Bioengineering: An Interface with Biology and Medicine
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Lecture - 15
Clinician's Perspective-II

Welcome to the MOOC NPTEL course on bioengineering an interface with biology and medicine. In the last few lectures, we have discussed about gene cloning, polymerase chain reaction and various DNA tools. What is so interesting to learn that currently the revolutions in gene and genomic technologies have started paying directly in the clinics, lot of patients are getting benefitted because of these genomic technologies.

In order to illustrate some successful examples and to give you message that how biotechnology engineering technologies have started making its impact in the clinics today we have invited a distinguish guest Dr. Nikhil Phadke to interact with you.

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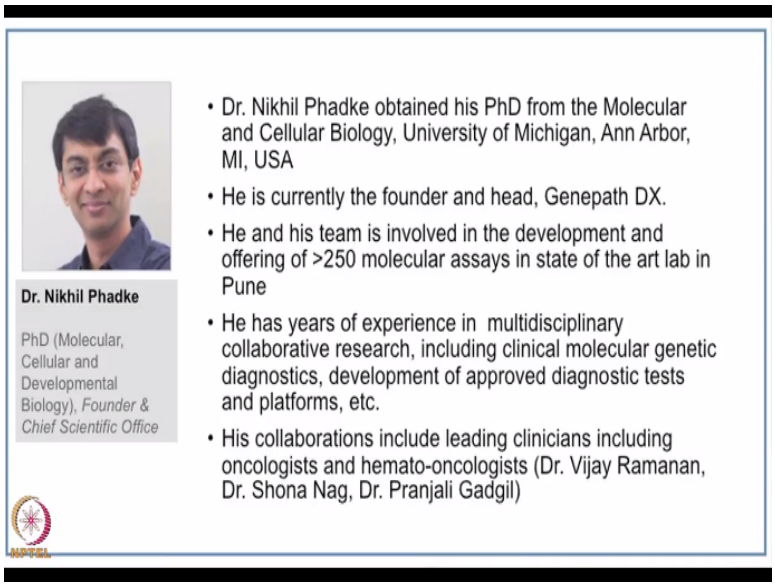

Dr. Nikhil Phadke obtained his PhD from the Molecular and Cellular Biology, University of Michigan, Ann Arbor in USA. He is currently the founder and head of a company GenePath DX in Pune. He and his team is involved in development of over 250 molecular assays in a state of the art lab in GenePath in Pune.

He has years of experience in multidisciplinary collaborative research including clinical molecular genetic diagnostics, development of approved diagnostic tests and platforms. He

has various collaborators and therefore his projects involved in different areas of oncology and hemato-oncology as well as infectious diseases.

I definitely felt very impressed with Dr. Nikhil Phadke's work and realize that whatever the latest advancement we see happening in US clinics where patients can directly see the benefit of looking at the gene mutation, the sequencing analysis, those things Dr. Nikhil has brought very quickly back here and those have been started making revolution in patient care.

Today, it is great pleasure to have Dr. Nikhil Phadke with us who is going to talk about clinical and commercial applications of molecular genetic diagnostics, the HandyLab and GenePath DX story. Welcome Nikhil.

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Our story actually starts in a class very similar to this. Two of my colleagues who were the cofounders of the company one is from IIT Mumbai and one is from IIT Chennai which is it is really a personal story. I have been teaching classes in medical schools and international research and I am told that these are some of the brightest students in the country. So I am sure you are all going to be superbly successful at whatever you do.

It would be nice if you could also enjoy yourself by being while being successful and make a positive impact on society while doing that, so if all these things happen then it is absolutely fantastic. I will talk a little bit about myself. I trained at University of Michigan, Ann Arbor. I worked in the medical school there and in the science and engineering schools.

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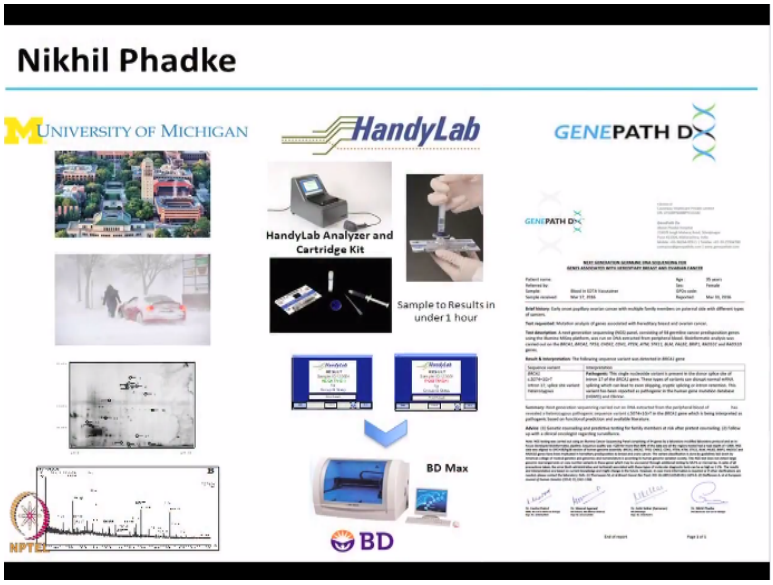

And Ann Arbor always has those pretty pictures up there in the brochures but really most of the time it is just full of snow. So we have two seasons, winter and construction anyway. So after working there for slaving day and night in the lab, I got together with a bunch of my friends, many of whom were from IIT and we set up a company known as HandyLab and we built a molecular diagnostic platform.

Molecular diagnostic platform that integrated engineering principles, science principles and medical principles all together. So really a melting of minds of different areas of specialization and after we were successful with that I came back to India and started a company known as GenePath, which is focused on doing pretty much the same thing that we did in the US but catering to the needs of Indian patients and Indian doctors okay. I come from a medical background.

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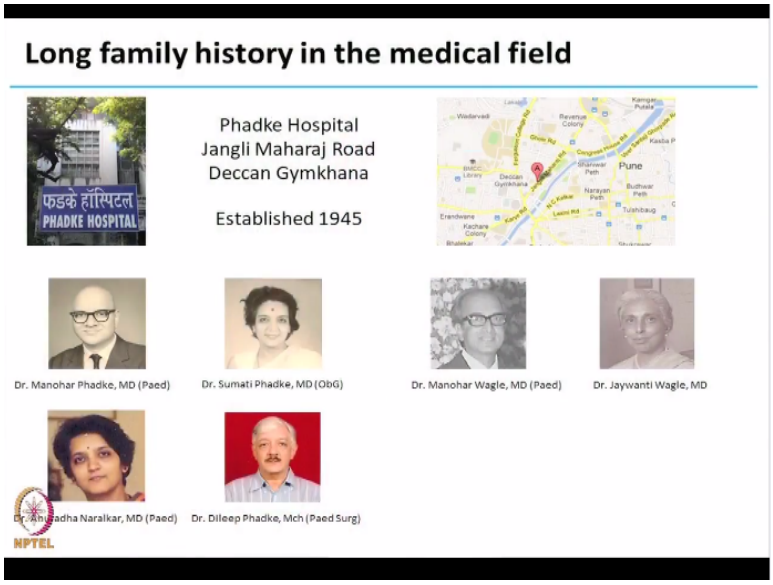

There is 4 generations of doctors in my family on both sides and we have a hospital that was started in 1945 in Pune and my lab is actually based out of there. So there was a motivation to get into medicine but not actually do day-to-day medicine the way they had been doing it. I was also interested in engineering, so nothing better to combine all those areas into one.

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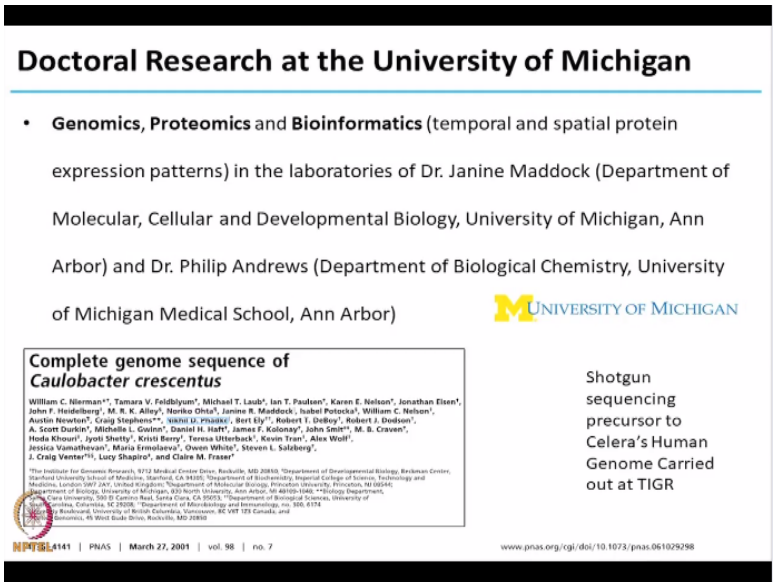

My doctoral research was at the University of Michigan and I worked on genome sequencing before the Human Genome Project was completed and if you look at the people on this, so there is Craig Venter there, that is me there, this is one of the projects that we did before the Human Genome Project was completed and we took almost 2 years to finish the sequencing of this genome.

It was a lot of fun but we were totally clueless at that point in time but the two years is an important part to remember. Anyway, throughout my PhD work, I did a lot of work on genomics, bioinformatics and proteomics.

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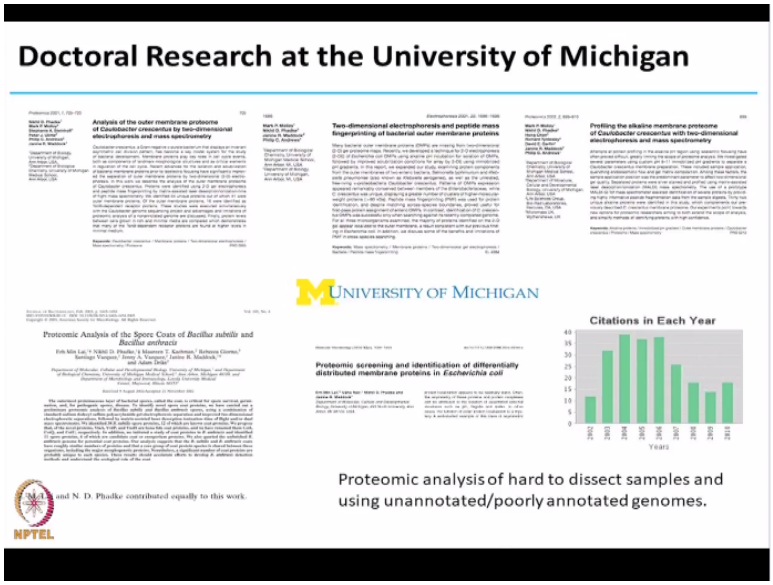

And the nice thing is that even after all these years many people are still quoting our research. It remains relevant even after all these years.

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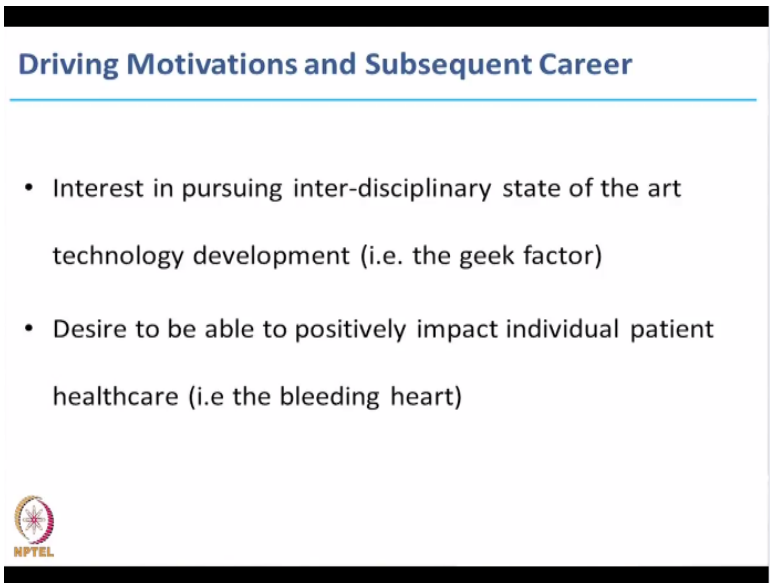

But after I finished my PhD a question that I had to ask myself and a question that you guys will be asking yourself at some point. What is it that really drives you and what is it that makes you really happy because you are going to spend more hours in a day doing that than not doing it. So find out what really drives you okay. If it is photography that is great, if it is

making boatloads of money that is great whatever it is but find out what it is and then go after that and if you need to take some time off to figure that out, do it.

So mine were I was really interested in interdisciplinary state of the art work, so I did not want to do just medicine, just science, just engineering. I liked all that stuff coming together. I am a geek basically and I also wanted to be able to impact individual patients and there is no joy like seeing a patient come up to you and say you know what sir I had no idea what was wrong with my kid but you guys have figured it out.

There is nothing like it, I think that is an amazing sort of motivation to keep you going through the day and the night. So these were my two motivations, they do not have to be your motivation. I am just saying figure out what yours is whatever it is is fine but take the time to find out.

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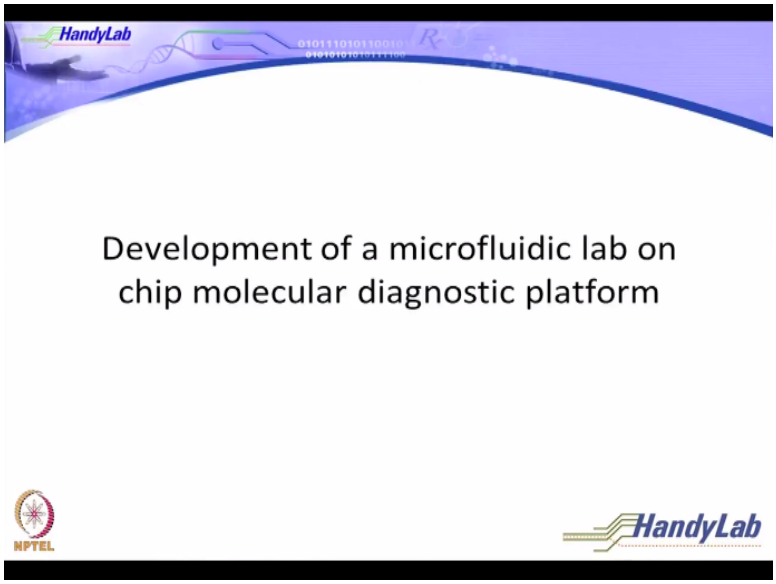

So the first thing we did was HandyLab. So HandyLab was developing a microfluidic lab-on a chip diagnostic platform. So lot of the stuff Sanjeeva has been teaching you about how you work with DNA, etc. These things are done in a lab and there are lots of people in the lab with lots of expensive equipment. If you look at a lab from the top, it is basically unit operations of fluid moving from one place to another.

So you take some liquid a blood sample, it goes into the labs. Somebody might heat it up or cool it down or centrifuge it, stick it on the machine you measure the pH or you measure the

conductivity or you measure the optical properties you combine it with something else. These are all unit operations.

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What we did was automate all those unit operations on a microfluidic cartridge that is a network of tubes and pipes inside a thin little chip. Unfortunately, I did not bring it with me today and the idea was to have a diagnosis in a completely automated manner at micro scale in a very low time.

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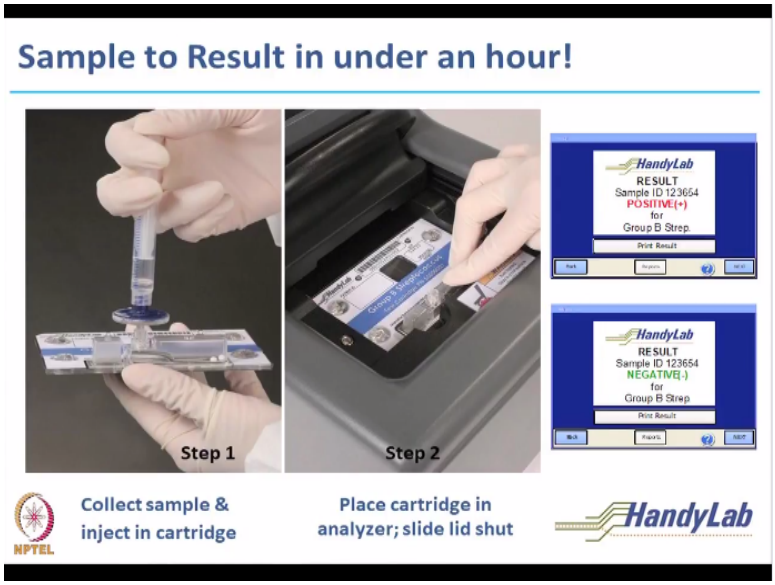

So one of the first test that we developed in the US was a test for Group B Strep. Now Group B Strep is an infection that infects pregnant women around the time of delivery and it can cause meningitis in the child and the child can die. The thing with that diseases that it can be

treated with a simple single dose of penicillin up to four hours before the child is born but the older test took 72 hours to do by growing it on a culture microbiological culture.

So you had to go in between 38 weeks, 37 to 38 weeks to get a swab, 72 hours later they would tell you if it is not and then they would give you antibiotics. Now you can lose the infection in that amount of time or gain a fresh infection. So that is a little bit of a problem. So we develop this test that gives a diagnosis in 45 minutes. It takes the sample, extracts the DNA from it, mixes it with all the reagents which are inside that cartridge, optics everything, built-in and you get a result positive or negative in 45 minutes okay.

So that to actually pull off something like that we needed lots of diverse areas of expertise to come together.

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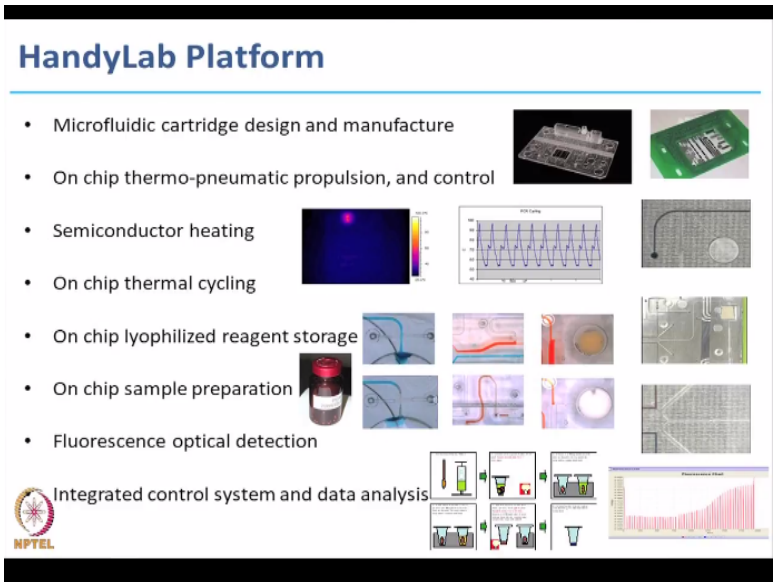

So one is microfluidic cartridges, designing them and manufacturing them. They have to be something that can be built for individual patients not something that only works at 1 million dollar lab. Patients have to be able to get it. All the stuff that we moved on the chips was through thermo-pneumatic propulsion and control. So that means we would heat up areas of the chip, air pockets above that would expand and push liquid.

By firing many of these different types of pumps on the chip, we could mix liquids together. We had gates and valves, so phase transition materials that are solid at one temperature but when you heat it they melt and then you push an air pocket through, so you can open a

channel that was closed and the reverse when you have a closed channel or you can close a channel by forcing in wax from the side.

So wax that is solid at normal temperature but liquid when you heat it, so wax gates, valves, etc. So this is like I do not know if you can see it on this particular video here but yeah can you see that that is the heating profile of the chip. So we are heating different areas of the chip, it is almost like one of those predator kind of movies but this is a thermal image of the chip and we are pumping different areas, mixing liquids together.

This is thermal cycling for PCR. Here is some liquids mixing, right there you can see liquids mixing on the chip. So basically what is happening in a big lab is now happening in an automated manner out there.

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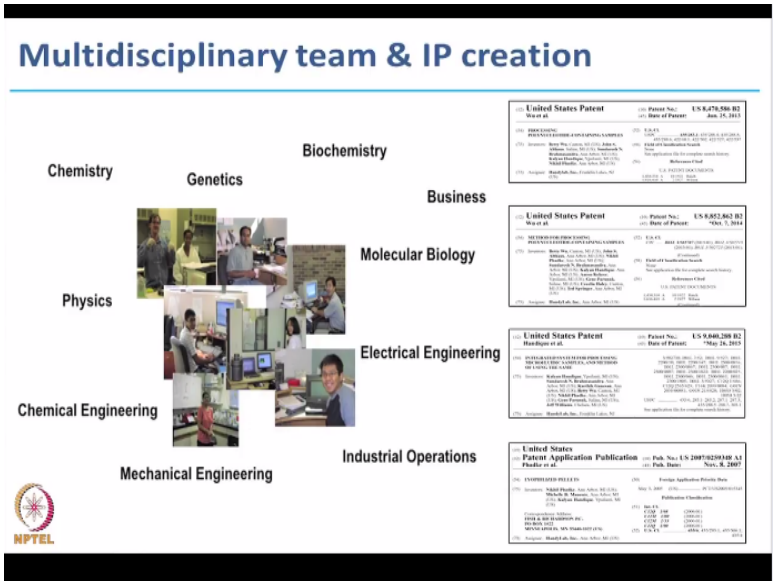

As I said again I keep stressing interdisciplinary, so people from physics, people from electrical engineering, mechanical engineering, chemical engineering, molecular biology, business people, genetics all working very tightly together to build all this technology. There was a huge number of patents. I have just shown 4 out here. Large number of patents that were generated along the way.

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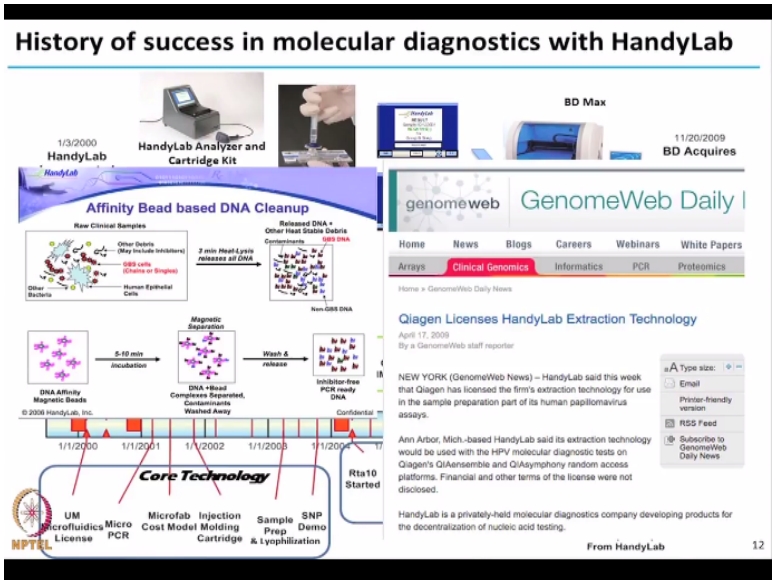

And we took nearly 10 years to build this, 9 years to build this. So hard work, there is no substitute for hard work but at the end of the day you can make to be polite a shitload of money. So it is well worth doing and if you are interested in this kind of work, there is a huge reward at the end of it. The technology that we created was globally valuable. So Qiagen which is one of the world's largest nucleic acid sample prep companies actually licensed our patents for their current generation of products.

So the idea is that people who are sitting here are going to make great things in lives in their lives and you know these are examples of things that you can do to be world leaders not just the best in India, you will be world leaders with the kind of work we do. So Becton Dickinson bought over our product after we took it for an FDA and then now market rate globally under the BD MAX brand name.

So having done that I wanted to continue doing that but to do something that was more relevant to our patients here in the context of Indian Healthcare and in India unlike in the United States patients pay out of their own pocket and so they cannot afford the same kind of things that they afford in US.

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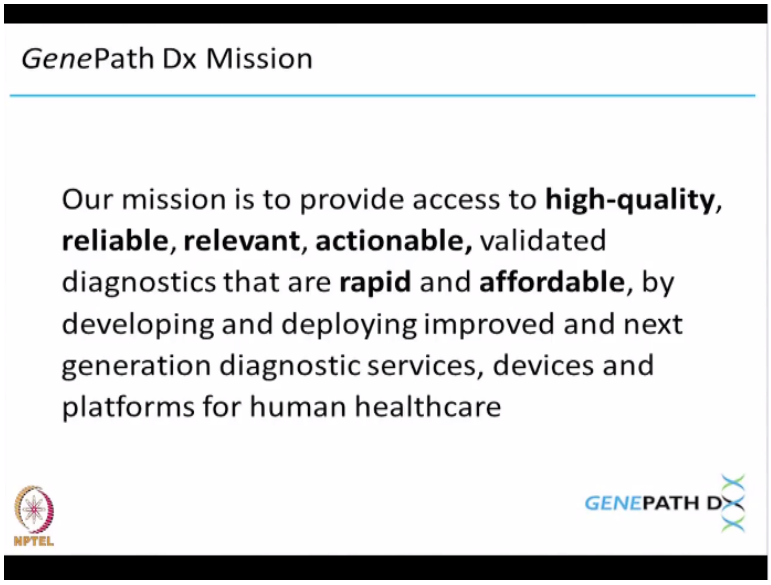

So we wanted the same high quality, it had to be reliable but they needed to be relevant to the problems here and they had to be affordable and so we wanted to start by offering services and then go on to building devices and platforms. So go back to building what we did similar to the US but do it in the context of India.

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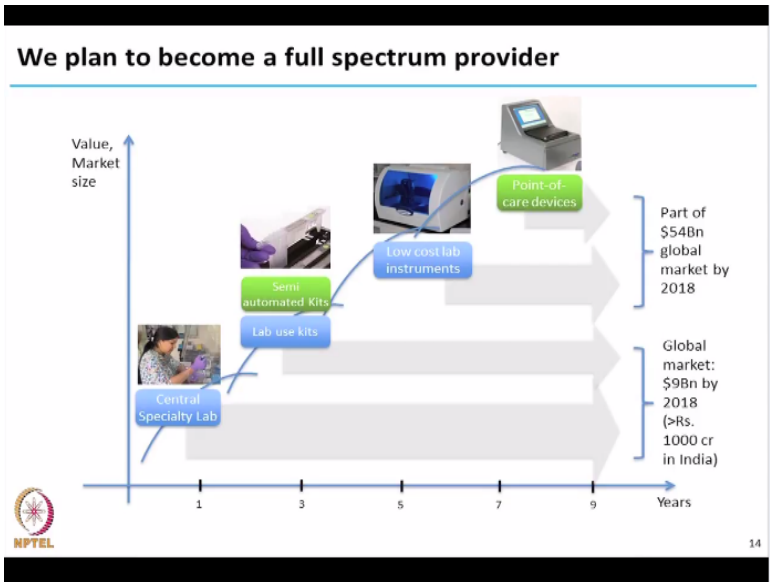

So our company in Pune is now in the service mode and we have just embarked into building devices okay. So service mode is there, it is a big market. We do this, we have over 350 molecular diagnostic tests in our lab right now but we are also starting to build products.

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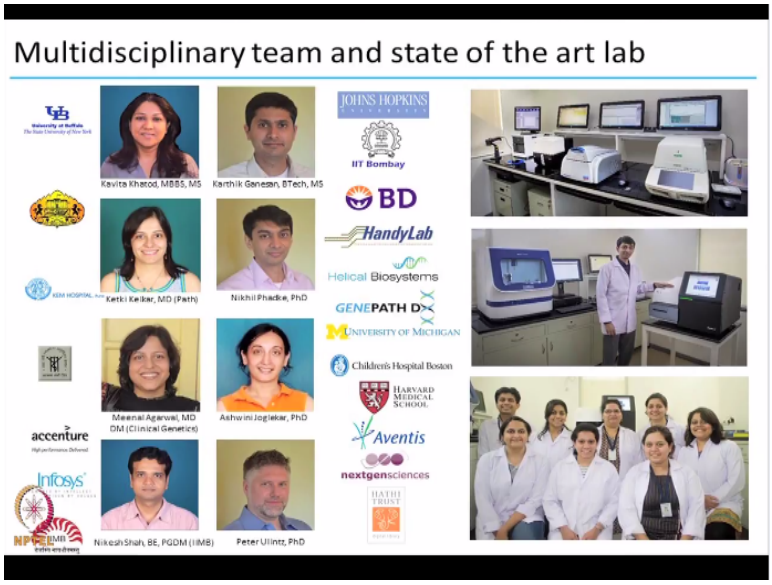

Keep stressing multidisciplinary but here is our team core team. We have MD DM people, we have PhD's. Karthik is from ITB and from Hopkins, I am from the University of Michigan, Meenal and Ketki are MD path and clinical geneticist, Kavita is a mixture of scientists and a clinician, Peter is a bioinformatics person. So we all work very, very closely. We also have PhD students in our lab.

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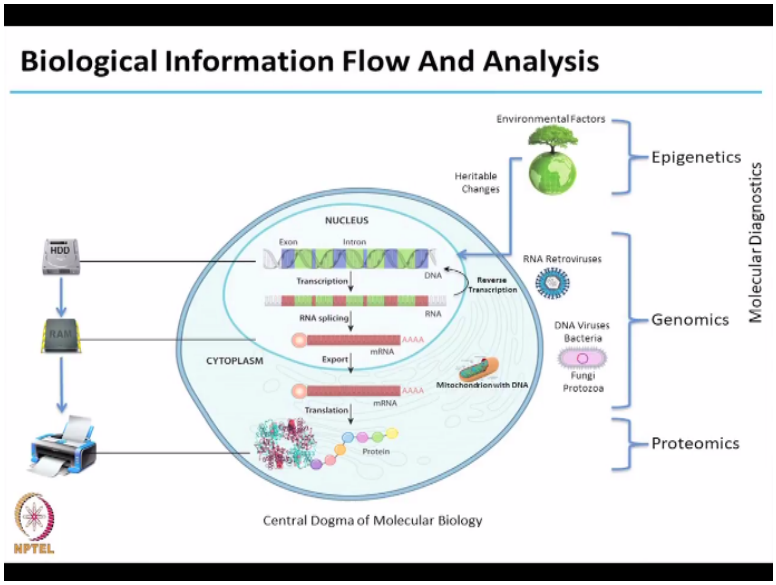

So now to get to some examples and a little background, so Sanjeeva has been teaching you about the basis of you know DNA and the basics of molecular biology. I will just go through it really quick. In human information systems, information usually flows in a direction, usually there are some exceptions. Information goes from DNA to RNA to protein. So DNA is like a hard drive for your system like you buy a new computer, all the information that you need is on the hard drive.

But when you want to use a program like I am using PowerPoint right now, the information that is required is taken up into the RAM. Similarly, in biological system the information that is required at a given point in time is taken up into RNA and finally the effector molecules like the outputs in a computer system like you have a printer or a display, similarly the actual work is done by protein molecules.

So information flows from DNA to RNA to protein. There are some exceptions; retro viruses like HIV change the game. They go from RNA back to DNA and mess up the system. We also have interaction with the environment through processes known as epigenetics and epigenetics actually reversibly writes through the DNA and modulates how the DNA is converted into RNA.

So there is more and more understanding. This is a very exciting area of research right now, so just keep that in mind.

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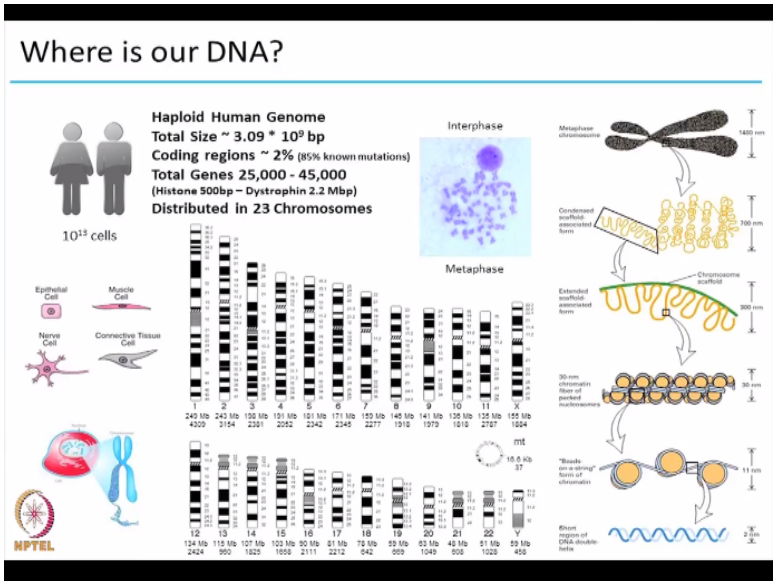

So where is this DNA and what is this DNA basically? So it is like an encyclopedia for each one of us okay. It is like an encyclopedia with all the information you need in your life essentially how your body is going to react to its environment and this information is a very long message. It is 10 to the 6 base pairs or 10 to the 6 letter of an alphabet and the alphabet has got four types of letters A, T, G and C unlike English which has 26.

Now this big long messages actually divided into 23 volumes from your father and 23 volumes from your mother. So think of it as an encyclopedia that is broken up into many volumes, 23 volumes from your father, 23 from your mother totally 46. Now each one of these volumes is a chromosome, so you heard of the word chromosome. The volumes are the encyclopedia of the chromosome.

Within every chromosome or within every volume, there are paragraphs that make sense and the rest of the paragraph that do not make sense. The parts of the paragraph that make sense are the genes. So we have about 25,000 to 45,000 genes in our system, so that many paragraphs in there. They are distributed amongst the 23 chromosomes and you have 2 copies of each of these chromosomes, one from your father, one from your mother except for the sex chromosomes.

Males have one X and one Y, females have two X's. The interesting thing is that in this entire encyclopedia only about 2% of it has known function today. This 2% is known as the exome and 85% of all mutations in our disease sort of the diseases that we know and understand are located in that 2%. So that 2% becomes very, very important to study. So every time a cell divides this entire encyclopedia gets replicated okay.

Six billion bases are getting replicated every time you make a copy of a cell. Mistakes will happen, we have machines to correct those mistakes but sometimes those machines fail and you have the mistakes carried over.

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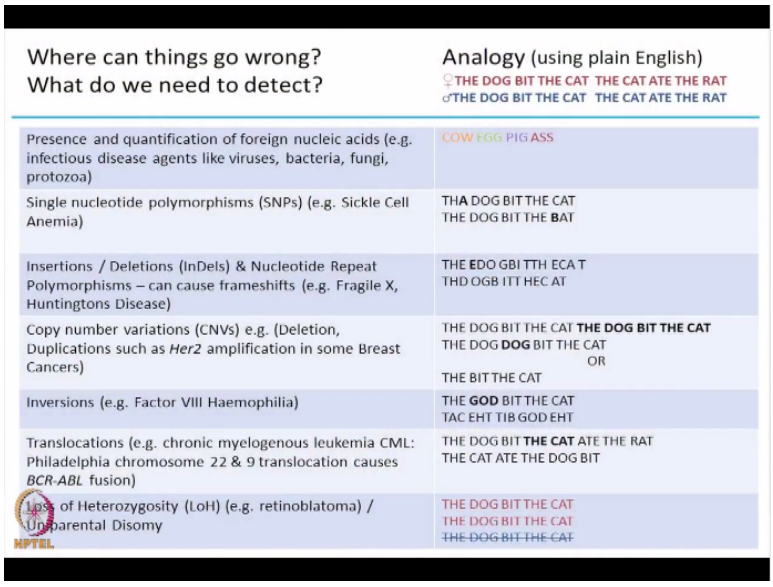

So what are the things that can go wrong? One is that so I have an English sentence, the dog bit the cat, the cat ate the rat. There is two copies of it, one from the father, one from the mother. This is how the sentence should normally look. You can one have an infection, you can have something coming from outside cow, pig, ass whatever that is not supposed to be in there, if it comes and messes up the meaning of the sentence.

So that is foreign DNA or foreign nucleic acids coming in. You can have spelling mistakes which have single nucleotide polymorphisms SNPs. This is when a single alphabet or a single letter in the alphabet changes. So in the top case you can actually understand the meaning of the sentence, so it does not really matter so much but in the lower one because the C has changed to a B, the entire meaning of the sentence has changed.

So this SNPs can be bad, they can be harmless depending on where they happen that is important. You can have insertions and deletions that is addition of a letter into the message, these are almost always bad. Conditions like Fragile X and Huntington's disease they are really nasty diseases, they are often caused by insertions and deletions and this is because when you say added the C there, the complete meaning of the sentence changes.

Similarly, when you remove something the meaning of the sentence changes, you cannot recognize it anymore. You can have copy number variations where entire messages or parts of the message are replicated. You can have inversions where the message is flipped over. You can have translocations which is like buying a cheap pirated book on the street, you are reading chapter 9. Suddenly it goes to chapter 22.

The sentence in itself makes sense but overall the story is making no sense. So that sentence makes sense makes a protein that does not really work well, hell breaks loose okay. Loss of heterozygosity is very complicated, I will skip it further. So there is a lot of mistakes that can happen, you need to use the right tools to figure out what the mistake is. Somebody will want to use the most complicated tool available and charge a huge (()) (17:51) but that does not make sense.

So the right diagnostician has to understand the context of the disease, the molecular and the clinical background and pick the right test that is going to be relevant to the patient in a clinical relevant time.

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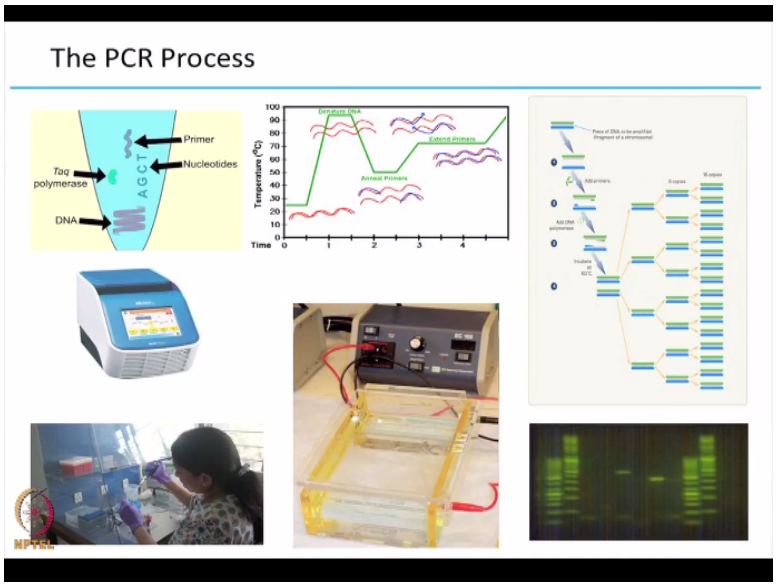

So the most all of our technology is based on two very major things. One is PCR, the polymerase chain reaction. Do you all know PCR? Have you all done it in this class? Briefly, so the polymerase chain reaction was invented by a total slacker known as Kary Mullis who got the Nobel Prize and everybody is still scratching their heads about why he got the Nobel and how he managed to pull off something so brilliant.

But it is basically a system for photocopying DNA, it is like a xerox machine, you take one molecule of DNA it becomes 2, it becomes 4, it becomes 8, 16 and in a very short amount of time half an hour 45 minutes you can make a billion copies of DNA from a single copy in a test tube okay and that is really the basis of almost everything that we do in the lab. The Watson and base Crick pairing that he talked about where A pairs with T, G pairs with C is exploited by the PCR process.

Again this class is too short a time to explain and introduce PCR but it is the cornerstone of everything we do.

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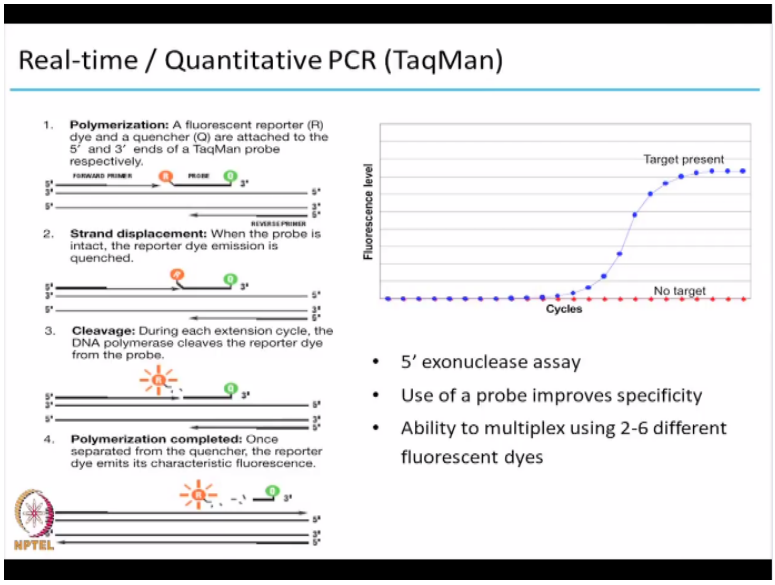

You can couple PCR with a fluorescence event and monitor it in real-time. So when there is more DNA you get this S-shaped curve. The more to the left this is the more starting material you had, so you can use that to detect whether infections were present, how much of it was present. If you had a mutation in a gene, what ratio of mutant to non-mutant, so this is probably one of the most important techniques that we use in the lab day-to-day.

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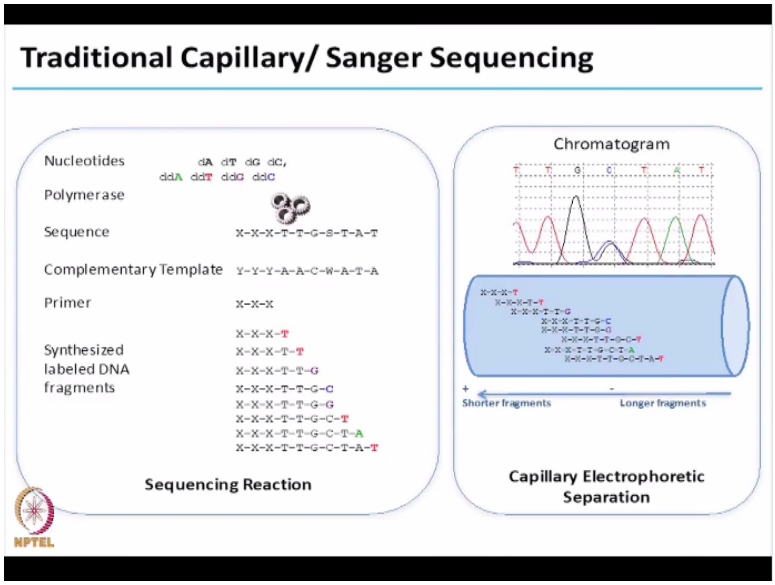

The other one is capillary sequencing. In capillary sequencing again was a Nobel Prize winning effort for Maxam and Gilbert as well as for Sanger and it is a way to read DNA base by base one by one.

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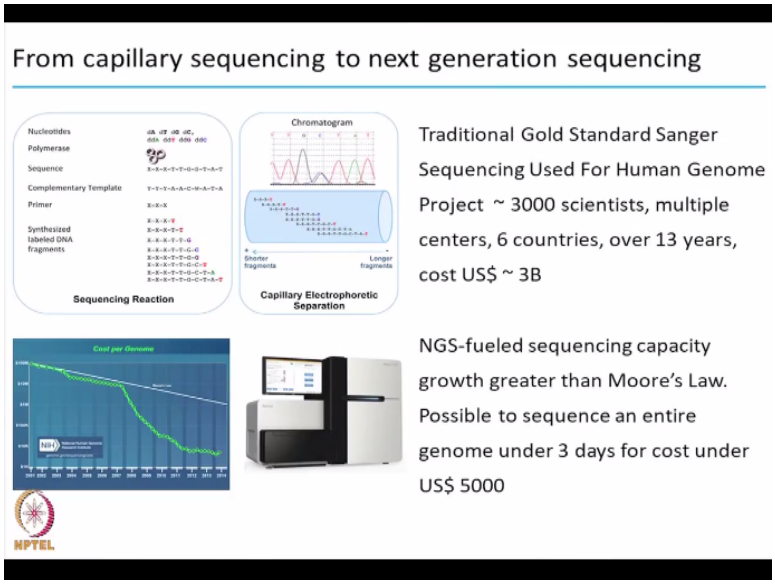

Now Sanger sequencing has been the cornerstone of our sequencing abilities for a long, long time and the Human Genome Project which everybody must have heard of hopefully was completed in 2002, it took 3,000 scientists, 13 years and a cost of 3 billion dollars to sequence the first human genome. Many countries across the world they were all working day and night these machines running by Sanger sequencing.

Today, it is possible to sequence a single genome in under 3 days for 5,000 dollars. This has put Moore's law completely to shape and this is because of a series of technologies known as Next Generation Sequencing.

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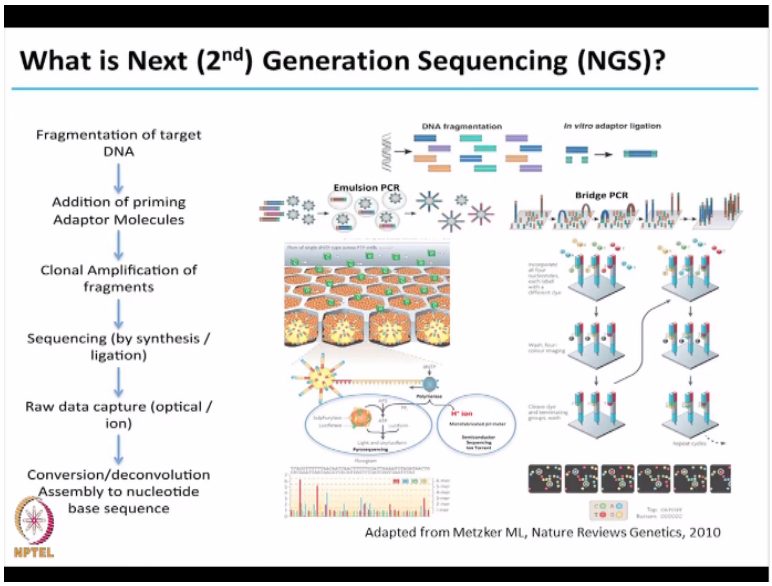

Again explaining Next Gen Sequencing is beyond the context of this class but it is a parallel process. So basically what you do is you take all your DNA, you fragment it into small

pieces, each small piece you clonally amplify and you sequence it individually. So our sequencers in the lab today can do 25 million sequencing reactions in parallel on a chip of this size.

And the newer models that are coming can be 10 or 100 times more powerful than that. So you break your DNA, you add adapter molecules to barcode them, you clonally amplify them, you sequence them in real-time, capture the data and then you put it back all together like a giant jigsaw puzzle. So because of the increase in computational power this has been possible but just think about it, 17 years ago what took 13 years to do, today can be done in under 3 days okay.

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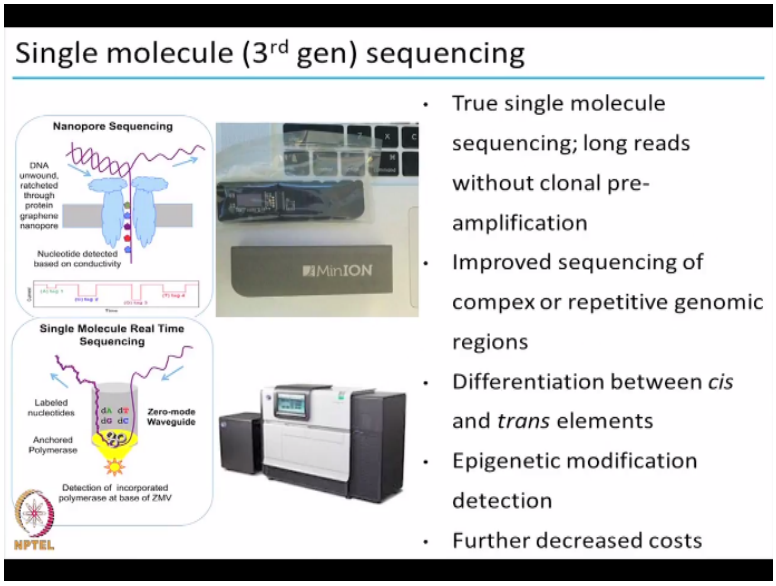

This is just mind blowing as it just boggles the mind and even that is only the start. Now we have this single molecule sequences and we have some of these in our lab which can take a single molecule of DNA, thread it through a nanopore and read out the sequence in matter of seconds. So they are going to further change this.

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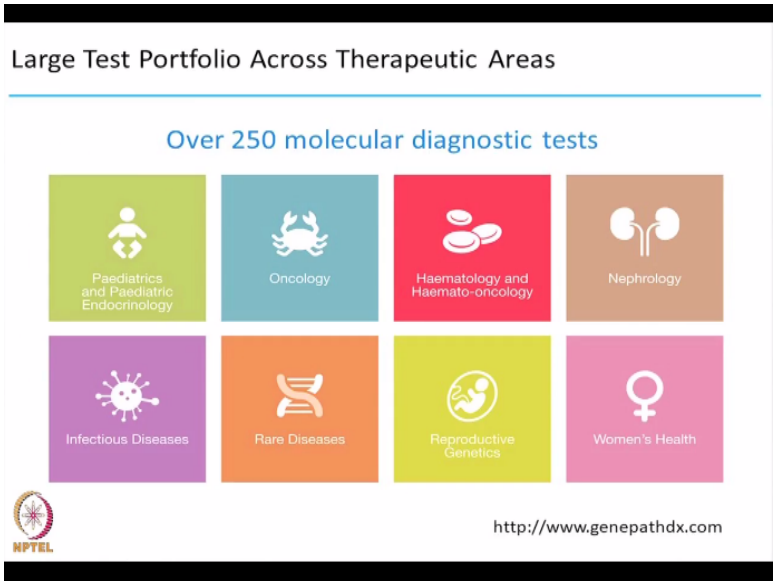

So we use all of this, now this is over 350 tests that we have but we use this for many, many different areas to actually manage patients and I am going to give you very few examples of where this technology is used.

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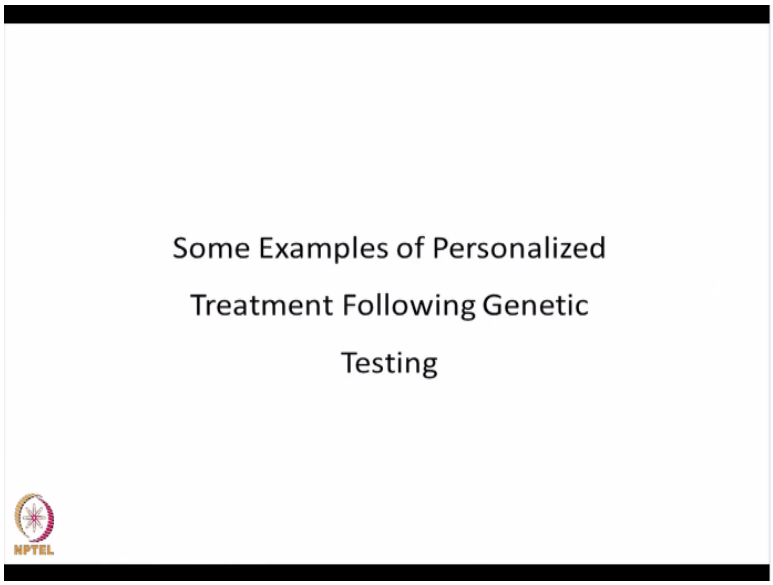

So one very common use is in hereditary breast and ovarian cancer okay. There are I told you there are about 25,000 to 40,000 genes and every time the DNA gets a cell divides the entire DNA gets copied over. Now there are some machines that prevent errors and BRCA1 and BRCA2 are two examples of those machines that prevent errors. So when there are errors, they fix it.

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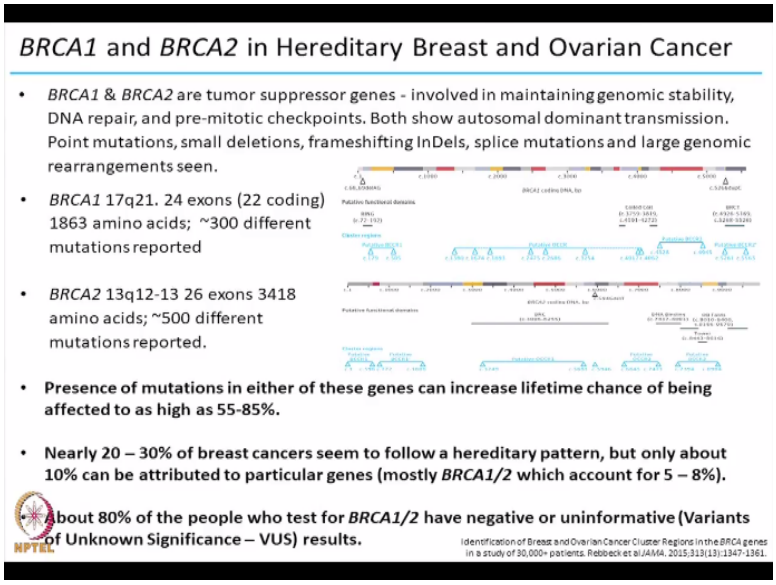

Unfortunately, sometimes these error correction mechanisms also fail and so if you have a mutation or an error in one of these genes you have as high as an 85% chance of getting cancer okay. It is a very, very high chance and nearly 20 to 30% of all breast cancers and ovarian cancer seem to follow that kind of pattern. So what are the advantages of genetically testing for this?

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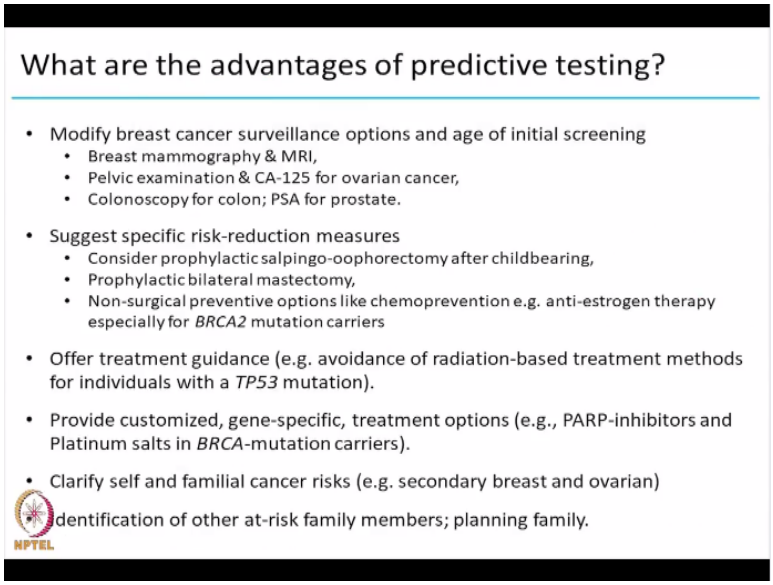

One is that if you know that you have this mutation, you can modify your surveillance options. So you can go in for improve surveillance and this is not only affecting women, men can also get prostate cancer, they can get breast cancer themselves which is far, far more dangerous in men than in women. So we can have specific risk reduction measures. So we can have salpingo-oophorectomies, we can have bilateral mastectomies and advanced surveillance.

Depending on the mutation they have to decide whether they need to get radiation or not get radiation. Once they have the cancer, we can change the therapy that they use, so if you have a BRCA mutation, we can use platinum based salts, we can use PARP-inhibitors which are very new exciting drugs.

Some of our patients who have been using them were in sort of almost terminal stages and now they have got a fresh lease of life and we can figure the risk to other family members also.

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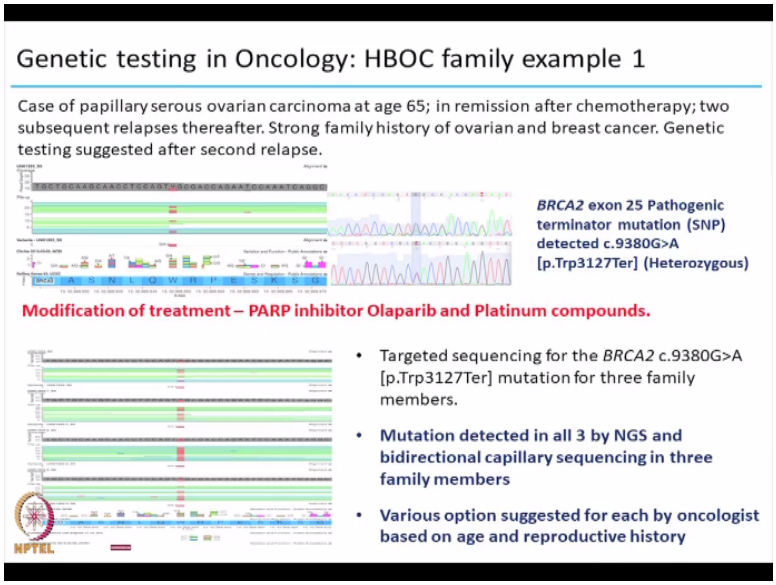

I would not get into details of individual patients because I think we are running out on time.

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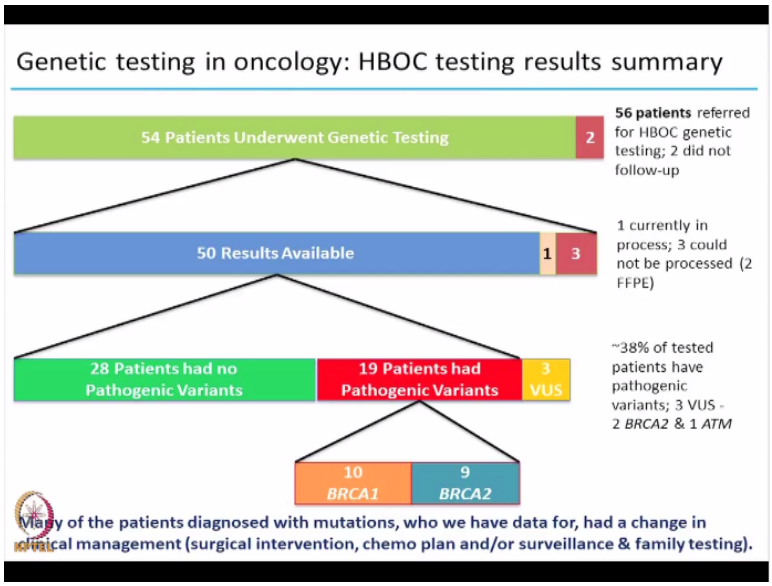

But just the summary is basically of all the patients we have tested nearly 40% of them had a change in clinical management after they did genetic testing. So you do the testing and what you would have done by the old method of medicine is no longer done, they would have some new treatment options.

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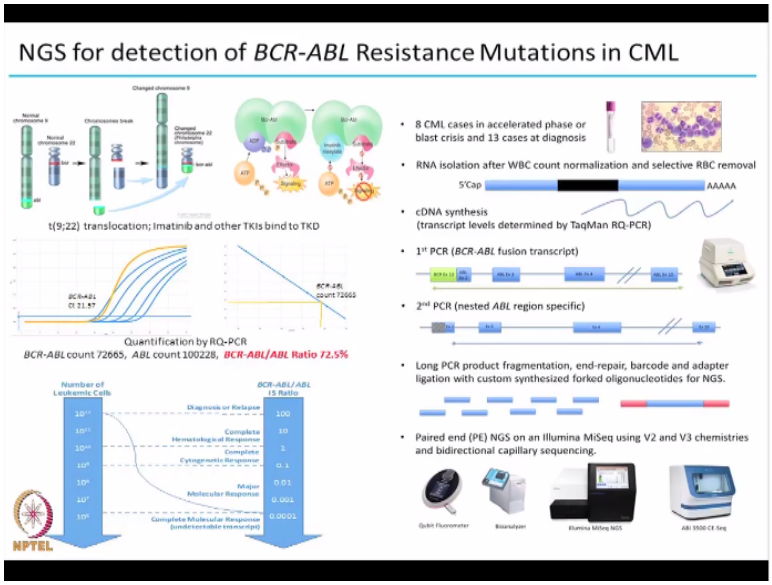

Another very nice example, use of genetic testing is in CML which is chronic myeloid leukemia. Chronic myeloid leukemia up to 15-20 years ago used to kill over 90% of the people who got it. Today, thanks to a drug known as imatinib, 98% of people have a normal healthy life. It is absolutely like magic but to prescribe imatinib, you have to show that your chromosome 9 and 22 have fused and we have a genetic test to do that.

We test the patients; find out if they have that fusion, if they have that fusion they get imatinib, every 3 months we monitor them to make sure they are responding correctly to that drug. As I said 98% of patients do very well but there are 2% of patients who do not do well.

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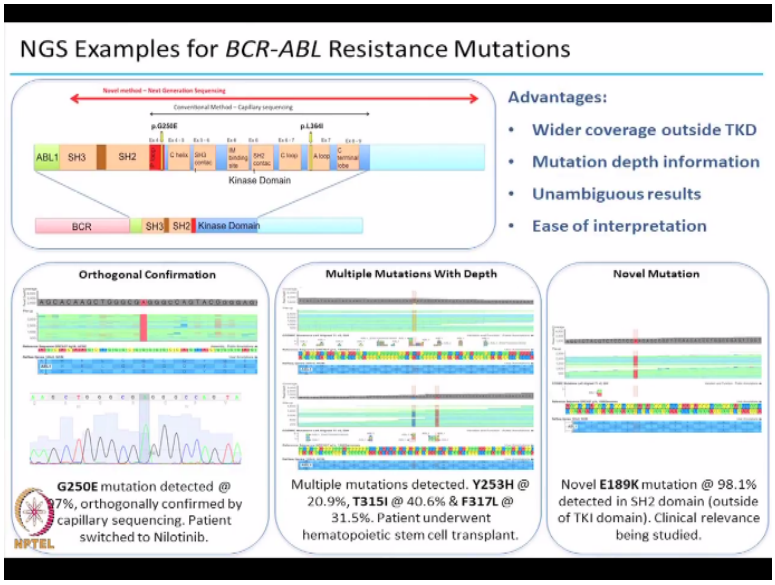

In that case, we sequence that translocated gene, we find different mutations in them and based on those mutations we switch them to other drugs. So some patients are switched to imatinib, some patients switched to dasatinib, some patients have multiple mutations and for those you need a hematopoietic stem cell transplant but this genetic information is actually being used for personalized medicine on a day-to-day basis and that is pretty, pretty remarkable.

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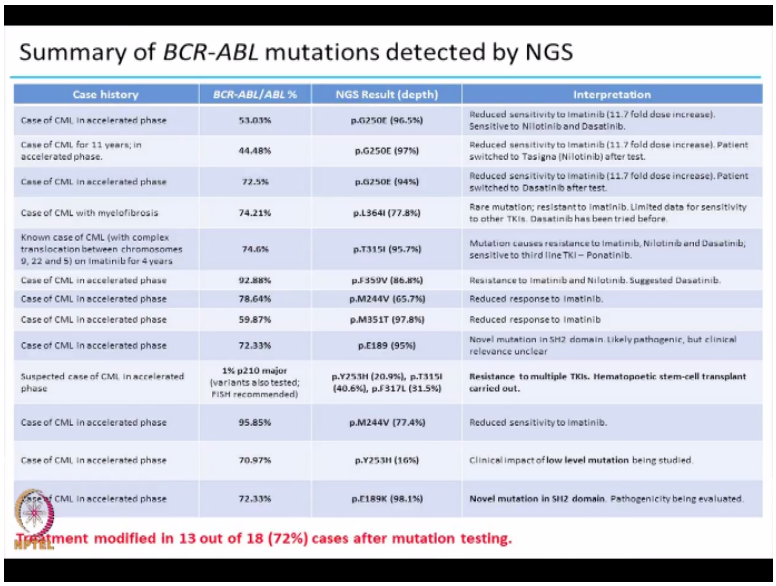

So I give you an example from solid tumors and example from leukemia blood cancer.

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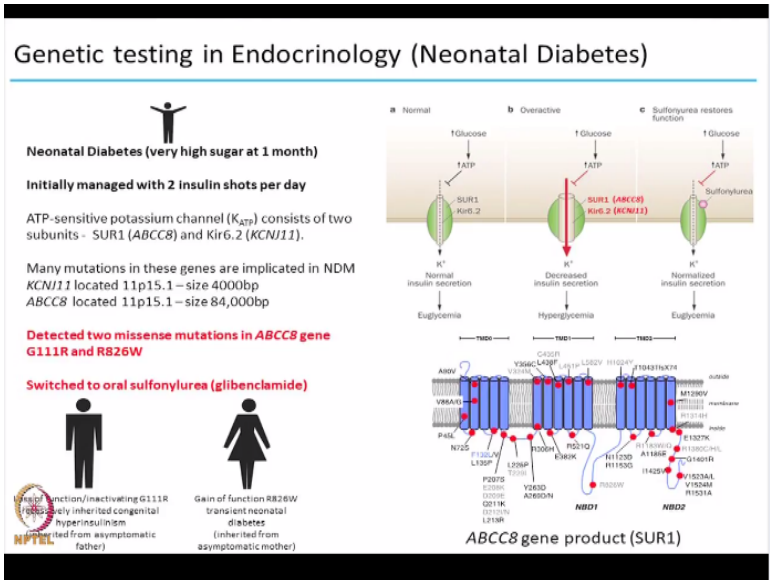

This is also used in endocrine system, so here is an example of a 2-month old baby, very high sugar at one month, initially being managed with two insulin shots a day which is very, very painful for a you know family of a small child. We went ahead and sequenced and found that there were two missense mutations in this ABCC8 gene. We knew how this gene behaved, so the child could be switched to an oral drug sulfonylurea.

The child stopped the insulin dosage and now is being orally treated, doing absolutely fine. So this is an example of this in action.

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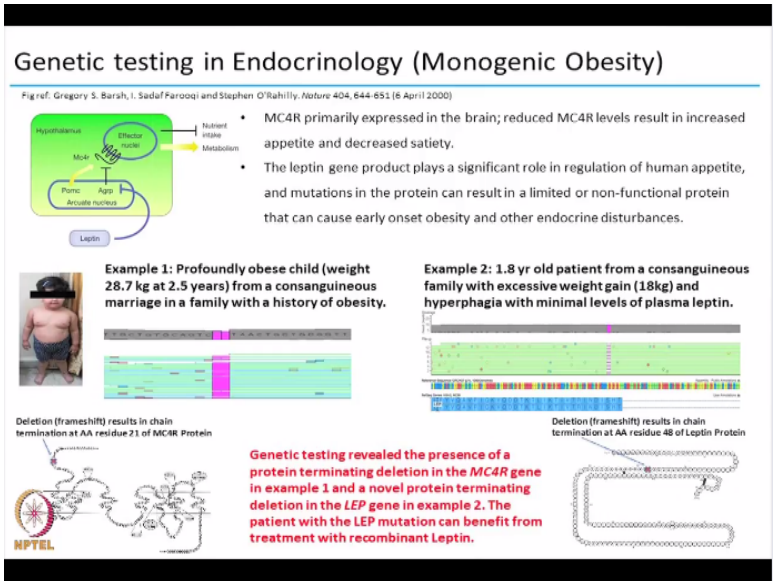

Another example in endocrine, two obese children profoundly obese 28.7 kilos at two-and-a-half years, another one 18 kilos at 1.8 years went in and sequenced the MC4R and leptin genes. One patient has an MC4R mutation. The other one had a leptin mutation. Recombinant

leptin is now available, so the child who had leptin mutation we could actually start that child on recombinant leptin and that child is doing fine. So there is an example of that.

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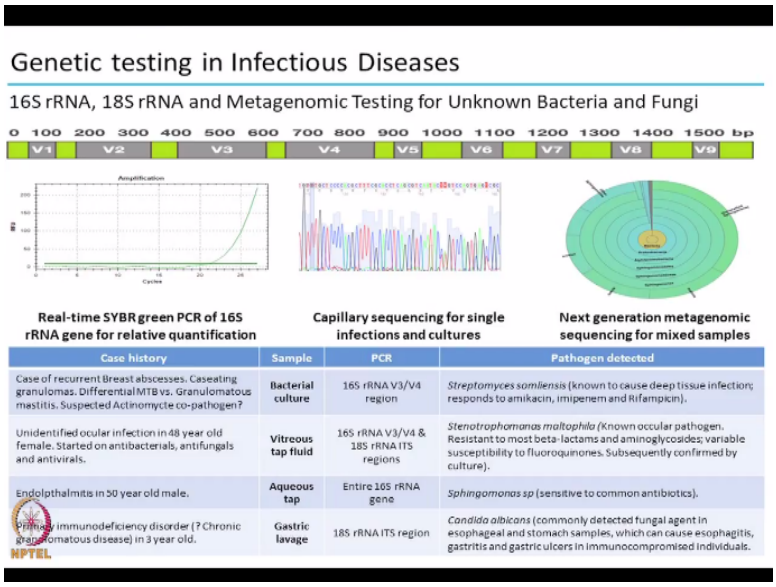

Completely switching tracks, we also do a lot of testing for infectious diseases using the same kind of DNA techniques. Here is an example of a patient who had an eye infection okay. They were worried that this person would go blind, so they started the patient on antibacterials, antifungals and antivirus.

This is like throwing the kitchen sink at the patient. In about 12 hours we showed that it was not a fungus, it was not a virus but it was a bacterium and within 48 hours we showed it was Stenotrophomonas maltophilia. This means that the drugs that it has a different drug reaction profile, so they had to switch the drug, patient got fine but they would have missed that because they only pick that up after 5 days by conventional cultural methods. So these genetic tools are really, really important.

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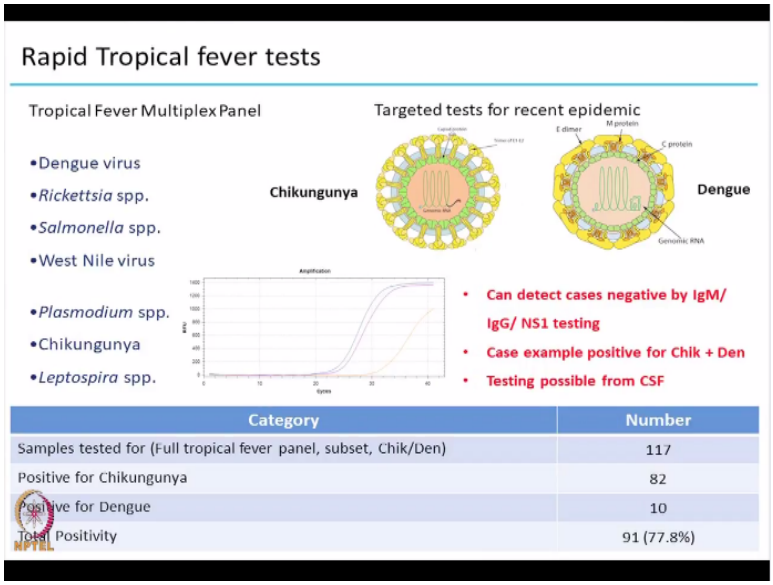

Recently, there has been a spate of Chikungunya and Dengue in the state. In Pune, it is an insane epidemic and again conventional testing was missing in huge amount, using DNA and RNA based testing we were able to show almost 70 almost 80% of our patients were positive and this can be diagnosed instantly like within a day after you get the infection. One of our patients was even Chikungunya and Dengue positive, so that is great.

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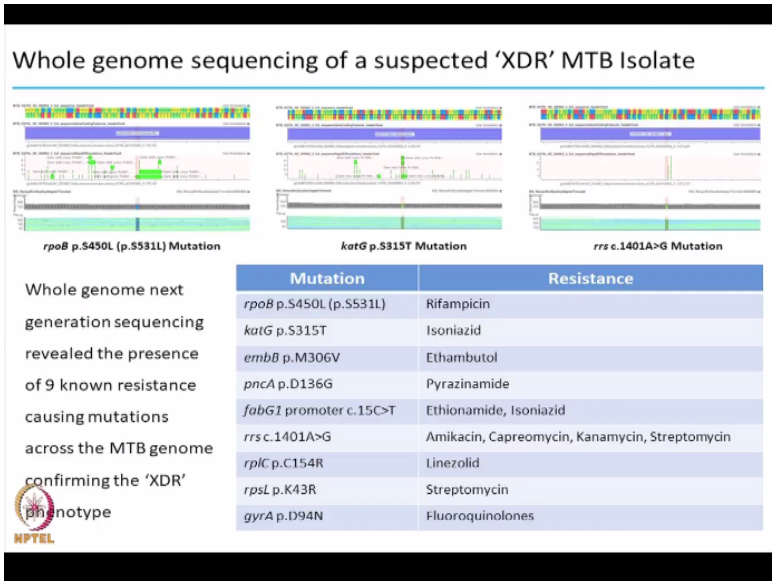

And then at the start I told you that when I started my career, we sequenced the genome of Caulobacter crescentus as part of my PhD thesis and that took us nearly two years to do. Recently, we had a patient from Hinduja Hospital in Bombay with extended drug extensively drug-resistant tuberculosis and they said can you do anything for us and said sure we can try and sequence the genome.

So for about 12,000 rupees we sequence the entire genome of that patient, we found 9 mutations that are corresponding to the XDR phenotype and we did this in under two days okay.

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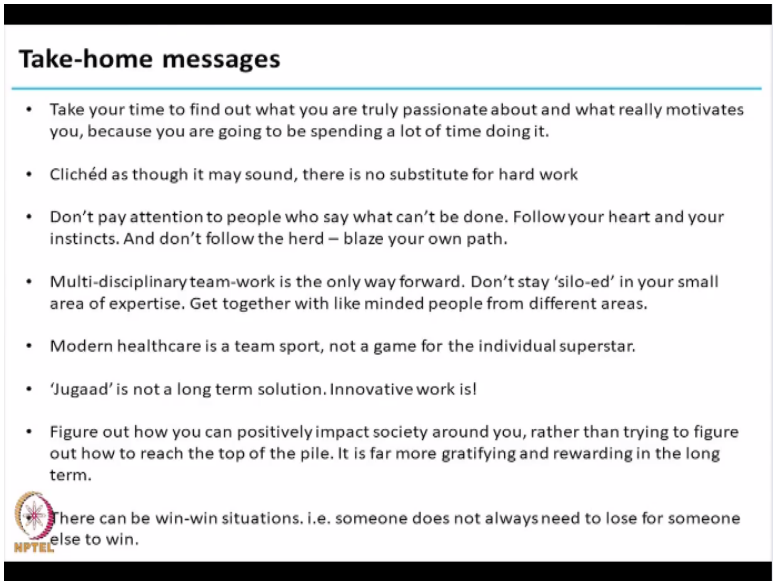

So what took millions of dollars when I was a student today can be done in days or like 12,000 rupees is like 200 dollars. So it is just incredible. So I just snapshots of examples I can go on for hours but I did not want to waste your time. I have some take-home messages for you guys before I go away. Take your time to find out what you are truly passionate about and what really motivates you because you are going to be spending a lot of time doing it and cliched as though it might sound.

There is absolutely no substitute for hard work and do not pay attention to people around you who say you cannot do something you can almost always do something. When we started building that chip, everyone said sample prep is not possible, we succeeded in doing it. Follow your heart and your instincts and do not follow the herd you know blaze your own paths.

Multidisciplinary teamwork is the only way to go forward. Get do not stay side out in your small areas, get together with smart like-minded people from other areas no matter what they are. Modern healthcare is really a team sport and doctors do not know this. They think they are all superstars and they want to score the goal themselves but you need a strong team, you have to all work together and Jugaad is not a long-term solution.

Everybody in India loves to do Jugaad but it just takes us back. You have to innovate and do real hard work. Jugaad might take you 3 places ahead in the queue but the entire line gets pulled backwards so that does not help and really figure out how you can positively impact society around you rather than trying to reach out how you can reach the top of the pile because in a long-run it is much more gratifying and evolving.

And there can be win-win solutions in my experience you know people always think that you have to screw somebody over to come up on top, you do not, everybody can win and be really successful, so I have learned that in my journey. So that is it really. Thank you for your time. Thanks.

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