mechanism and these are very available very compact manner now a days in order to you know do hybridization based array detection of DNA. If you look at the

backside for the electrical connectivity of this particular chip you can find out very easily how is looks like.

So, essentially these are all the different contact that the provide in order to provide the electrical voltage to different array of pillars or spots of a principle gold these pillar or post as you can see here, which are carved are manufacture in the top of the chip. So, this essentially is the reverse circuitry of this particular chip here, which gives you the connection between the various the electrodes and the surface and you can actually keep biasing them positive and negative depending on your requirement.

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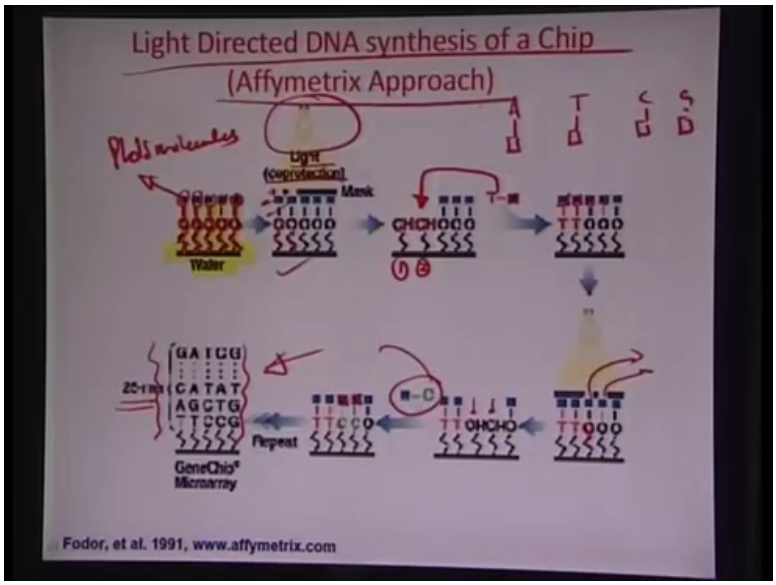

So, there is another approach which this company Affymetrix follows, so in this approach basically a beam of light is used to build capture probes molecule by molecule. In the way you do it is that you take a wafer and by the by this actually this technique was reported by Fodor at all back in 1991, which started this company of Affymetrix very, very know it if approach to be molecules.

So, you take a wafer and let suppose these are different photo molecule kept linker layers. So, there are two aspect in this particular layer one is that this blue dot here as you see are photo molecules and they are kept to over this linker layer, which essentially bonds the photo molecule two the sub strain. So, in this particular approach in the first step is that the some of the capping layers are first three moved they are uncapped and the way will do it by exposure to light.

So, you have this light source here and you do a light protection by using a mask we have been talk often a the mask is essentially kind of black and white transparency are a chrome coded hard substrate made up of glass are, which certain features and designs which would be transfer. So, wherever there is a transparent structure light kind of course, in to and decaps , uncaps this forty molecule layers. So, we have deprotected this linker layer here by using a light signal. So, you are deprotected the linker layer in this particular illustration.

So, once you do that you actually replace this linker one of the top linker layer by c h groups and use his particular probes to replace the thymine molecule which has this capping layer at one of it is ends. So, you have essentially unkempt this linker layers replace them with c h groups here using light reproduction you can asking step and then, the solution you do have these different adenine thymine cytosine guanine each with these water molecules the capping layer. So, take let say to have to grow the thymine in these two sights, sight one and two let say that is plan.

So, what you do is you actually use the thymine coded thymine conjugated molecular or this thymine conjugated capping layer to this particular molecule such that you replace this ch here by this thymine group and the arrangement actually does capping back of the opened pixel in this particular step once this is done. Again you can use again different mask with in expose in two other areas two other sides to open the linker layer again by decamping these two capping molecule.

So, kept molecules of water molecules and then you are again the same chemistry where in you have this o h bonded to this area in the way in this case you are applying a cytosine molecule. So, that this comes and bonds to these two sights here, which have been opened up and after bounding it again gets covered that this small capping molecule essentially you are doing a serious of bounding and capping simultaneously and when you keep on opening closing such capping molecular layer and keep on building with the whole library this rights here the illustrates a 25 library.

So, you have this c a t, so on up to G 25 in number, so it is like 25 library that you have built over each of these pixels in a different sense or in a different sequence. So, this is essentially is a again a series of masking and opening of the water molecular layer and conjugation with the certain group, which again brings in a water molecular layer and then doing the masking process again open in other sides you are building a library essentially planning you are opening and closing of photo molecules accordingly.

So, that is what the light directed synthesis approach of affymetrixes. In this particular case though as in contrary to the nanogen approach the affymetrix vendor actually supplies with these chips which have been developed in the laboratory and they do have, which is the provided with the particular microchips takes about the library and the capture probe information in different areas of the particular chips.

So, this is again as very, 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 to this particular capture sequence of the capture already synthesis their end in a sense.

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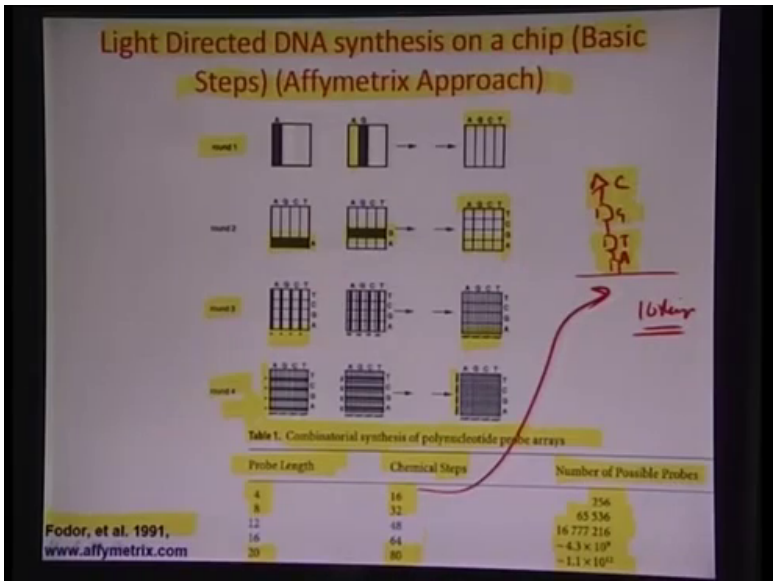

What you can do this synthesis is that you can actually try to build at dense combination of chemical steps or integrate of chemical step ten combination of chemicals steps to build really a long capture probe arrays. So, here as you as seen in round one array essentially opening and closing of the different layers using masking process that first a gets sutured, then the g get sutured and then c gets sutured.

So, there are four steps essentially here in round two you may actually decide to go you know horizontal and then try to make a crises cross arrangements that you have a, now you know combination of a let us say and a with an here a g with an a here c with an a here or t with an a here. So, this is layer one this one is layer two in the third step around key you can do a layer three from this end and so and so forth, then in round four you could again do the layer from this particular n which is dotted here.

So, what I would like to just a kind of set here is that you know the various layers of certain capture probe could be just drawn by rotating the direction of using or you know the direction of placing the mask with respect to the wafer surface and that way you could have a dense integration of all these different molecule layer in one over the other. So, table one here really talks about and this has been again borrowed from this paper photo and solve for listed in this symmetric website essentially.

So, the combinatorial synthesis of a poly nucleotide probe arrays would essentially mean that. So, many problems let say length of four would essentially take about 16 chemical steps and the number of possible combinations that are available is 16 times of 16. So, it is a 256. So, essentially what it means is that when you are actually having a probe length of four. So, you are trying to build a sequence let say A T G and C 4 probes. So, for these 4 probes you have to perform 16 steps as you can see here.

So, the first step can be from this end it is to remember it here. So, the first step could be form this end the second could be from this end the third could be from this end and fourth could be from this particular end. So, you are essentially rotating the a that you can align the mask with respect to the wafer and you are essentially going through 16's steps in order to get the first layer the second layer the third layer and finally, the fourth layer of the molecule, so it can be.

So, if you want to build like you know can array with 4 different layers all to the surface you need 16, 16 steps for these 4 layers and the number of combinations that you can have is essentially if you assume sixteen possibilities in sixteen chemicals steps you have about 256 steps number of possible probes. So, similarly if the prone length is 8 the chemical step is 32 the number of possible probe is 65536.

So can see here that the way that this combination of different sequences increasing tremendous it can go us to all most ten to the power 12 with probe length of only twenty we should involve about essentially 80 chemical steps to get realized. And therefore, it is a really of vast utility the library can be ah the very diverge and also it can develop large number of possibilities just with a few problems on the particular surface. So, light direction synthesis in a way this very simplistic way of understanding how you could capture different combinations all together on a single surface different molecules.

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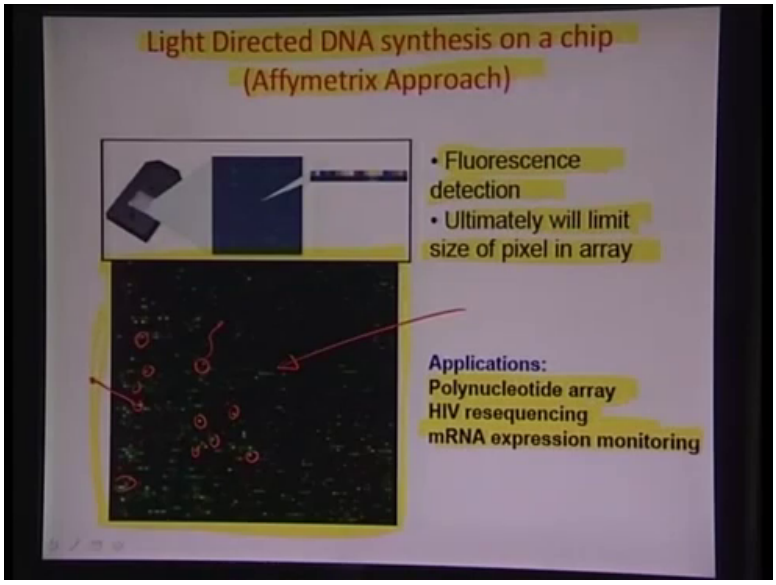

So, all these gene steps where it is a symmetric or nanogen essentially would look in the products forms and something like this is essentially the read out of such a chip. If you can see here there are these a fluorescent pixels which have open and close that various regions of this particular chip which essentially be signifies the binding of the target DNA molecule. And therefore, the capture probe, which may have been in this region would have corresponded to the target.

Similarly, and this particular region would have corresponded to the target similarly and you have idea of how what would be this sequence in the capture probe if you could already know what is the existing capture probe which is here. So, you would give an idea of the target the target molecule if you can know or if can have prior information of what is there is the sequence of the capture probe. So, that is how a real affymetrix read out would look like.

So, ultimately fluorescence detection is the basic means of finding out whether binding has happened or not and ultimately this fluorescence detection would be able to limit the size of the pixel in the array. Because, you cannot really go on synthesizing small smaller and smaller just because of the fact that you have enough in fluorescence intensity for the detector to be able to tell you independently whether it is a pixel or which has opened up a signifies the presence of the fluorophore.

So, if you go on reducing this size there may be a drastic loss in intensity and the detection no long may be able to sense. So, that is a limitation point for designing the pixel size final pixel size although using fabrication would have probably taken out much lesser in term almost orders of would less in the in the existing size. So, the detection in process the limit on the final size of the array and these are immensely utilize now a day's these different a build gene

chips is using various approach, which is in various laboratory related experiments poly nucleotide array, HIV sequencing, MRNA expression monitoring.

Some of these protocols very often in great need of such gene chips to tell you accurately rapidly quantify how much amount of you know what is what can be the sequence on the target or what can be the concentration of the target. So, one more information that you get out of this persons read out is a fluorescence intensity in the fluorescence intensity can signify what is the really binding level in a particular capture probe.

So, if the intensity crosses the certain threshold only you take that data in that case and also beyond that the threshold whatever change in intensity is recorded is also change in concentration of the target molecule. So, therefore, the florescence intensity the providers in addition to the yes no type answer sequence an idea of what could be the concentration of the particular target sequence. So, that is exactly what the gene chips essentially do for gene and analysis.

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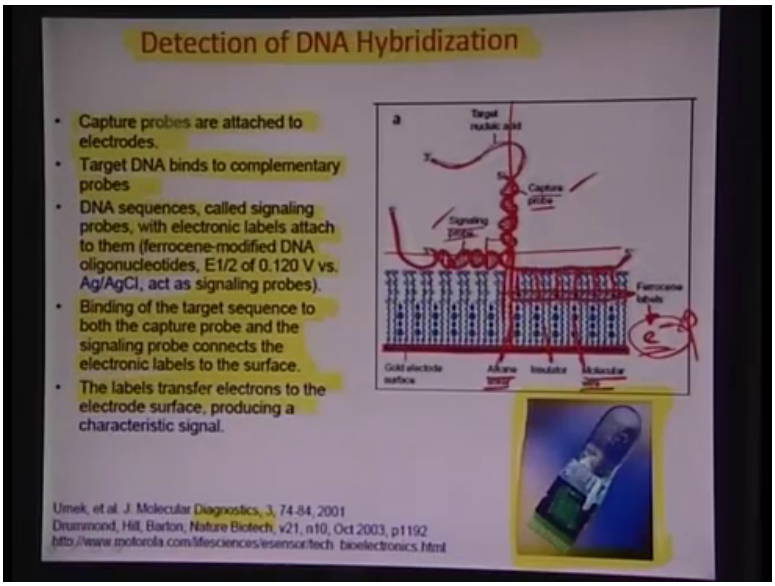

So, there are other protocols names for detection of certain sequences of DNA this one approach here that we would discuss. Next is essentially a chip, which has been develop by Motorola and this was supported also in this case nature by technology journal back into two thousand three and here this is what the chip really looks like. If you look at the whole platform this is what the gene chip really is and here at the detection again is based on a label which is a more like an electro chemical label.

So, the capture probes are attached to the electrodes in the first step and this here if you look out is really the capture probe which is mean mount this blue stain here is a capture probe in bound all the way down to this or Alkane Linker . So, the substrate preparation in this particular processes very critical the surface is prepared in the manner. So, that you have molecular wires. So, in this particular illustrations here as you can see there are the surfaces not really plain surface it is a pretty complicated there is a gold electrode cell surface, which is shown that the blue here.

So, this is essentially the gold electrode surface there are alkane linkers which are in this particular area since later there is a molecular wire which is their inside these alkane linkers. So, the molecular wire essentially are the conductors. So, here is an alkane linker which is an insulator and in parallel to it there is a molecular wire which is a conductor. So, it is conducts like a it is like a you know 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 and if you can see here and the signaling probe this is essentially is the signaling probe this is to make this thing a little more clearer. So, this is essentially is the signaling probe this other end this complementary and of the dna it has a signaling wide this called a signaling probe is, because it varies from three prime to five primes side of the DNA and it contains an electro chemical label called ferocin probe.

So, what a ferocin label typically would do is it essentially is an electron transfer agent it transfers electrons from the ferocin label where an enclose proximity to an electron in this particular case the label if you can see is bonded molecularly to this signaling molecule like this. So, you have a capture probe here bonded to one of these alkane linkers, which is an insulator here and you have a signaling prove here which has a ferocin probe and which is lines some where around in the surface.

So, this is the situation now let suppose we have a target nucleic acid which we want to detect and very intelligently this is the system can pick this up by aligning the signaling probe flat to the bed of the molecule from nano wires and getting the ferocin very close to the molecular nano wires let see how. So, you have a certain sequence in the capture probe and certain other sequence in the signaling probe now this sequence is can be altered in the manner that large dna molecule let say large single stand DNA molecule can bind to both the capture probe as well as the signaling probe.

So, let suppose this three prime to five prime molecule is the target nucleic acids it is a single standard DNA molecule and it has probes complementary to 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 half way through the capture probe and half way through the signaling probe.

But, there is another effect which it leads two words generate you know when this kind of a binding happens there is almost always upper perpendicular angle between the signaling probe direction and the capture probe direction. So, when the binding event happens the binding event kind of ensures that these things are just perpendicular like this. So, this is the capture probes that standing a pride and this is the signaling probe and they are just perpendicular to each other.

So, it gives a some pressure to the ferocin molecules with adjust simply lying on this wire of brushers which has an alkane linker which is essentially insulating nature. So, as the pressure is given because of this perpendicular alignment suddenly capture signaling probe the ferocin level label kind of it is a to a little bit depth crosses the alkane lean linker layer and comes very near this molecular nano wires which are a little shorter then the alkane linker and therefore, this single electron transfers are rapidly recorded.

So, as a they are recorded it gives an idea of a that dna has bound both to the capture and signaling probe of this is a little complicated process and essentially there are few reasons why this kind of a technique technology works very well one is the specificity of detection. So, if you think about the case when there is a dna molecule which has a sequence which is aligning not only to the capture probe, but also to a signaling probe to consider this electron transfer effect or event to happen this is a much better selectivity over just normal hybridization has a which has a 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 have always be in the buzz word in the industry because of the fact that you can align the very well with micro processes. So, that can do automatic measurement data analysis signal acquisition so and so forth. So, therefore, micro chip architectures to always have been associated with some kind electro chemical or electrical detection.

So, this third process of this signaling probe also false and line which is same in dna because these two reasons this is very well known architecture, which is currently available for dna detection Motorola is company which actually cells some of these chips for a laboratory

usage. So, essentially in the summary there is a target dna which binds to complementary to probes in captures probes are attached to the electrodes dna sequences called signaling probes with the electronics labels attach to them.

So, ferocin modified dna oligonucleotides E 1 by 2 at or E half emf generated in this particular examples about 0.120 volts and this essentially ferocin modified dna essentially acts is a signaling molecule by emitting electron and binding of the targets sequence to both the capture probe in the signaling probe connects the electronic label to the molecular nano wire, which is their on the surface and it can do the electron conduction on the table transfer electrons to the electrode surface producing characteristic signal which would give an indication whether of the dna that you are looking at the target dna has a base pair complementary 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 market. So, I have been probably able to kind of take you through at least some of the few intricate gene chip arrangements that commercially available. Now, let us look into are kind of a totally different aspect of how to sequence at dna and this reaction is also popularly known as the Sanger's reaction develop by assign this call Williams Sanger in 1975 for, which he won the Nobel prize it is one of the first steps in identifying the sequence of information that is their on a DNA molecule. And this is also very foundational for the field of a molecular biology or diagnostics to have developed it kind of emerged from this sequencing activity.

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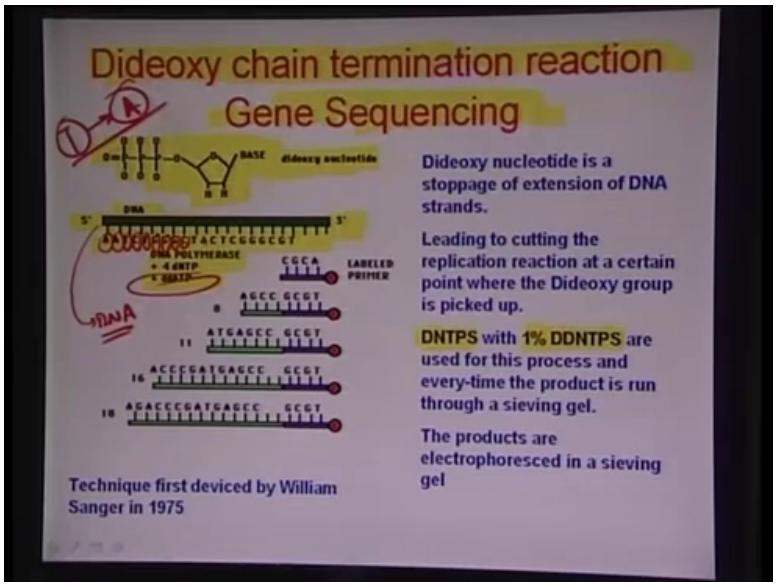

So, let us look at how the this reaction has been designed in step by step manner. So, to do this thing first what we need to certain find out is what all of the constitution of this reaction. So, let say we are trying to sequence this particular dna product which is five prime to three prime. So, this kind of dna structure and let say you want to find out what follows what in terms of these molecules. So, as you seen here this molecules a ts c t g gg cn so and so far is this sequence information that is really not visible at the outside when you have this DNA molecule with you we want to find out what is there in the sequence looking at this molecule is a whole.

So, this reaction is also known as the dideoxy chain termination reaction dideoxy chain termination reaction is the gene sequencing. So, let suppose as we were talking to before we want to detect the sequence on this particular molecule the single standard dna molecule which you are seen here from five prime to three prime. So, you need that the following for detection you need a dna polymerase you know already how the dna polymerase is work such an enzyme which kind of zips the dna open.

Then, try to suture the two daughter strands to make two complete strands by fixing olygoneuotides form the solution one by one on the five to three prime direction and from on the three to five prime direction it does. So, by producing fragments the okazaki fragments which we discuss in about the last lecture. So, that is what the dna polymerases is to need a dna polymerase for this as well as you need the neucleotides A T C and G independently is called dntp.

So, when you have four different dntps and then you have dideoxy chain terminated let us suppose atp in this particular case. So, it is a dideoxy chain terminated nucleotide also represented as this here this is the chemical structure of this nucleotide. So, the advantage of the dideoxy chain terminated nucleotide in such a reaction is the as and when this particular nucleotide times to certain region of the chain the reaction terminates that is why it is called chain terminating nucleotide.

So, whenever this dideoxy the dideoxy group essentially prevents the first further development of suturement of the sugar molecule to the next linkage it gives an energetically least favor favorable configuration. And so therefore, whenever there is a dideoxy group which comes and sets at towards the end of the chain the chain does not replicated any more kind of terminates there. So, we use these as an idea for stopping the reaction in a control

manner as in when very require to stop, now also in important to mention here is that this dideoxy group has some kind of .

So, it what is important to meet would tell you was that you know particularly of this dideoxy group in this particular case it has the certain concentration with respect to the DNTP. So, the dideoxy chain or the dideoxy all nucleotide is of certain ratio in comparison to the actual DNTPS's which are present without the dideoxy probe. So, normally in all Sanger's reaction processes the DNTPS's as we popularly know them the dideoxy nttp is about one percent by volume of the DNTP's this is a standard which is followed in the reaction design.

Now, interestingly when this kind of a reaction proceeds and let suppose we have a ddatp as in this particular case the DDATP. So, there would be fragments produced from this DNA wherever there is wherever there is a binder molecule t we know that the thymine times to that adenine from earlier knowledge about the dna. So, whenever there is a thymine molecule some where placed in the chain. So, the adenine molecule some where place in the chain that may be a point of cleavage or cleaving the molecule as advent the dideoxy times with the thymine on it on the particular a.

So, suppose there are fifty centers with the nucleotide a on the parent strand on the dna molecule and this dideoxy chain terminating thymine probe which is present as one percent of the normal DNTP's that kind of binds whenever it binds to any of those fifty sites for A it terminates the change. So, the n result of this process of reaction is a set of fragments of a of change which are terminated each at the adenine. So, it kind of gives a location to us of the adenine on the particular base pair on the particular parent’s strands of the dna molecule.

Now, let us suppose we have four different such reactions with d dideoxy dtp dideoxy CTP dideoxy GTP each of them are one person of the normal DNTP's and these four reactions are all independent in four different wires. So, what is going to happen is that wherever there is a presence of a g molecule would be terminated and fragments would be developed in the reaction vessel which contains the dideoxy g molecule.

So, similarly when there is a fragment present of let say the adenine there is a when there is a when there is a n group or group that we are trying to discuss zyber is guanine in that case we can produce the fragments of wherever they go line was present in the parental chain by putting it with one percent d CTP or a dideoxy you know CTP cytosine nucleotide. So, if we take all these orders and run it on a gel something like this.

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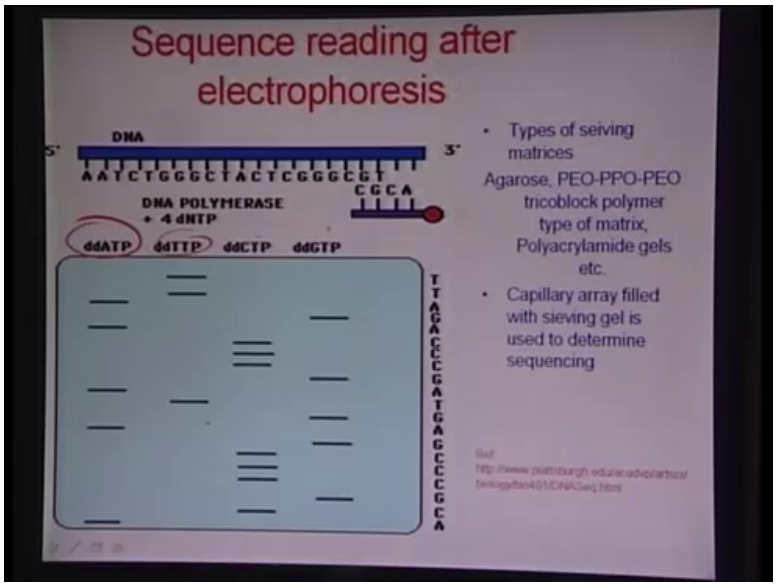

So, what happens is wherever there is a chain termination on the ATP would develop based on sizes this fragments, which we can later on glow up by using florescence for the DTTP case there can be fragments in this manner. Similarly, for the CTP and GTP and if you do the overall read out will be able to get the position of what follows what in this particular chain. So, I am going to kind of take this ahead a little bit later we have a almost come towards the end of this particular lecture.

So, the next lecture will talk about this Sanger process a little bit more in details and try to see how micro scale architecture can be kind of develop with respect to Sanger reaction to do rapid sequencing.

Thank you.