Interactomics: Basics and Applications

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Lecture – 51
NGS Target Enrichment Workflow for Exomes, Targeted Panels and Beyond

Today you will hear again from Dr. Mukesh Jaiswal who is another application scientist works in the areas of Next Generation Sequencing Technologies. He will talk to you about whole exome sequencing kit for investigating rare diseases. This is probably going to be the last lecture on the NGS technology and it is application and while today's lecture is not going to cover much of the basics of NGS, but it is definitely going to give you more information about possible applications from these platforms.

So, let us continue on this lecture today and then we will try to conclude what we have learned out of these NGS based platform from basics to the applications. So today we are going to talk about the investigating the rare disease and it is treatment with the agilent solutions. So, that is site is. So, I am going to cover about what are the rare disease and how basically it can be diagnosed by the NGS solution and how we can basically give the treatment to the patient right.

So, we have some solutions, we are basically you use agilent solution for the diagnosis of rare disease and it is its treatment part. So, it going to cover some background of rare disease and then I have got talk I will some part of CRISPR Cas, how basically it can utilize for the treatment purpose.

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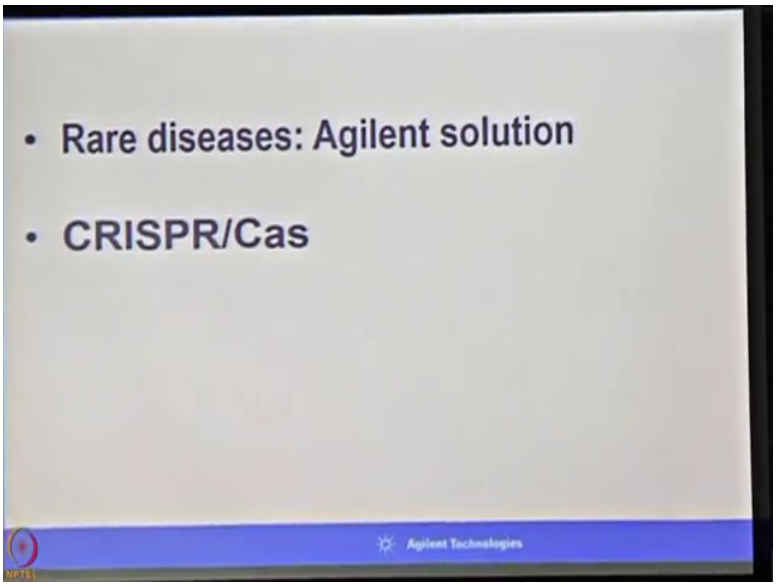

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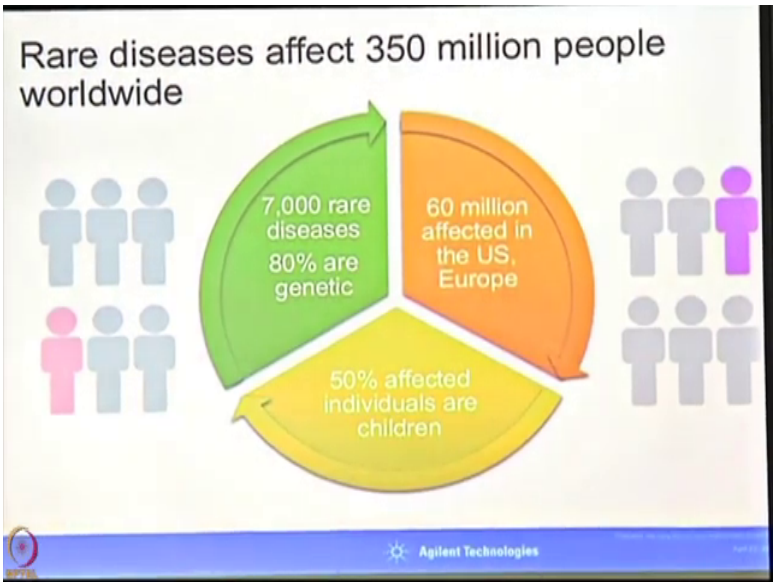

So, what are the rare disease? Rare disease is basically it is a rare disease it is affected 60 million people in US and Europe. So, it is a big number it is quite big number and 7000 rare disease basically known in till now and 80 percent reason of that is it is genetics it is something wrong in their genetics. So, yes please.

Student: Here I am talking about 60 million affected in the US and Europe.

Yeah.

Student: There any (Refer Time: 02:40) body got anybody.

I am coming to next slide. So, I am coming to the next slide. So, this is like some worldwide I am telling. So, now, I am going to next slide we have I have Indian data also. And a 50 percent affected childrens are basically affected for this disease.

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So, coming to Indian scenario. So, ICMR the Indian Medical Council of Research launch this registry 2017 and they said that around 70 million peoples in India is also suffering for this rare disease.

So, it is a quite big number and that is why they launch a project ICMR registry where you can basically write a grant to them to work on the rare disease what is the problem how you can diagnose that thing was a treatment part of that. So, in 2007 April 2017 they launched this project and so, these are the key objectives of this ICMR registry.

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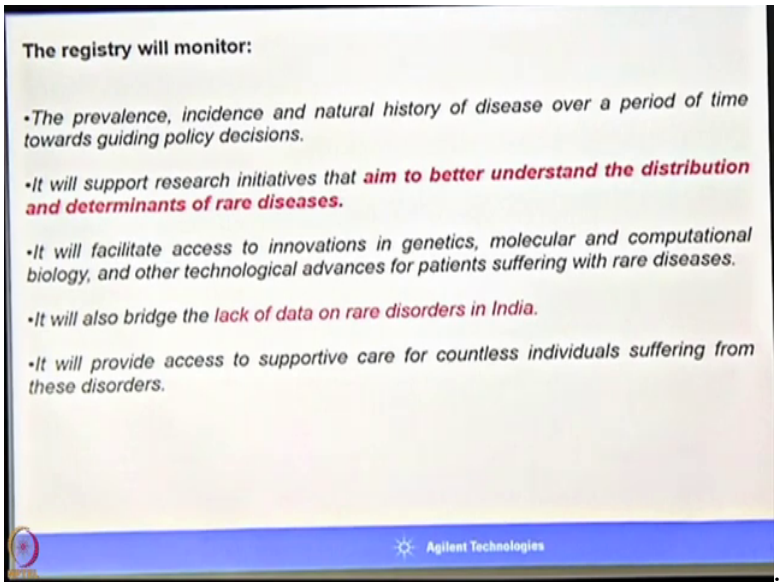

So, it main objectives that to understand what is the problem of rare disease what is the causation of that and how it can be how this data can utilize for the treatment of the rare disease. So, these are two main objective of the ICMR and so, it launched and I think it is available for the grant application also ok.

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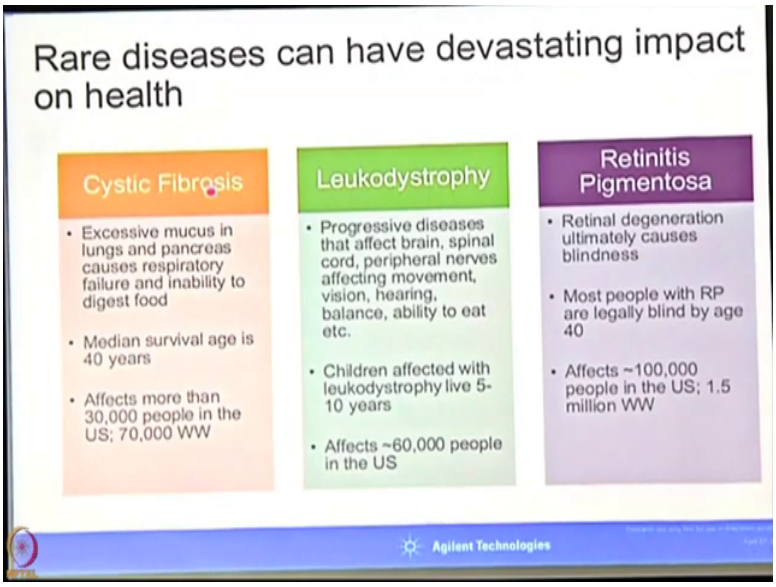

So, I am going to talk about some of the rare disease which is existing worldwide is it is worldwide. So, let us come in the first cystic fibrosis; this one so, this is the this is a disease basically, a where is the excessive mucus is deposit on a lung or pancreas this causes the respiratory failure and inability in the digestion part. And the median survival rate for this disease is 40 years right and the worldwide is a 70000 patients are known worldwide. Then leukodystrophy; this one, and this is again the progressive disease affect the brain spinal cord and the nerves system.

And this is basically childrens basically affected of with the disease and 5 to 10 year basically childrens and around 60000 childrens are affected worldwide. Retinitis pigmentosa; this is another disease and it is affected it is causes the blindness and it basically the survival is like

40 year and around 100000 or 15 million 1.5 million worldwide patient are known for this disease.

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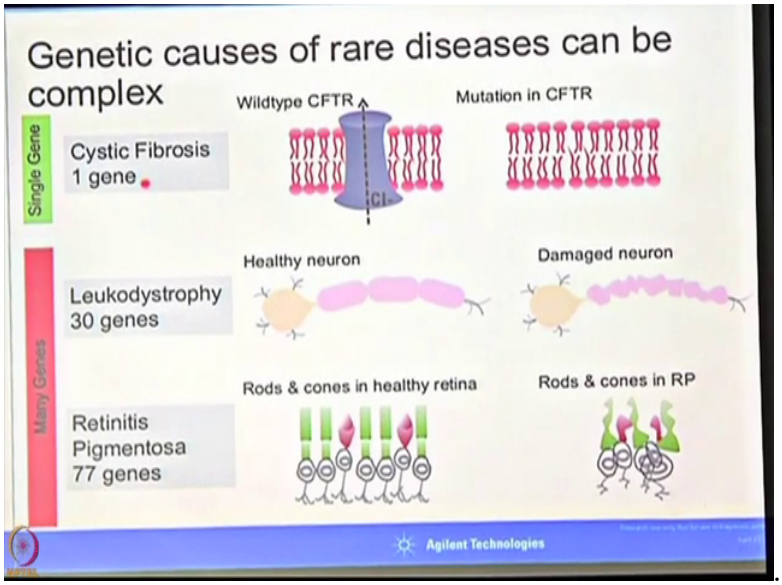

So, what is the cause of this? What is the cause of this? The cause of this basically sometime it is affected by the single gene or sometime it is affected by the multiple gene right. So, if it is like the first one, cystic fibrosis is only the one gene that is CFTR they are several nutritional CFTR and then this transporter, transporter is basically disturbed and that is caused the cystic fibrosis.

So, in this cystic fibrosis only one gene got affected right. But if you see this these two the multiple genes are affected. So, it is very difficult to identify when the multiple genes are affected. So, here like 30 genes are affected here and is in this disease is 77 right. So, problem

is that this cause the damaged neuron and here in the retinitis pigmentosa it is cone cell and the rod cells are basically disturbed right.

So, these are the disease and there are multiple genes are basically involved in there some more which is including the ICMR project basically. So, this is childhood ovarian cancer ovarian cell carcinoma endometriosis and there are multiple genes basically involved in this disease also.

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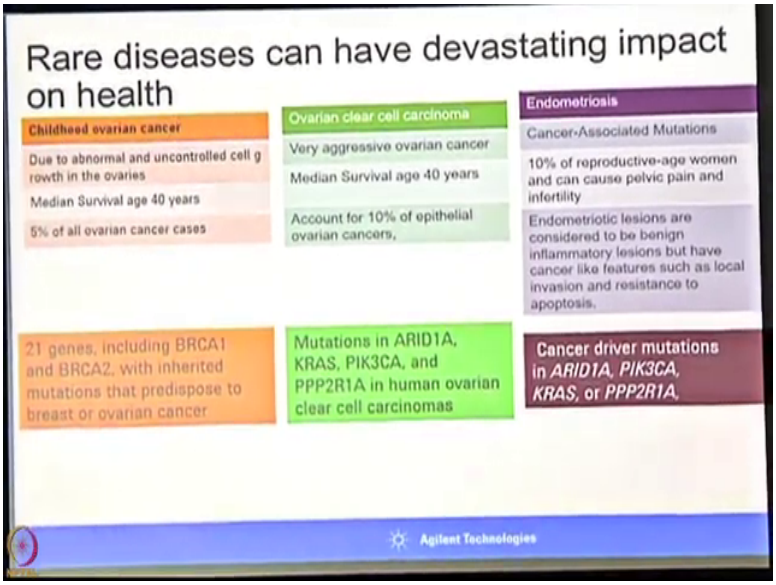

So, this is also incorporated in a ICMR registry. You can go on their website you can basically look what are the rare diseases.

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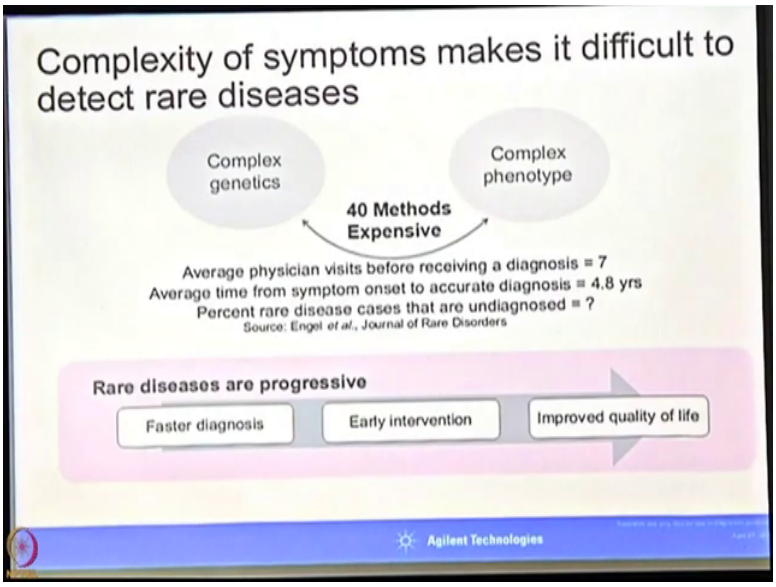

The challenge is that is their diagnostic. How to diagnose this disease right. So, if you go the regular process of a diagnosis of this one, this one is pretty expensive and doctor basically takes at least 8 year to diagnose and they go like 40 different methods to diagnose this test. So, because the complexity of disease is not like one gene it is like multiple gene they are affected right. And there is the basically they take at least 7 year and 40 method they use for the diagnosis.

So, that is a challenge it is pretty expensive it take time, but for the treatment purpose, if you have early intervention of this disease. You know the cause of early, very early then, the very early diagnosis then you could you can do early intervention and then improve the quality of the life. So, that is, this is a challenge, but if it take a lot of like 8 year to diagnose only it

would be difficult right. So, in so, that is why the that is why it is very important to diagnose the disease in very early stage.

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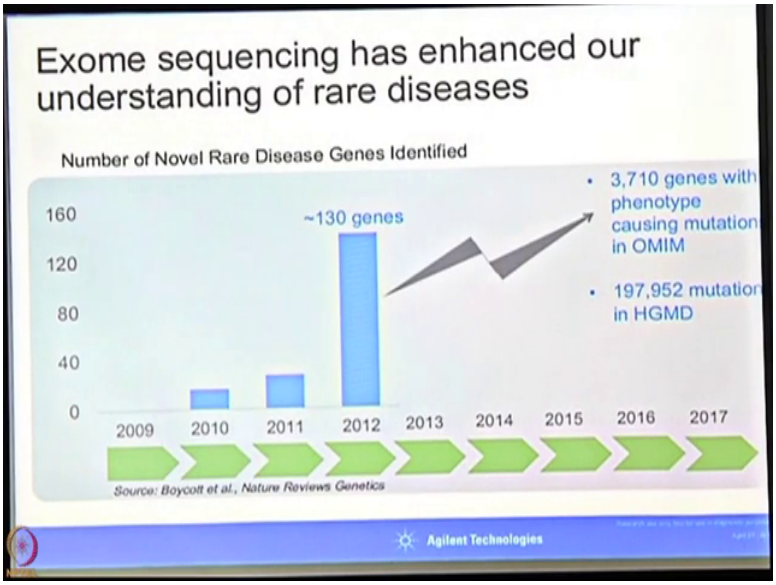

So, right now if it is if you see this rare disease. Now 2012 when the example I just started it was only 130 gene, now 2017 it is more than 200 key mutations are known for or the rare disease. Basically, this is because of the more advancement of the exome panels.

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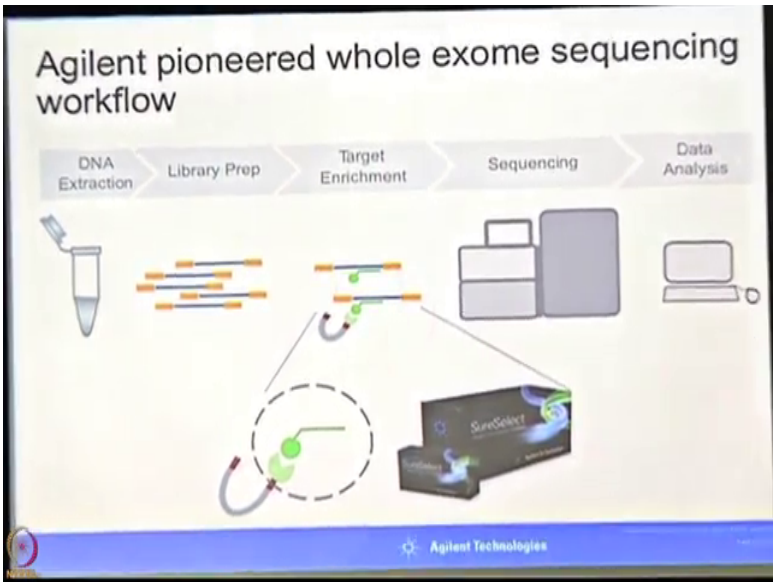

So, it is you need to extract the DNA from the patient right and then go for library preparation, library preparation and then target enrichment. After seeing the libraries basically perfectly fine you can sequence and go for data analysis this is kind of one workflow basically you can use our exome panel for the diagnosis of the rare disease.

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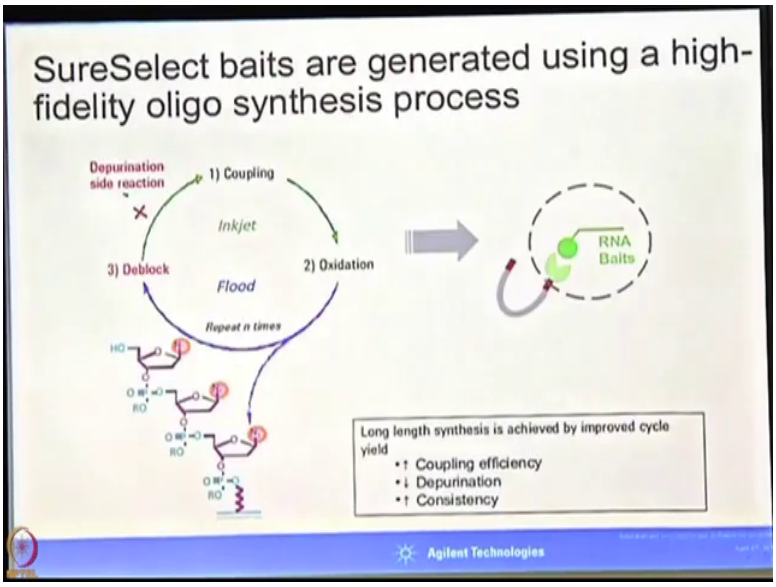

So, the challenges is always there, but I would talk about a why the Agilent exome panel is more better actually in sense because we make RNA baits and this it is oligo baits basically. And these are because we make the RNA bait they have the better RNA, DNA hybridization and these are the high fidelity basis which we make by the inkjet technology. We make this bait by the oligo.

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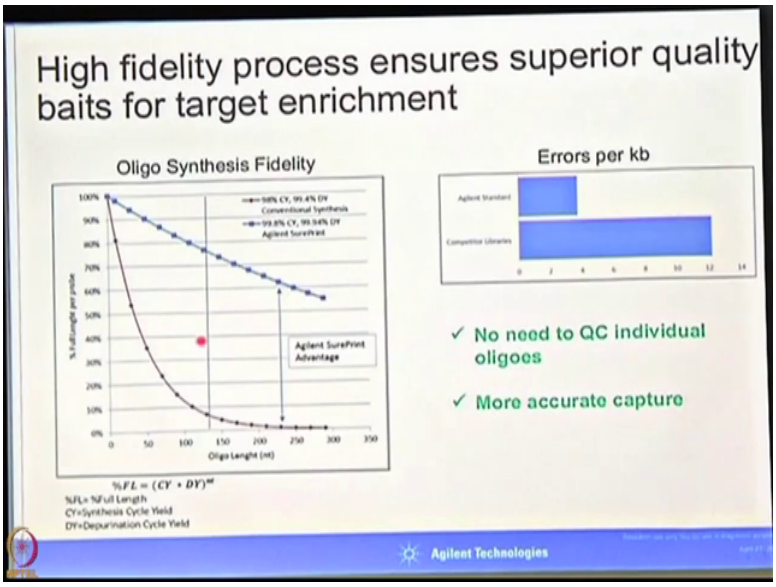

So, this is the our high fidelity basis if you see the error rate in the base in the probes basically is very low in agilent is like one or two error basically in 1 kb, but others have lots of so we have the high fidelity probes basically which are biotinylated it is used for the making the libraries.

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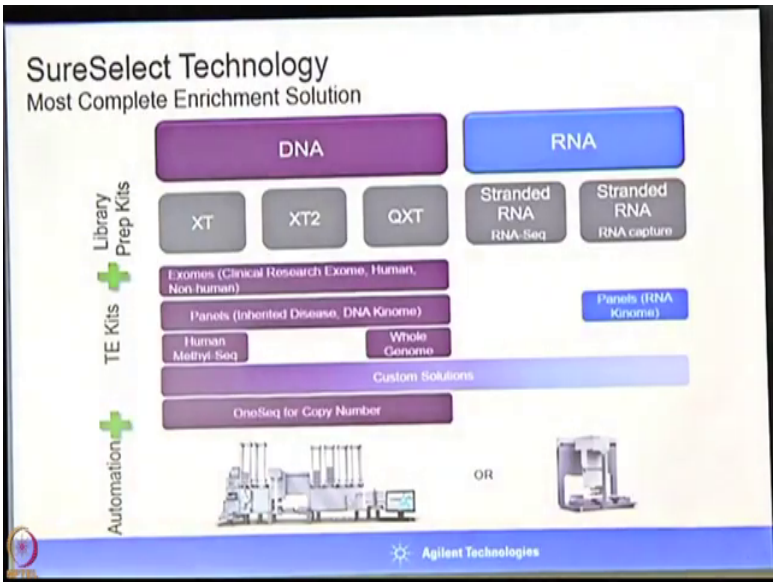

So, this is the different ways you can make the exome libraries. So, starting material always with the genomic DNA right we have three different ways to make the libraries for the exome sequencing; one is XT, XT 2 and QXT, XT is basically it is this one.

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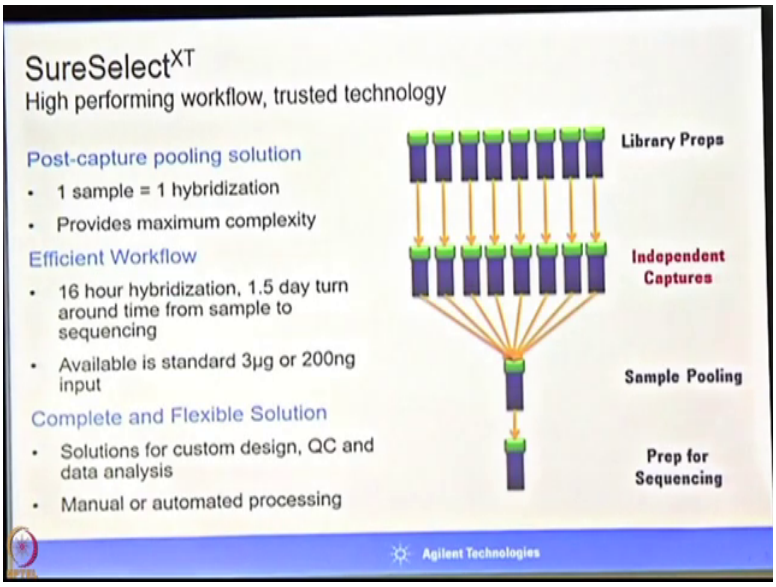

If you have a different patients 8 patients right. You can make a individual library from each patients independent capture and you can pool while sequencing right. When you go for sequencing you can pool this sample and go for one sequencing grant. So, that is the XT preparation of the exome preparation.

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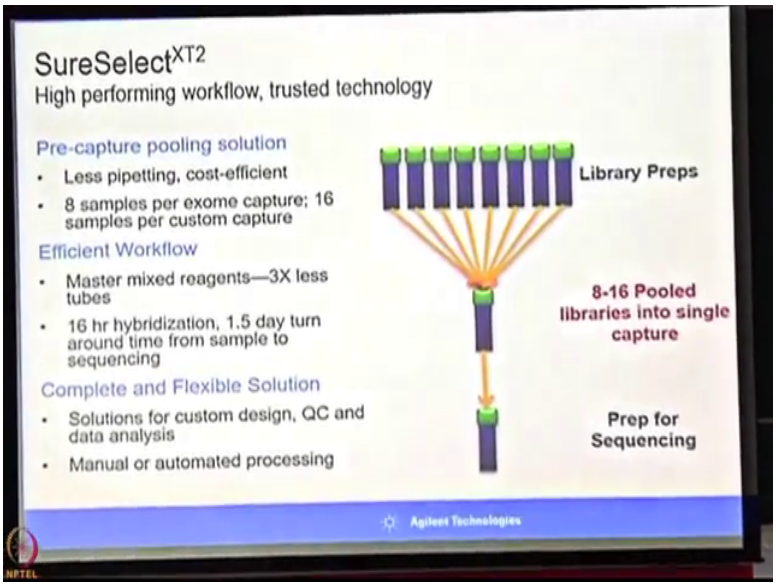

Another if you want to do the some comparative study you can barcode the patient sample and pool itself 8 to 16 in one pool you can followed by the capture and go for sequencing. So, it you can compare between the patients also that is XT 2.

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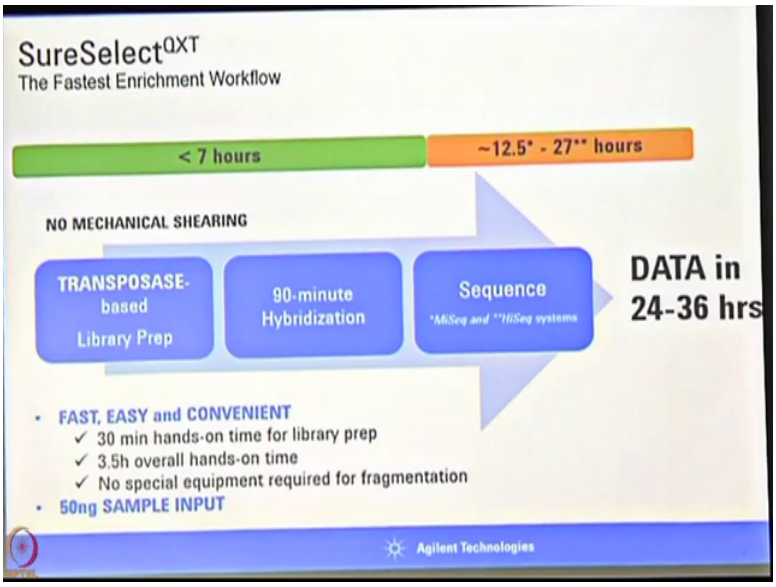

And another if another is library preparation is based on enzymatic sharing. So, you can use transposes enzyme for the to make the library specific transpose enzyme then hybridization followed by the sequencing. This is the fastest way you can make the libraries for the exome sequencing ok.

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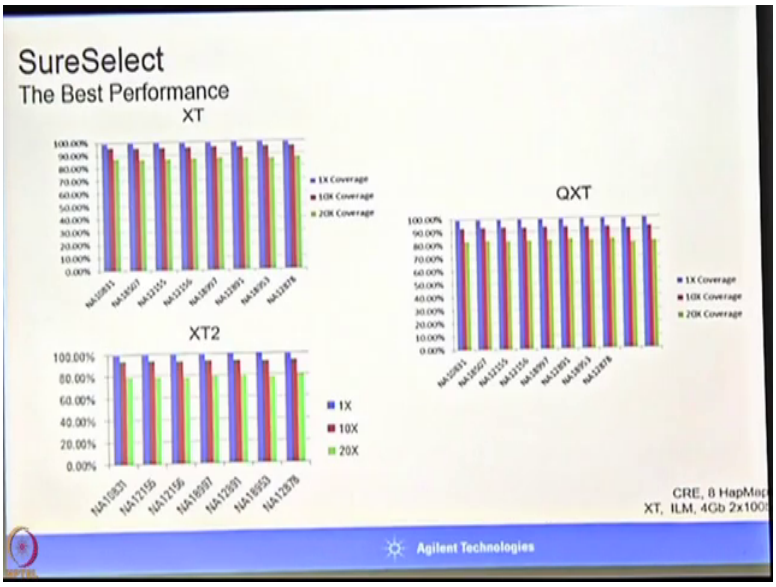

So, performance for all these mattered to make the exome library are is pretty good and they get they get very good coverage more than a 95 percent coverage all the matters.

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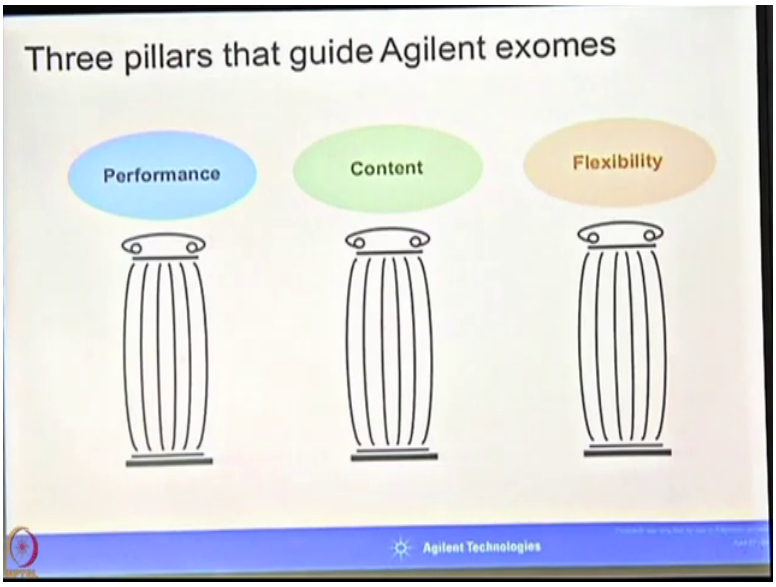

This is the three pillars basically agilent works on basically, performance of the exome, library contents and the flexibility. You work from decades to improve the performance content and the flexibility of the kids basically.

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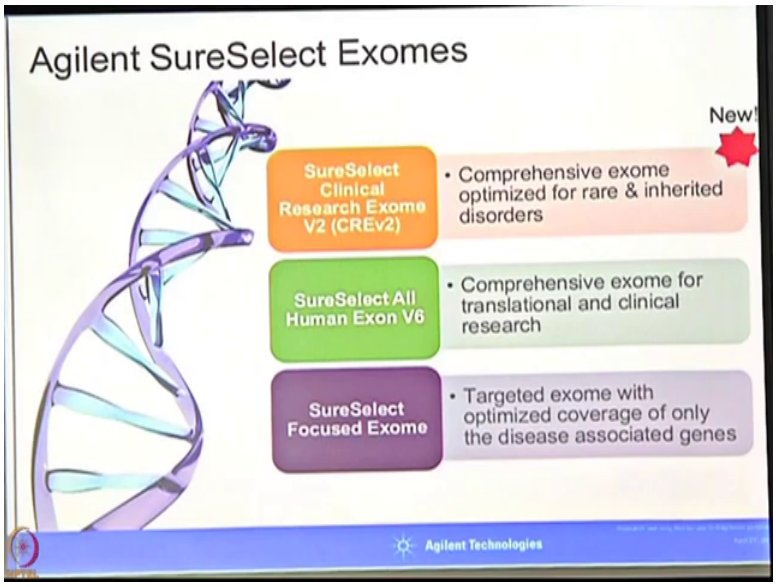

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So, if you see more of most of the rare disease basically not studied by this panel is called a clinical research exome panel. And this exome panel contains the all the exon reason and also the panels or on the intronic part of the reason neatness in a this which basically associated with the inherited disorder we have the latest exome panel V 7 this is mentioned V 6, but we have now V 7 and that basically covered the all the translational and the clinical research panel it cover whole exome.

Another we have the very small panel of focused exome, basically it is covered a diseased citizen. But most of the; most of the rare disease basically which I talked before it is basically I use for the clinical research panel.

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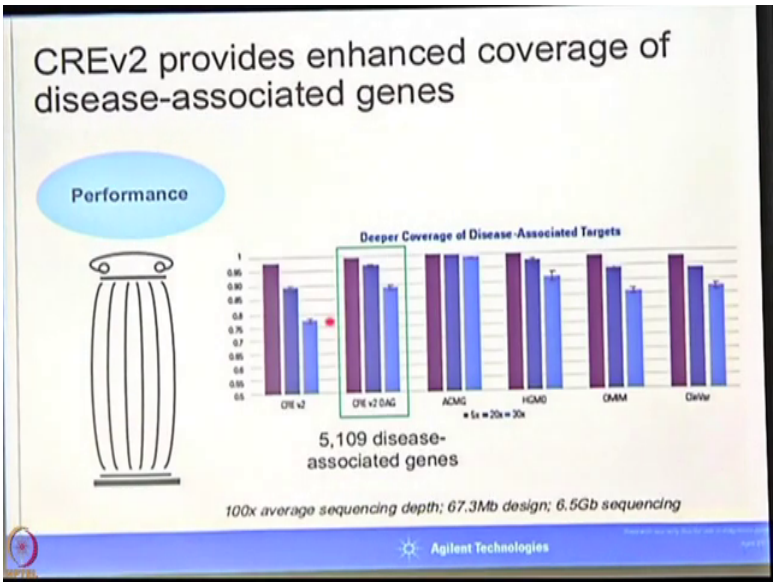

So, if you see the contents performance of this one with this clinical research exome panel they have like 5000 gene is basically it deeply covered with the diseased citizen with the clinical exome.

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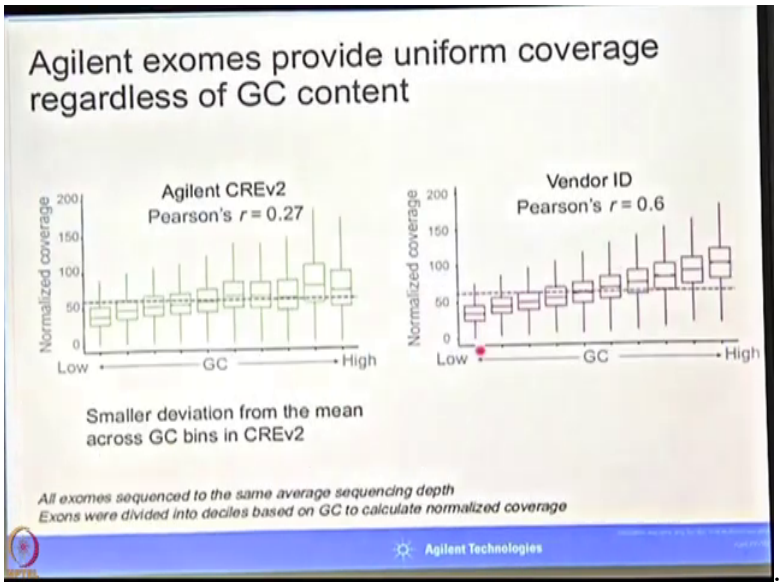

And the challenge is start when you go for the diagnosis of rare disease, the most of the mutations are present in the GCs region. To make the library for the GCs region is always challenge so, but in with our clinical exome panel the performance in the GCs region is very good. It is very uniform the preparation of the library when you go for the GCs preparation.

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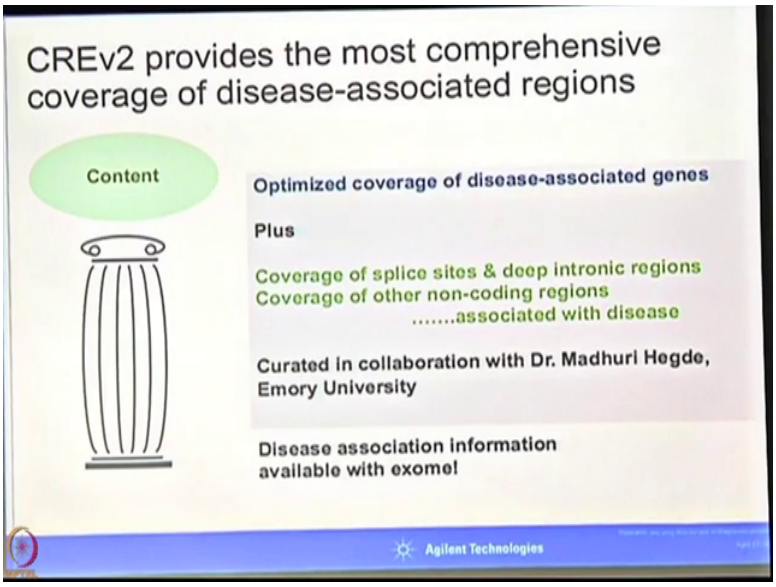

So, content basically what is the content on the for the probes basically it is basically designed by the Dr. Madhuri Hegde from Emory University and they make the basically the probes which is covered all the disease associated decision exon and intronic part which covers maximum rare disease parts.

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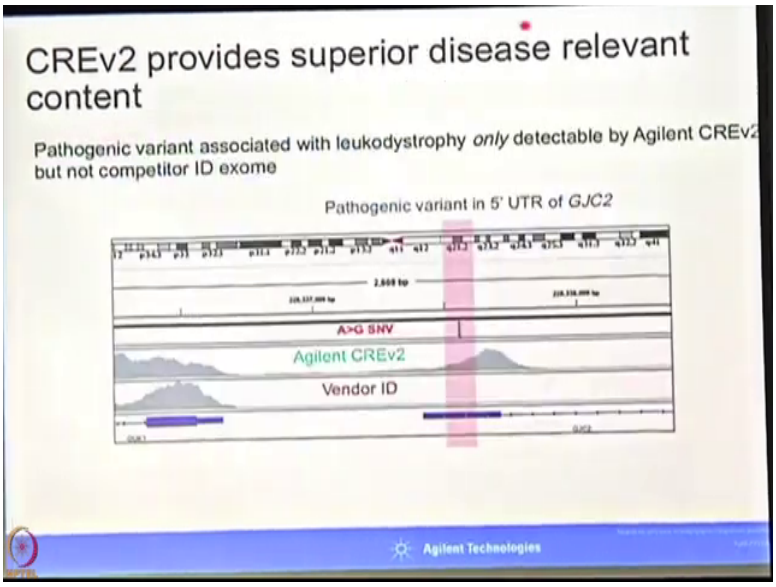

For example, this is the leukodystrophy right and the this pathogenic variant it is GJC 2. This gene basically, if you see compare because this is the GC rich region and if you this compare with the other vendor you do not see any coverage. There is no coverage for this gene and if you see the clinical exome panel we covered this part also to be able to read the 5 prime UTR variants with this disease. So, in this disease this is the kind of pathogenic variant and you can easily dictate by the clinical exome panel.

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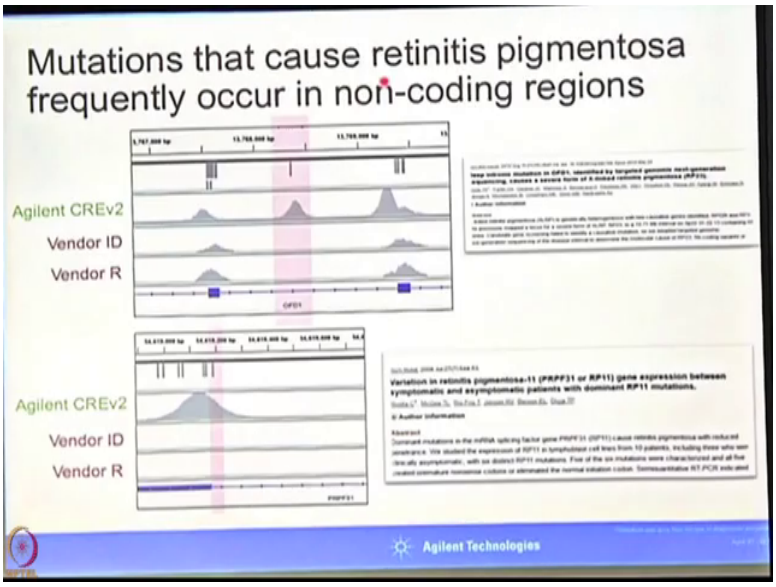

Another retinitis pigmentosa; if you see two other vendors this a CFD one gene is not covered in this part because this is a intronic part, your exonic part. Intronic part are not covered right, but if you see; however, is fairly covered that part that means is detection of that mutation is very easy on that then, if you see this region again is well covered by this. Now this is these are all non coding region, these are well covered with the with our clinical research exome and most of the pathogenic variant basically in for rare disease is present on non coding region.

So, it is fairly covered with the clinical research exome panel. So, if you see overall in the clinical research panel we cover all the clinical variance pathogenic regions and it covered mostly like 98 percent regions are covered and others basically has low coverage.

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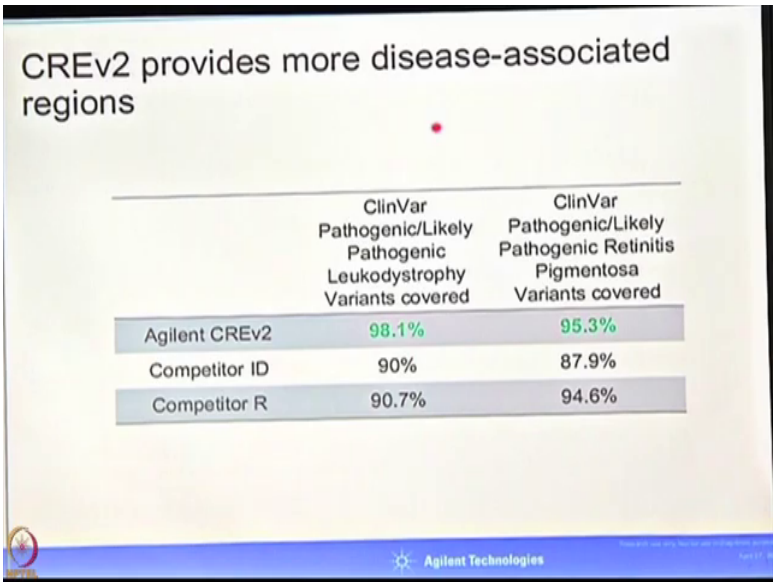

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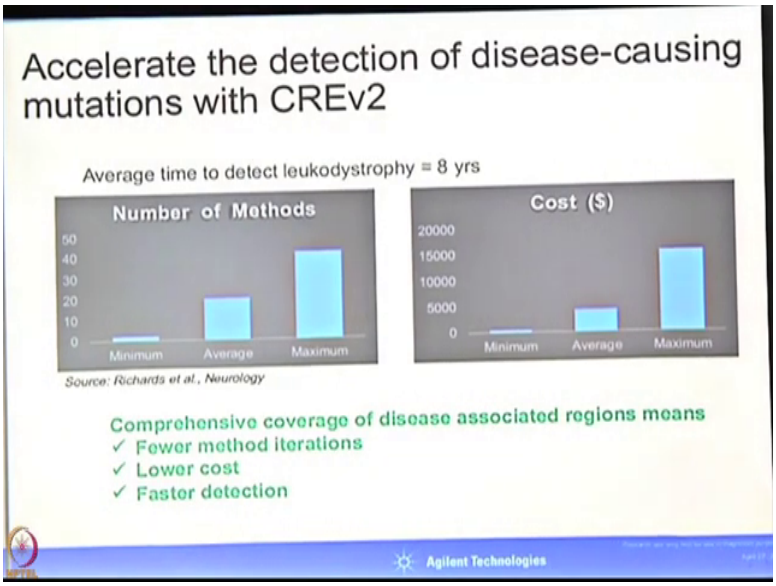

So, if you think about when you when the doctor going to diagnose this leukodystrophy, it take 8 years right by the normal mattered and the average test basically they do around 20 30 test to do to diagnose for this one I take 8 years right. And the cost is goes up like it is like 20000 dollar right.

But when you do one simple test our clinical exome panel easily identify this mutation and that is basically for a leukodystrophy and it is very cost effective right and a.

Student: Cost (Refer Time: 16:46) there is a there is a patient called like (Refer Time: 16:49).

So, it is just going to cost like 15000 rupees right. So, one test costs like 15000 rupees for exome panel.

Student: It still the chance like might be somewhere then there is just a chance that everybody somewhere disease and (Refer Time: 17:03).

Because it is covers the intronic part exome part definitely it going to detect that thing, but detection rate is faster right and it take less time. So, doctor is start their intervention much earlier with that like suppose this is our content we, but we does not make this content in vacuum. We do the research and we make we make the probes for cover all those reason right, but sometime when you did some new experiment right and some part basically you think that this is the part maybe pathogenic part and it is missing right and you want to incorporate that part in your panel.

So, it is very flexible, we can customize the panel according to your requirement also. Suppose, any gene and it is not a basic the intronic part is not covered and if you want the interested I want to cover this part also we can basically add this panel incorporating your panel. So, that is about flexibility. So, we work on three parts performance and the contents always optimize year by year and then flexibility if you want to add more right.

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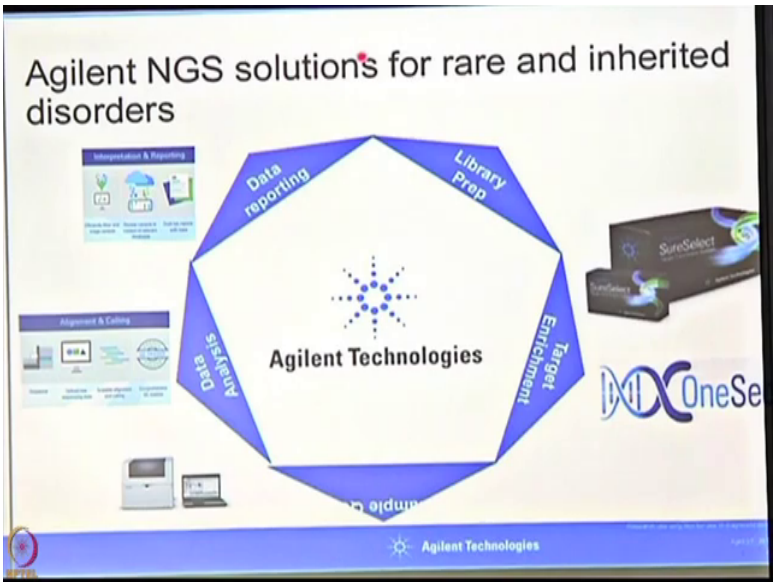

So, it is very simple workflow that I told it start from the library preparation, then we make the targeted panel by the probes right and then data analysis and reporting. Whole workflow basically take 3 to 4 days and it is easy to identify the kind of challenges for a diagnosis of rare disease right.

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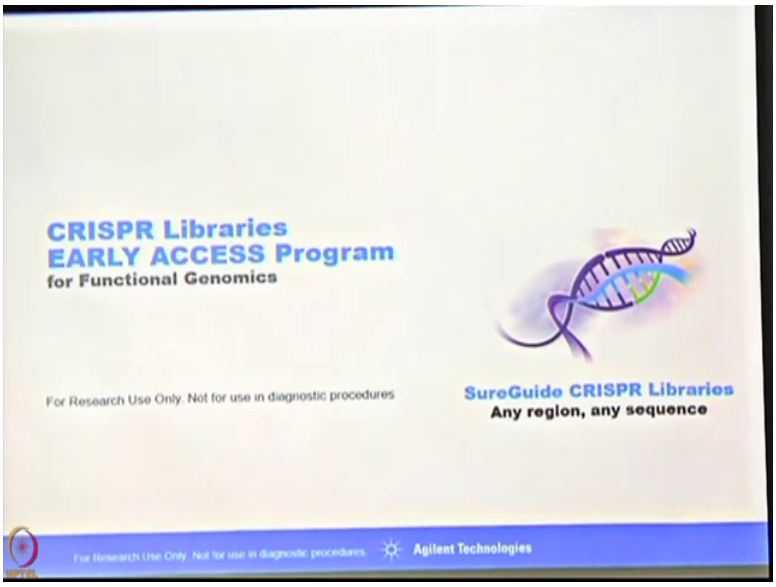

So, now this part is kind of over like if you get some kind of mutations right in any disease not only in rare disease of course, cancer right and, but it is multiple mutation and you want to solve this problem for a treatment purpose.

So, we have a tools called as the CRISPR Cas way basically you can do the gene editing right. And to solve to do fix that gene for a treatment purpose. But this is very early we launched some libraries for a treatment, but it is very early stage. Let us start with that what is the CRISPR Cas, this allow you basically to allow the mutation to correct maybe or gene editing. So, it is based on the guide RNA right.

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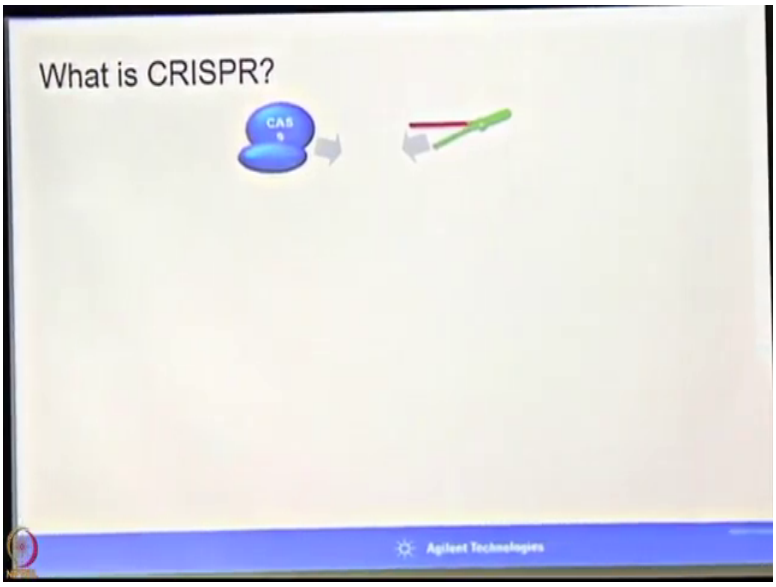

So, this is the guide RNA, this one and this is the site of recovery recognition and this is the Cas 9 enzyme. When this become active they attach and it goes to the pam side like this.

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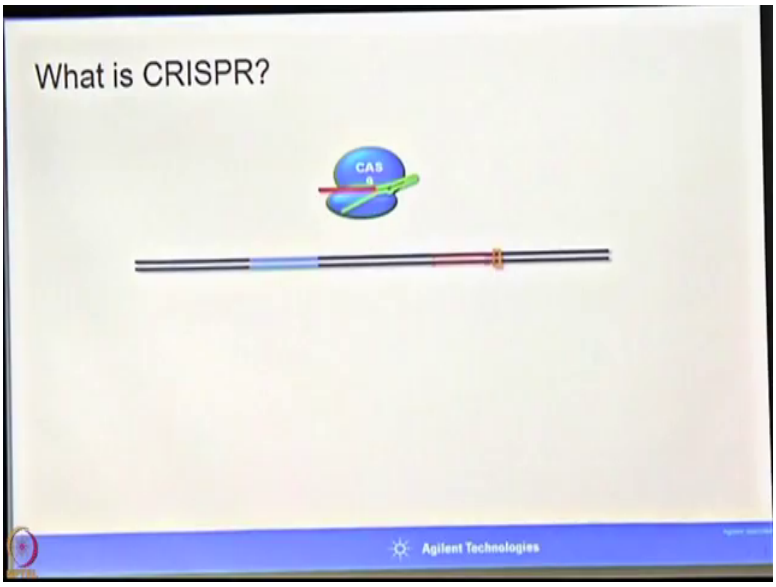

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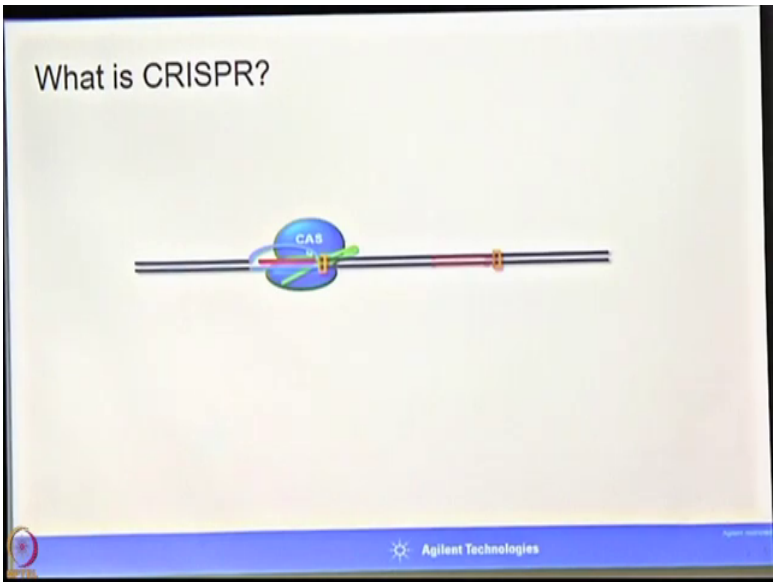

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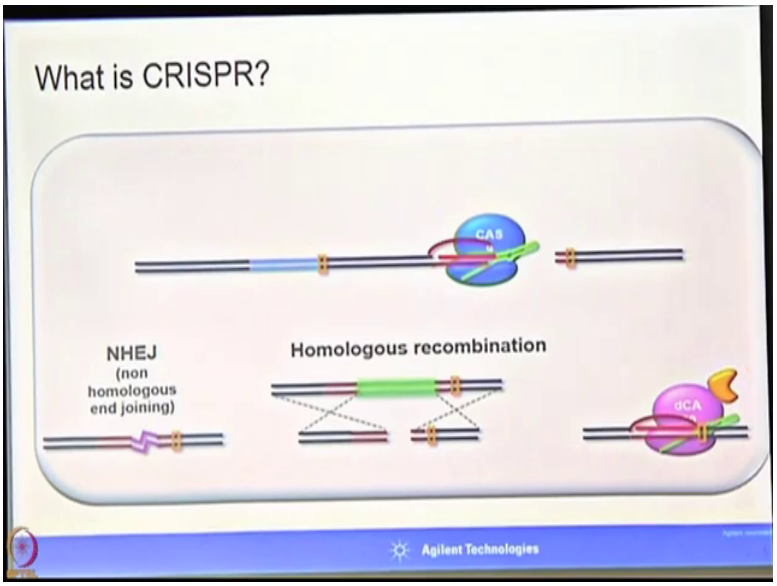

If it is identical or hybridize on that like this. It is identical with that it creates a link right this Cas 9 enzyme when this guide RNA is specific to that it create a link. Now it is allows you 3 possibilities. One possibility is that just leave like this and body repair body system our cell system basically go for non homologous end joining and cause the knock out of that gene right it is called here is a knockout.

Second is the homologous recombination if you want to do the gene editing and you some have homologous sequence you can incorporate this homologous sequence in that right. Third part this is the deactivited Cas 9 enzyme this yellow color part it might be activator or deactivator depending upon the activator it can induce the gene suspicion or reduce the gene suspicion.

So, it is allows the whatever mutations basically you got from the exome panel right, you can basically try to correct or you can do the gene editing for the treatment purpose. So, that is whole story exome panel and what is they follow up?

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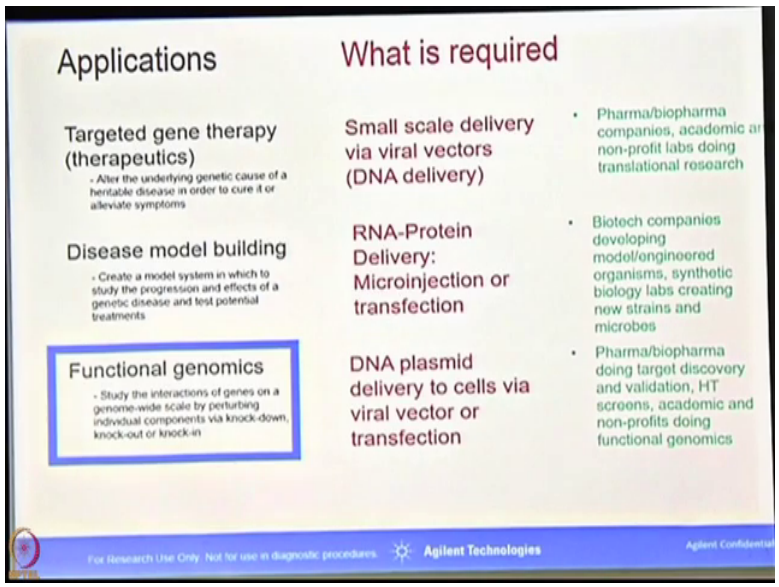

So, we are working on the functional genomics were basically we can try to edit the genes in a large scale like and it might be any knock down knock out and knock in anything and try to edit the genes to solve the problems.

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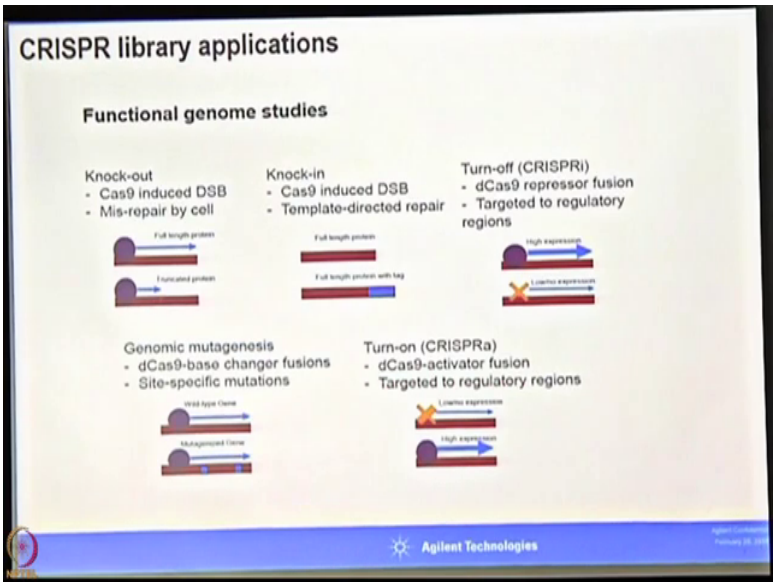

So, this is the if you study the functional genomics. So, very first prospect is knock out right. If it is knock out means guide RNA breaks that one it makes the truncated protein right. So, your protein not going to work is truncated protein. Knock in means is basically it going to add some tag on that on the protein turn off means if it is a high expression, this is the; this is the repressor fusion if it is a turn off means it is the lower expression of the gene this is the genomic mutations mutagenesis here basically it is a site a specific mutation you can create by the CRISPR Cas.

Suppose you got some mutation and you want to solve that mutation right. You can change a base by base by the mutagenesis right. So, this way you can correct that SNPs you got right, you can correct that part. So, this allows you to site a specific changes with the Cas 9 and if

you want to do some genes are basically low lowly express in sub disease you can basically induce the expression to the higher level right.

So, it can induce the gene expression. So, you can do multiple function by the Cas 9, you can induce the gene you can repress this in gene expression or you can do the site specific changes in the gene right that is allow you five different possibilities do.

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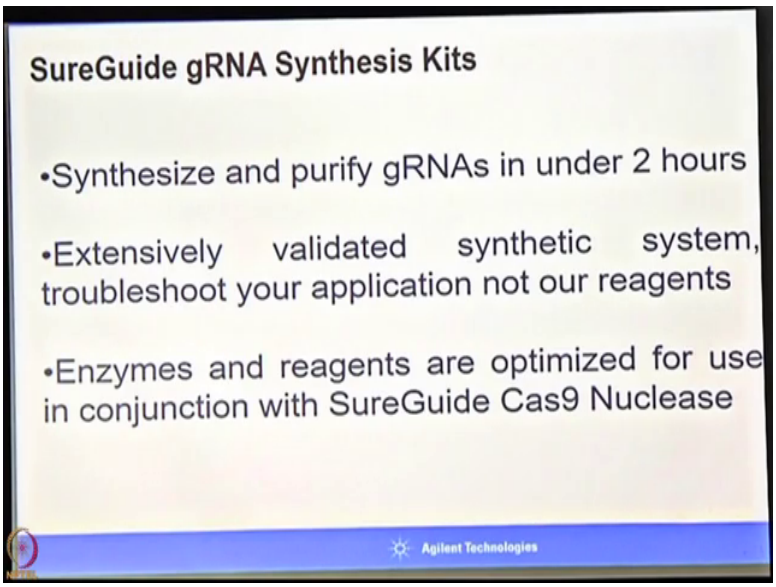

So, now the question is that, suppose you like in cystic fibrosis right. You work on single gene and there maybe 1 or 2 mutations you got with the exome panel right and you want to fix that problem right. So, there is two ways; one way means if one gene and few mutations. So, basically you are going to use 4 to 5 different guide RNAs not more than that right. So, for that one you can make the guide RNAs in your lab.

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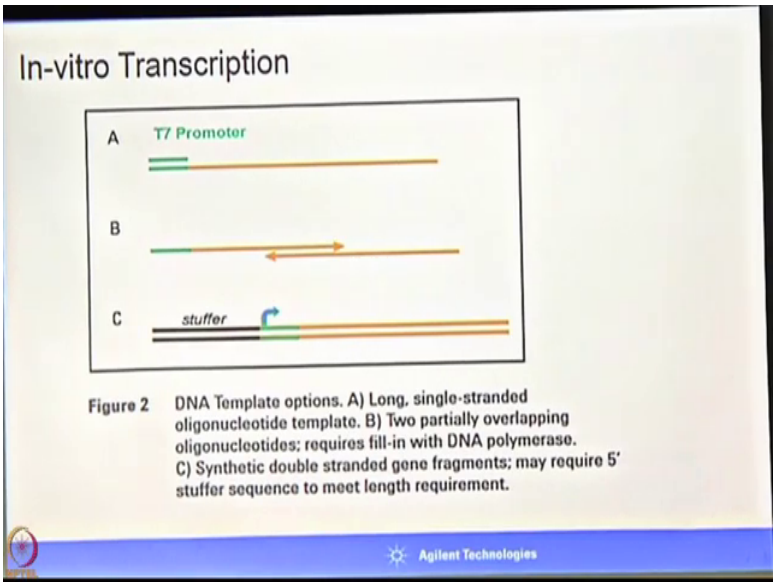

So, suppose this is the target basically you are going to you going to make a guide RNA for that one only. So, this is the targeted panel target you want just add the T 7 promoter on that right. If you add the T 7 promoter on that and go for. So, the kids basically what they have a T 7 primers and go for in vitro transcription to make a guide RNA for in vitro transcription you will make a guide RNA which is the reverse complimentary to the target which is going to be reversed complimentary to the target.

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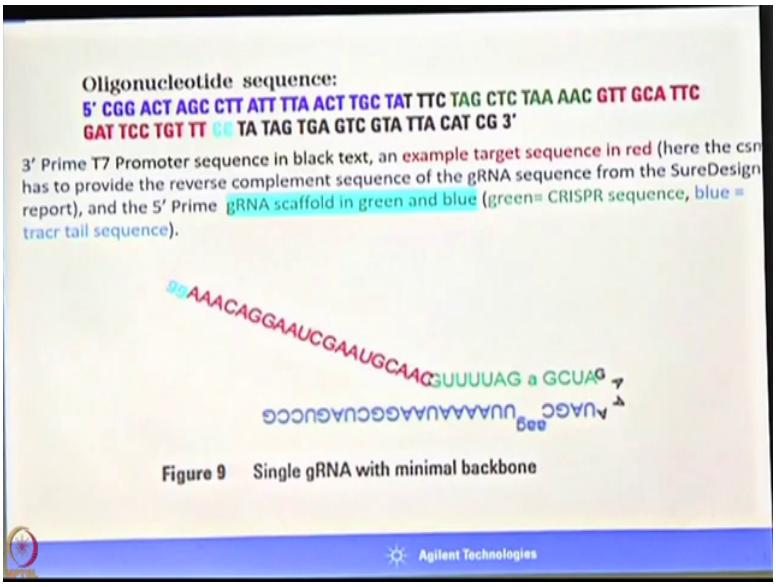

So, this is the kind of guide RNA, this is the red part basically is based specific to the target to the part where you interested for and this is the backbones minimum backbone.

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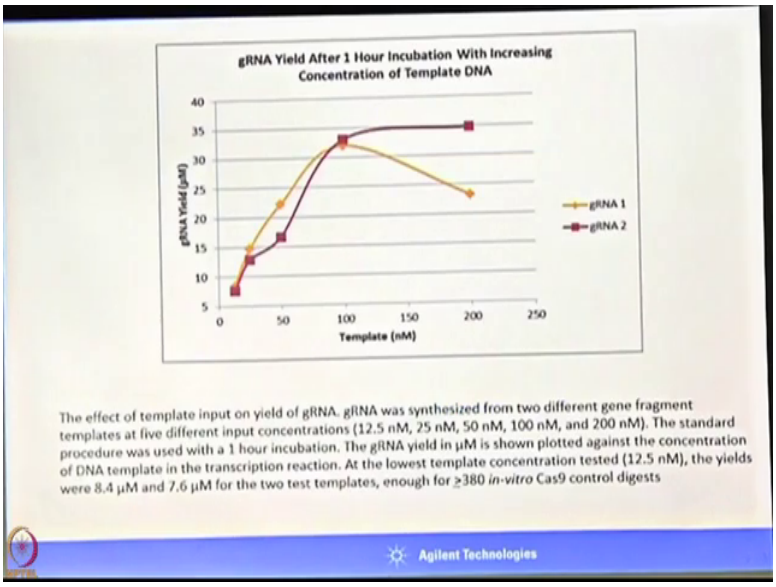

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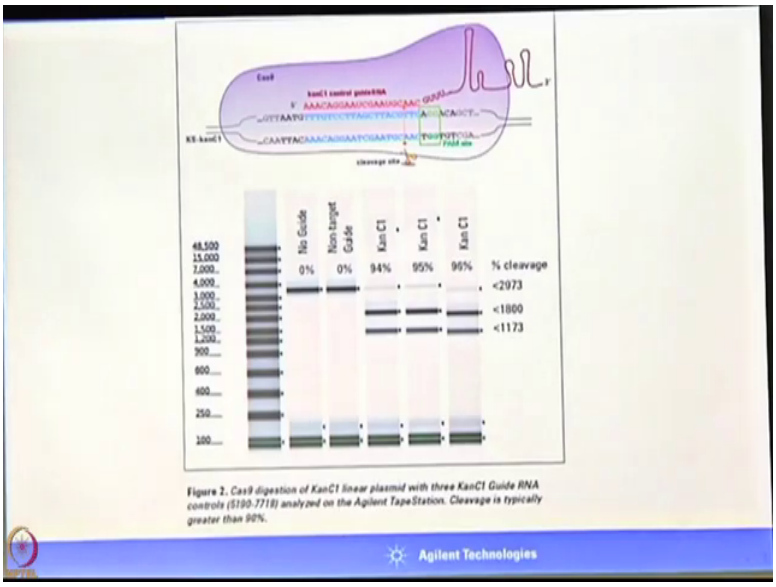

And this when this going to bind on the target gene, this targeted part in the presence of Cas 9 enzyme this gene basically cleaved in 2 part. If you verify this one in the presence of Cas 9 this gene basically cleave off right. So, this allow 3, 2 5 different possibility again basically it going to maximum time you going to knocked out the gene right. So, if it is your genes in numbers as 1, 1 or 2 gene and you want to use the you can make a guide RNA on your lab also right.

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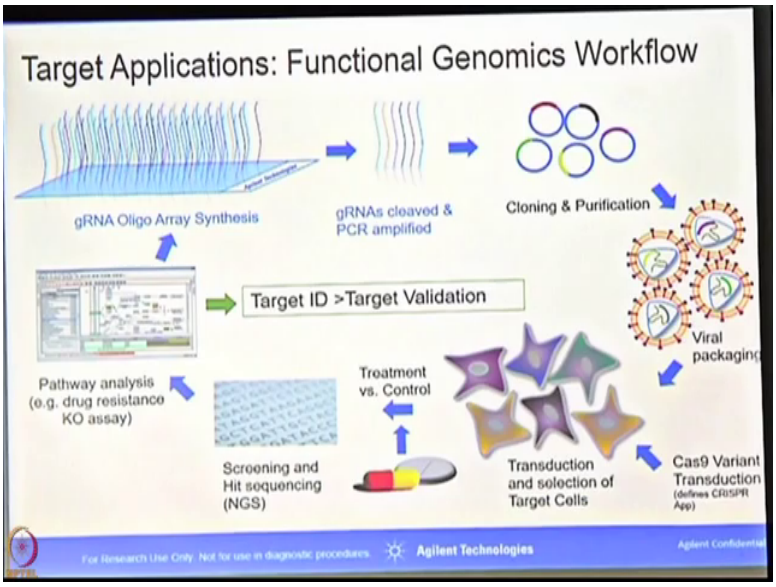

But mostly it is not a case, is not a case there are multiple genes are going to is involved for the for any complex genetic set genetic right like retinitis pigmentosa 77 gene is involved and mutations is like more than 1000 mutations are basically involved there right.

So, what do we do? We make a guide RNA for that panel right. And this is totally custom thing is we do not make we make some catalog, but we totally depend on the users. So, we make a guide RNA on a slide we cleaved that one from the slide do the PCR amplification and pegged on the viral particle noise ready to transfect in the cell system. That much guide RNA basically which is for that mutation right it going to ready for transfection and you can use for the therapeutic opportunity for this right. So, this is the this is.

Student: In that so, the target cells are actually cell culture.

Cell culture.

Student: And then?

So, it is a viral particle these are cells basically to transfect directly into their.

Student: Cells are then.

Transfect with these guide RNA.

Student: Right.

They already have a Cas 9 enzyme. So, it create the means Cas 9 is there right. So, it going to cleave that part of the gene or is depending on what you design basically.

Student: So, those cells could be there.

Yeah.

Student: Then how (Refer Time: 27:12) about (Refer Time: 27:13).

So, see I told you this is in very.

Student: Yeah.

This is very early stage. Again to do this thing you need to do lots of screening lots of NGS work to identify it is going to work or not. So, this is just a thought and guide RNA is it is we can synthesize for you, but again the protocols and how are you going to translate to the actually patient is very it is in very early stage. This is like 10 20 gene you can make in your

lab, but it is more than 1000 gene in high scale then we can make means it is for the high scale not other by you can just make in a lab just your any target at.

Student: (Refer Time: 28:00) specific.

Hm.

Student: (Refer Time: 28:02) these also specific target.

Yes, specific target just add the T 7 promoter on that use T 7 primer and do in vitro transcription that is inside the guide RNA see. It is very straight forward protocol, but yeah again you cannot make the 1000 guide RNA for multiple gene right. In that approach you can use this these guide RNA strategy and try to solve the problems, but I would tell you this is not easy this is very very hard right it is very hard because if you see this going to be like 1000s of the guide RNA and going to transfect in the cells right.

Now, you are going to go give some treatment by drugs right and then you need to go for screening protocols day by day and again you are going to verify this thing going to be work by the validate or not by the NGS. But of course, this is the thought and you can basically try to solve that problems.

So, in that cons contest Agilent is happy to collaborate with the peoples who are interested for to make a guide RNAs right.

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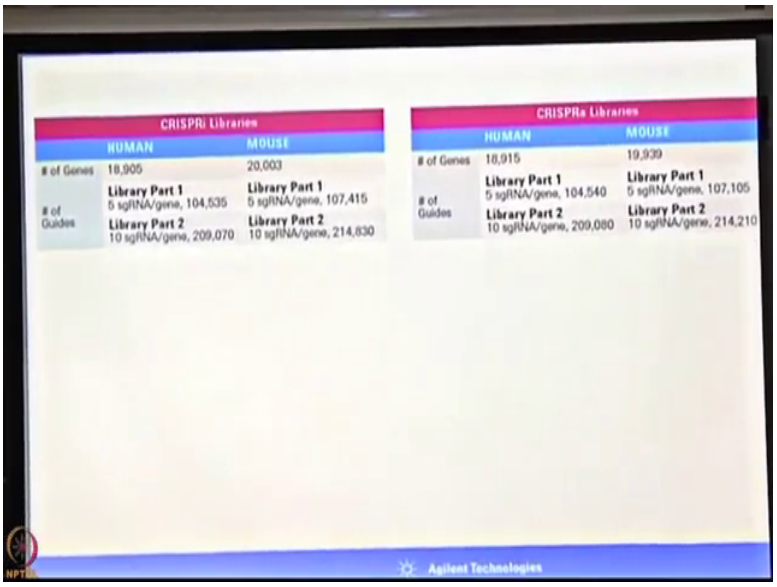

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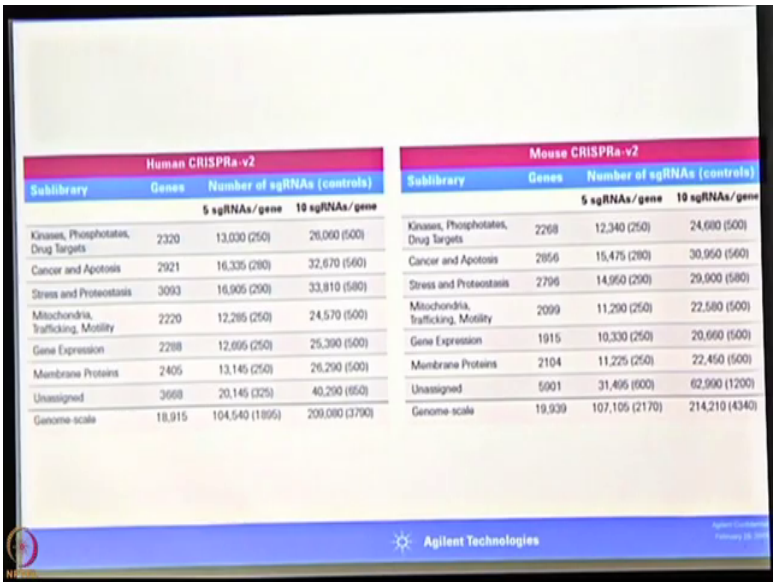

And we already had some guide RNAs of different disease like if you have the cancer panels, you already have the these are the genes and these are the guide RNA we make already have some. And they are multiple panel like mitochondria gene expression, protein membrane, these are the panels we already made, we already cataloged that panel. You can use that one, but again the how it going to work and how it going to screen it is little difficult task.

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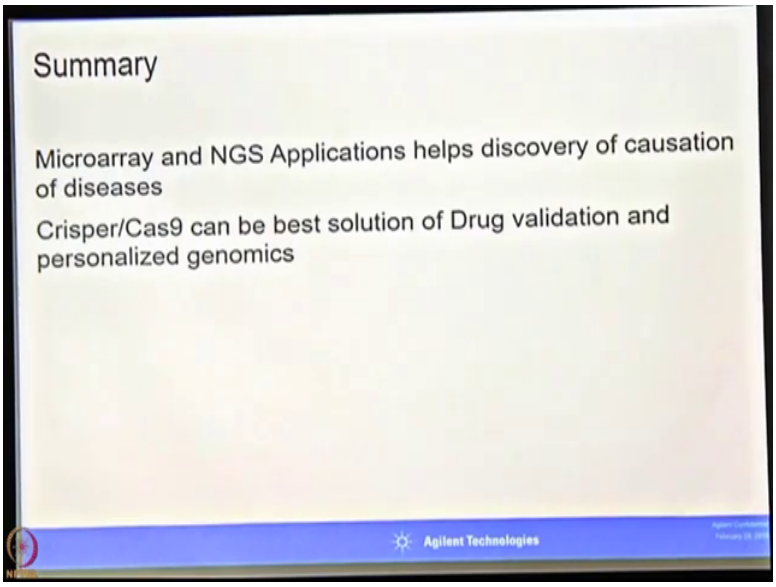

So, in summary means you can use the NGS application to identify the causation what is the cause of that and I gave the little brief idea how the Crisper Cas can be best solution for the drug validation and personalized genomics. So, this is something you can use the NGS application to identify the problems and Crisper can might be used for the treatment part right.

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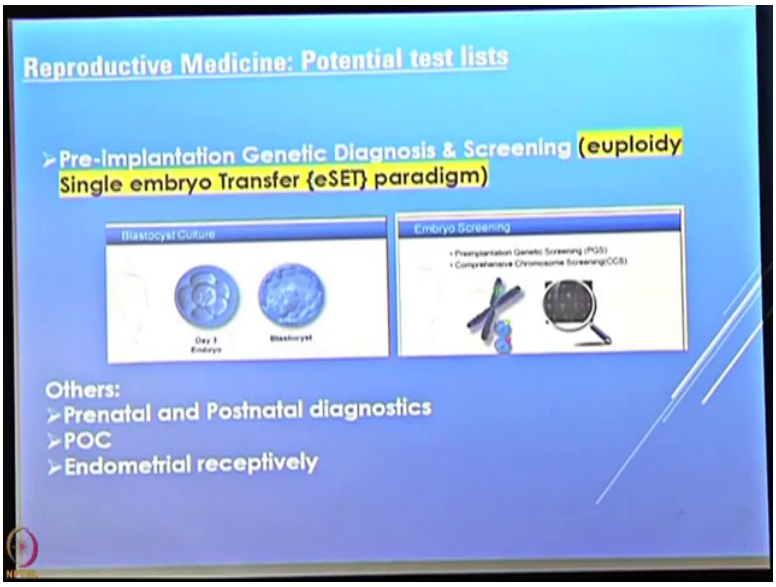

So, now I am covering 5 to 5 slide from the IVF segment. So, in reproductive medicine the most challenging part in the IVF is that aneuploidy in the embryos. Whenever couples go for the IVF and they do the in vitro fertilization and 70 percent are the embryos are basically aneuploidy right. And so, what doctor do? At least in India what doctor do they looked the good looking embryos identified it and basically implant like 3 to 4 embryo, sometime 2 sometime 3 depends.

So, the challenge is that when doctor do this thing if the good looking embryos aneuploidy not going to implant. So, you IVF cycle is fail if it is 2 embryos are a good. So, both going to implant, it going to give twins. If it is 3, good embryo it going to 3 kids right. So, it is a challenge you get either 1, 2 or 3, there is no; there is no control on that right.

So, we are discussing with the IVF Clinic and working on a single embryo transfer paradigm means; check the embryos they are good enough they are euploidy embryo not aneuploidy and only one single euploidy embryo is basically go for the further IVF cycle and for the implantation.

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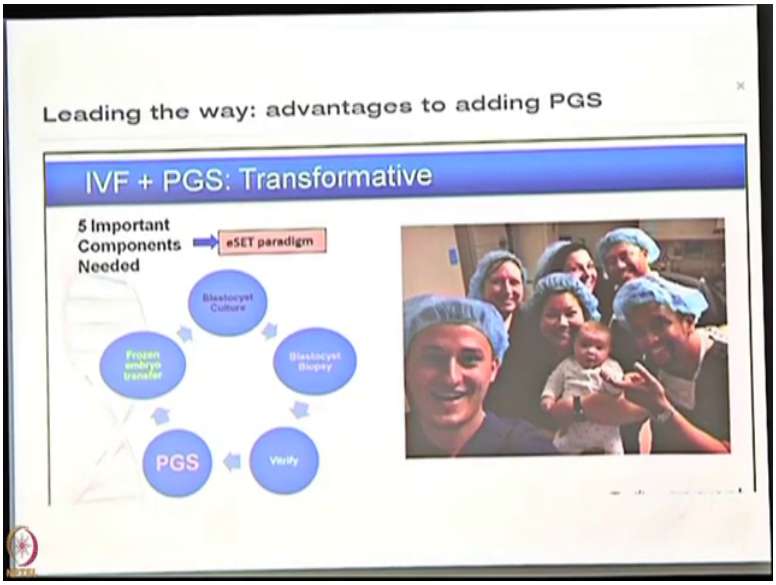

So, we are discussing this thing with single embryo transplant. Doctors basically do they do the blastocyst culture and they do not do they do the biopsy and frozen on the embryo, but they skip this part PGS preimplantation genetic in India and after vertific frozen these embryo taken and go for the IVF. So, they use three to four embryos and directly implant. What we are talking to doctor do the pgs identify the good embryos, once good embryo and implant process for the implantation. So, you so, doctor the couples get going to get only one kid right.

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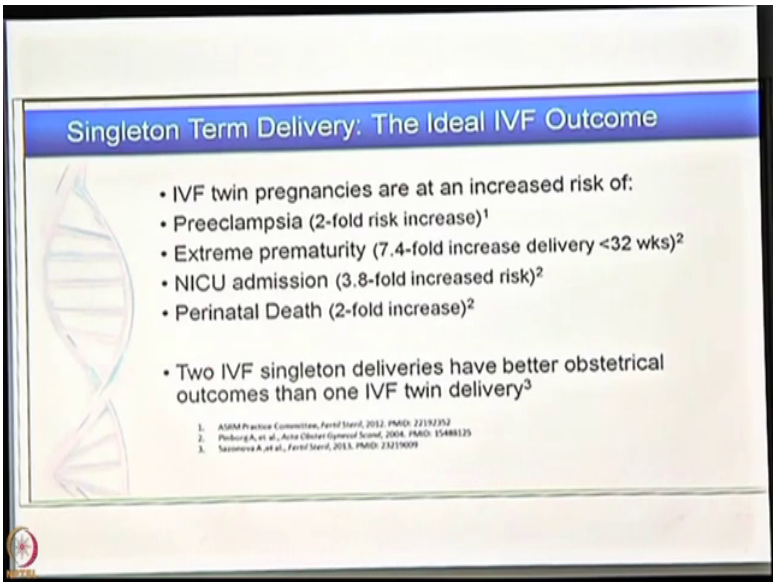

So, when we talk about the problems if they have they with the pregnancy goes with the 2 twins there is a lots of problem with the preterm labor preeclampsia and it is high rate of the prenatal death in the twins right.

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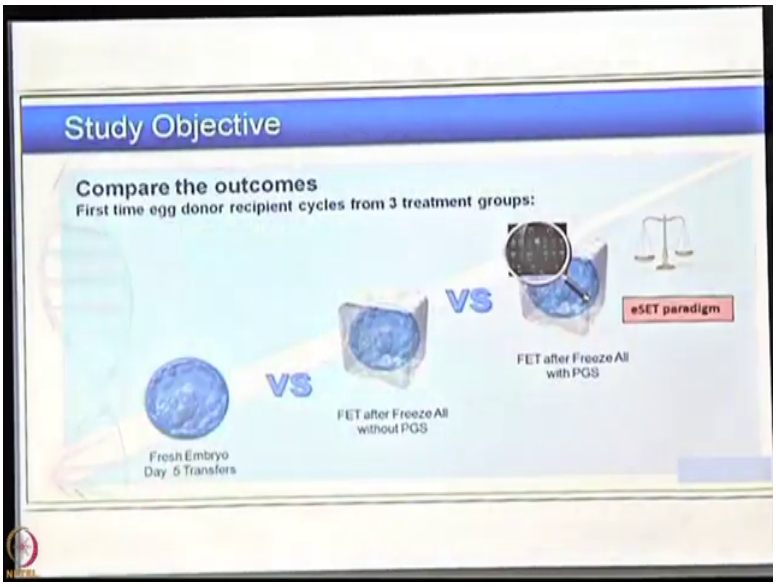

So, that is why we talk to doctor go for this test means you can go for frozen with that do the PGS, PGS means; you just go for the screening of all 24 chromosome identify all the chromosomes are good enough identify the euploidy embryo and then go for the IVF.

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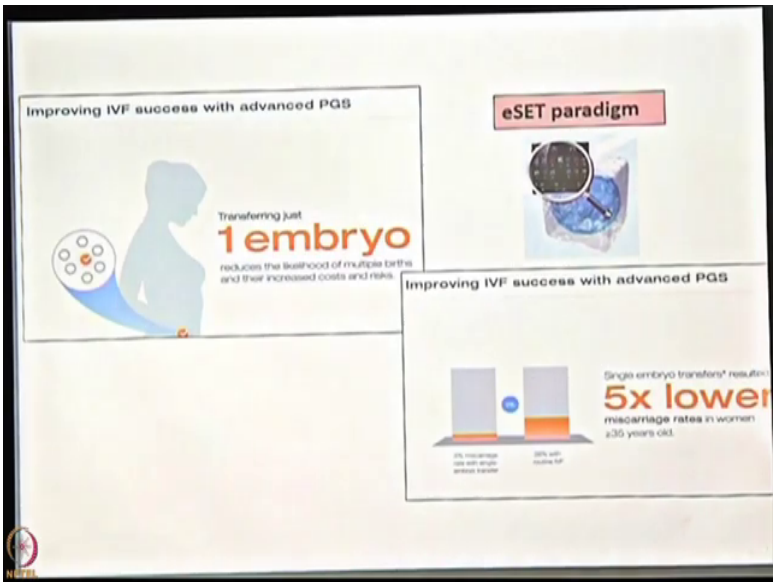

So, how basically they do by transferring the one embryo basically it also cost effective for the couples also it is 5 time less pain basically to grow the kids right.

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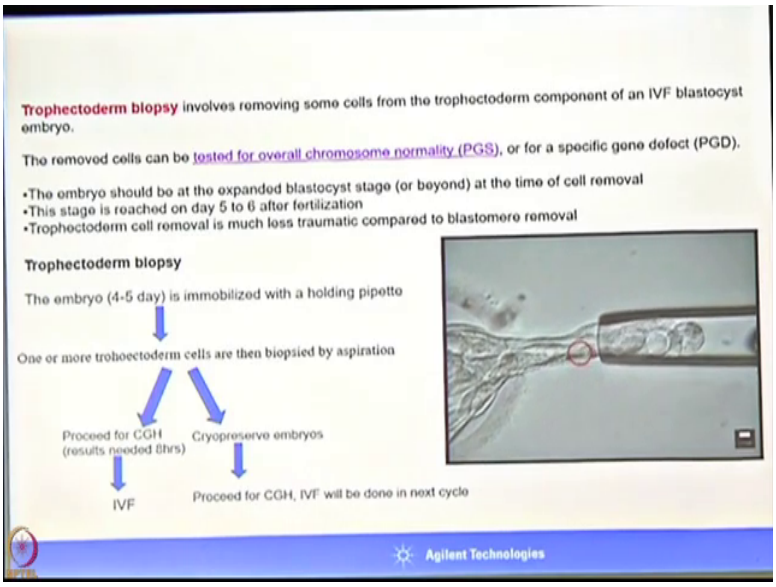

So, how they do? This test is basically done by the biopsy of the embryo. So, this is the embryo.

Student: (Refer Time: 33:49).

And this is the; this is the blastocyst and they take the one cell of the embryo only one cell of the embryo.

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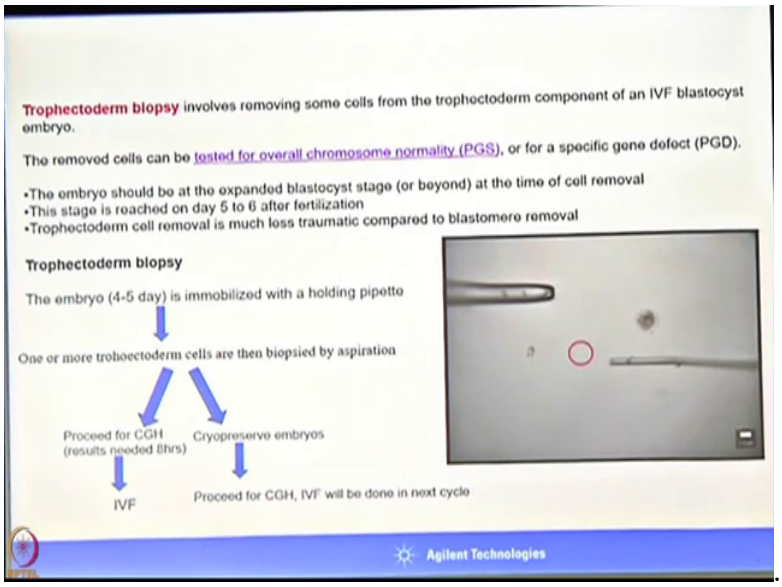

Student: (Refer Time: 34:02).

So, this is one cell they collected.

Student: (Refer Time: 34:06).

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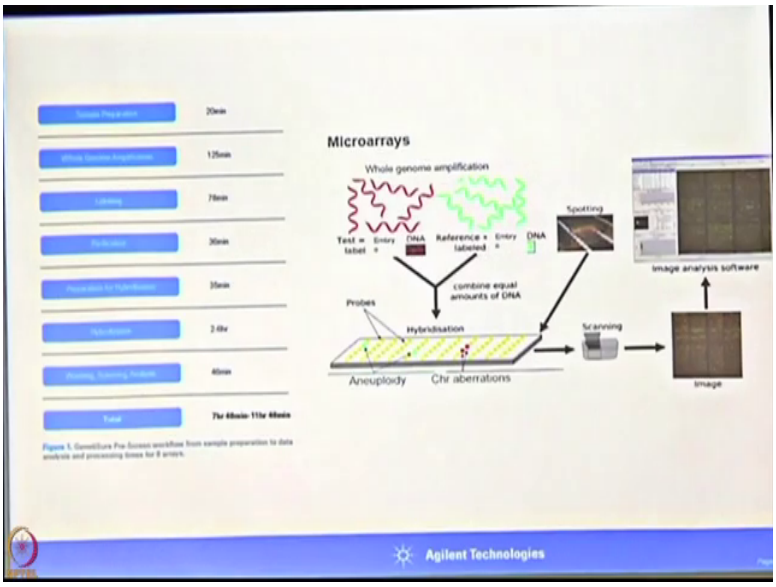

So, when this biopsy is done, one cell is collected from the IVF embryologist and for that because one cell has a very low amount of DNA you cannot do anything with that. So, we do we do the whole genome amplification to increase the quantity of DNA right. And we labeled with this DNA with the psi 3 and psi 5 dive right and hybridize on a micro ray slide and go for the analysis right.

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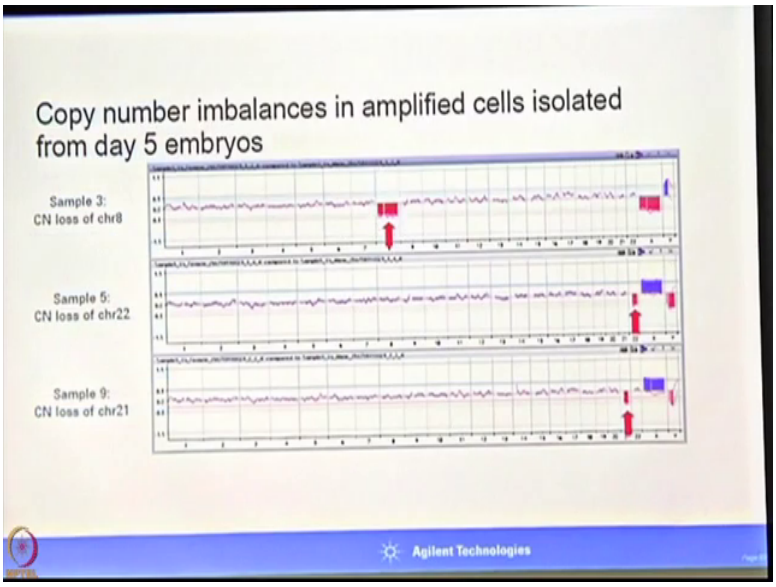

If they some mutations, this is the mutations. If some mutation on the chromosome number 3 or chromosome number 20 22 or deletion at chromosome number 21, it shows that this embryos are aneuploidy and do not process for the further IVF right. So, you can identify this by adding the PGS test you can basically identify the aneuploidy in 8 hours and after do this basically the success rate of the IVF basically increase.

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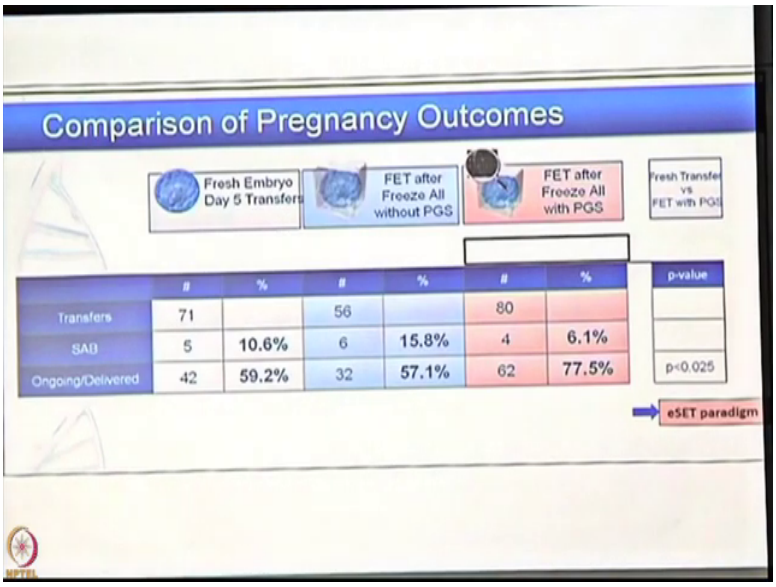

When they do not do any test the success rate going to be 80 percent with this single embryo transfer paradigm right because he identified the all the chromosome where is that where is a problem if they deletion do not process for the IVF.

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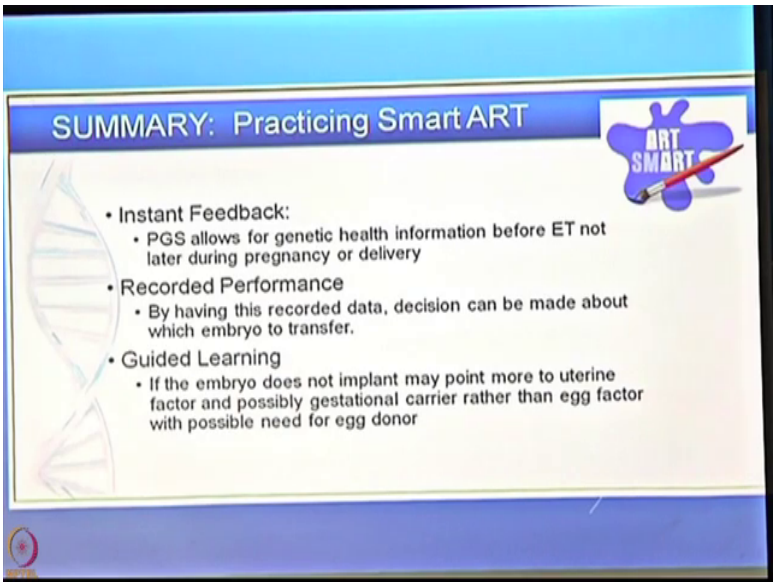

So, this is the smart ART right before to doing a implant process for the aneuploidy just do the pgs validate this embryo are good enough and then go for the IVF cycle right. Thank you.

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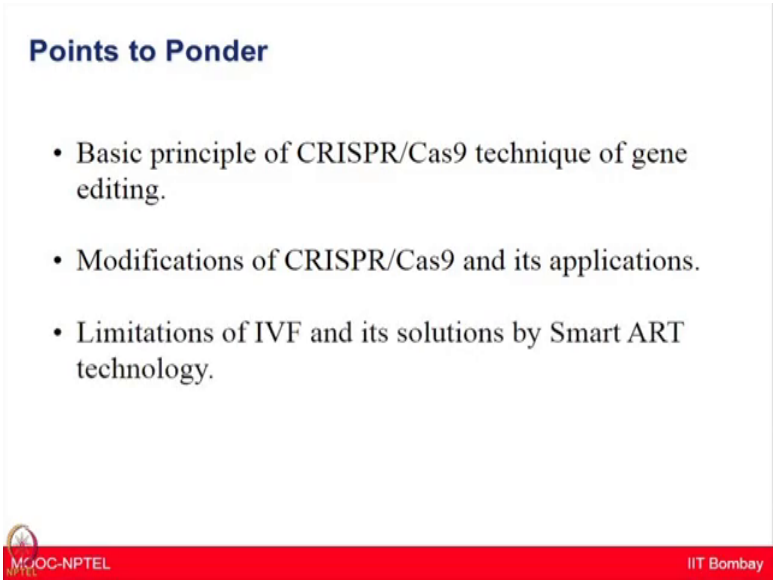

All right. So, I am sure after listening today's application based lecture, you must have found this very interesting and you saw that how the whole exome sequencing kit can be used for diagnosis of rare disease. And how the results can be used to choose the right treatment. Absolutely just one of the success stories of many things which can be done on various type of NGS based platforms. Dr. Jaiswal also briefly gave you an idea about CRISPR Cas technology which is one of they much talked about gene editing technologies available.

And I hope you have enjoyed not only today's lecture, but also the series of lecture which we had in the last couple of days and week about NGS technology platforms. And this is one of the revolutionary technology which is really transforming the way we have seen the medicine and clinics are really you know getting revolutionized with much faster pace of assists coming to the clinics for the patient care.

So, your understanding and your knowledge about these applications and these novel technology platforms are definitely going to be very useful. And I must say there is a wealth of data available now from various type of genome sequencing projects. If you know what you are looking for you can do a lot of data analysis from yourself. I will give you one instance one example, the cancer genome at least TCGA is one of the good resource for looking at the you know patients cancer data available. And while they publish that work couple of years ago in science and nature series of papers published.

But, what is more important when they made data publicly available for 1000s of patients genome data then their meta data analysis from that data many people have looked at very specific type of questions, what do they impact of giving genes in patients survival for example. Or looking at a specific pathways and you know maybe 100s of papers are actually published just by looking at data alone not by generating data right.

So, what I want to convey you is that you need not to generate the patient derived genome sequence data just for the sake of looking at everything biologically. You, if you are interested you can just go download these datas use many of the publicly available software and resources analyze in your own manner and then probably you can get some very meaningful and new information even possible just by looking at these data for addressing certain questions.

So, I hope some of these exposes what we are trying to provide you is going to really make you more comfortable and also make you more enthusiastic and motivated to really take lead forward. Well, I will thank you to stop today's lecture, but we will have more exciting things to continue in the next lectures as well when we talk about a new resource for you which is human protein atlas.

Thank you.