INDIAN INSTITUTE OF TECHNOLOGY

NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING

(NPTEL)

C-DEEP
IIT BOMBAY

Applications of Interactomics using Genomics and Proteomics technologies

Course Introduction by
Prof. Sanjeeva Srivastava

Lecture – 36

NGS Target enrichment workflow for exomes, targeted panels and beyond

(Refer Slide Time: 00:26)

Welcome to MOOC course on Applications of Interactomics using Genomics and Proteomics technologies. Today you will hear again from Dr. Mukesh Jaiswal who is one of the Application Scientist words in the areas of mission sequencing technologies. He will talk to you about whole exome sequencing kit for investigating rare diseases.

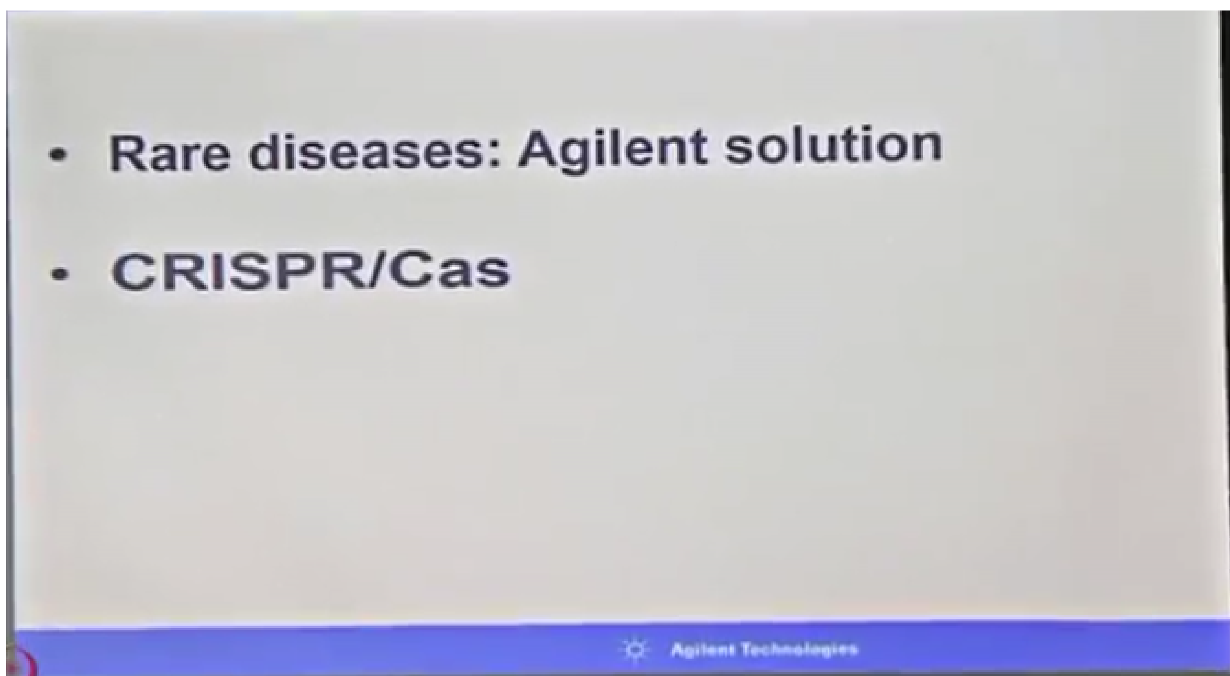
This is probably going to be the last lecture on the NGS technology and its application, and while today's lecture is not going to cover much of the basics of NGS, but it's differently going to give you more information about possible applications from these platforms, so let's continue on this lecture today, and then we will try to conclude what we have learnt out of the NGS based platform from basics to the applications.

(Refer Slide Time: 01:26)

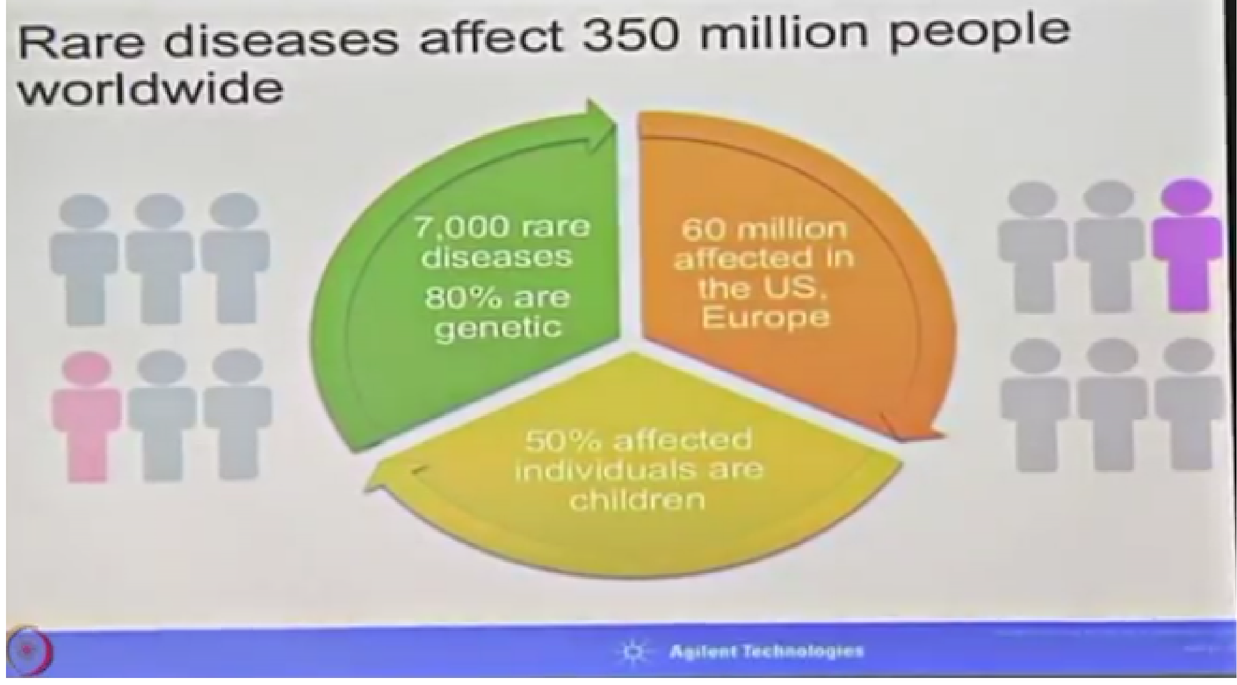
Dr. Mukesh Jaiswal
Application Scientist
Agilent Technologies

Dr. Mukesh Jaiswal: Okay, so today we are going to talk about the investigating the rare disease and its treatment with the Agilent solutions, so at this side is a, so I'm going to cover about what are the rare disease and how basically it can be diagnose by the NGS solution and how we can basically give the treatment to the patient, right, so we have some solutions where basically you use Agilent solution for the diagnostic of rare disease and its treatment part.

So it going to cover a sum background of the rare disease and then I got to hold some part of Crisper Cas,
(Refer Slide Time: 02:24)



how basically it can utilize for the treatment purpose, so what are the rare disease?
(Refer Slide Time: 02:26)

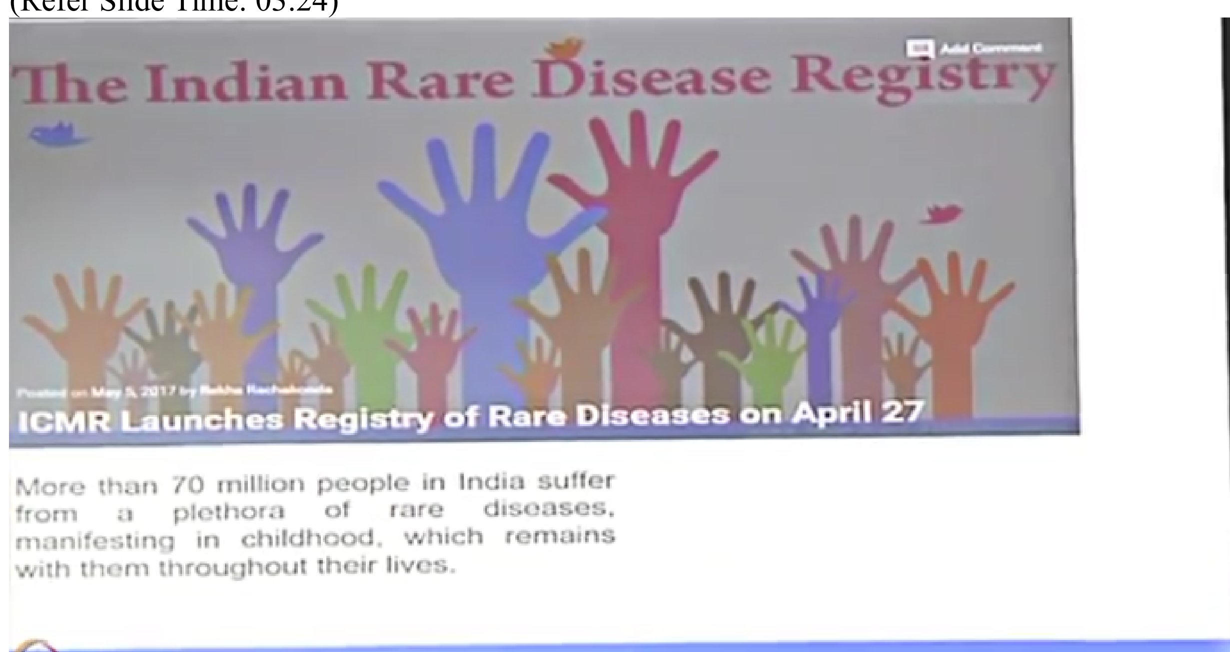


A rare disease basically, is a rare disease is affected 60 million people in US and Europe, so it's a big number, it's quite big number and 7,000 rare disease basically known till now and 80% reason of that is genetics, it's something wrong in their genetics, so yes please.

Unidentified Speaker: We are talking about 60 million affected in the US and Europe _2:59_

Dr. Mukesh Jaiswal: Anyhow I'm coming to next slide, so I'm coming to the next slide, so this is like some worldwide I'm telling, so now I'm going to, next slide we have, I have Indian data also. And 50% affected, children's are basically affected this disease, so coming to Indian scenario, yeah,

(Refer Slide Time: 03:24)



so ICMR there, Indian Medical Council of Research launch this registry in 2017, and they said that around 70 million people in India is also suffering for this rare disease, so it's a quite big number and that's why they launch a project ICMR registry where you can basically write a grind to them to work on the rare disease, what's the problem, how you can diagnosed that thing, what's a treatment of part of that?

So in April 2017 they launch this project, and so these are the key objectives of this ICMR registry,

(Refer Slide Time: 04:00)

The registry will monitor:

- *The prevalence, incidence and natural history of disease over a period of time towards guiding policy decisions.*
- *It will support research initiatives that* ***aim to better understand the distribution and determinants of rare diseases.***
- *It will facilitate access to innovations in genetics, molecular and computational biology, and other technological advances for patients suffering with rare diseases.*
- *It will also bridge the lack of data on rare disorders in India.*
- *It will provide access to supportive care for countless individuals suffering from these disorders.*

Agilent Technologies

so it main object is that to understand what's the problem of rare disease, what is the causation of that, and how it can be, how this data can utilized for the treatment of the rare disease, so these are two main objective of the ICMR, and so it launched and I think is evaluate for the grant application also, okay.

(Refer Slide Time: 04:30)

Rare diseases can have devastating impact on health

Cystic Fibrosis

- Excessive mucus in lungs and pancreas causes respiratory failure and inability to digest food
- Median survival age is 40 years
- Affects more than 30,000 people in the US; 70,000 WW

Leukodystrophy

- Progressive diseases that affect brain, spinal cord, peripheral nerves affecting movement, vision, hearing, balance, ability to eat etc.
- Children affected with leukodystrophy live 5-10 years
- Affects ~60,000 people in the US

Retinitis Pigmentosa

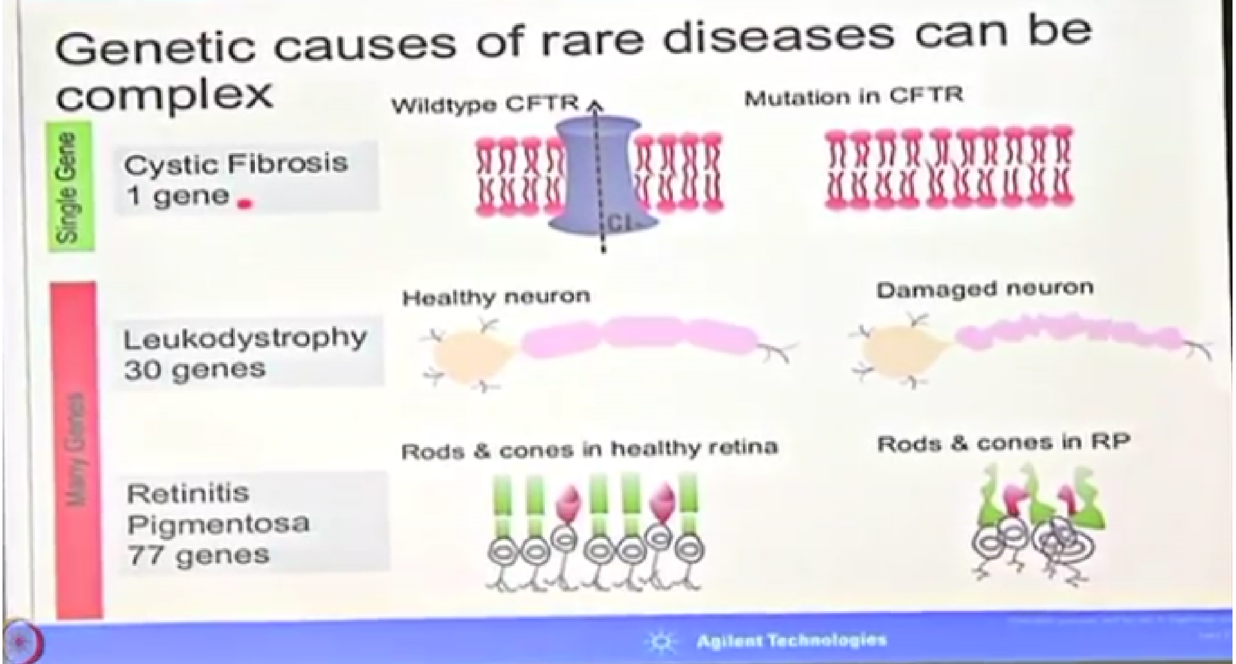
- Retinal degeneration ultimately causes blindness
- Most people with RP are legally blind by age 40
- Affects ~100,000 people in the US; 1.5 million WW

Agilent Technologies

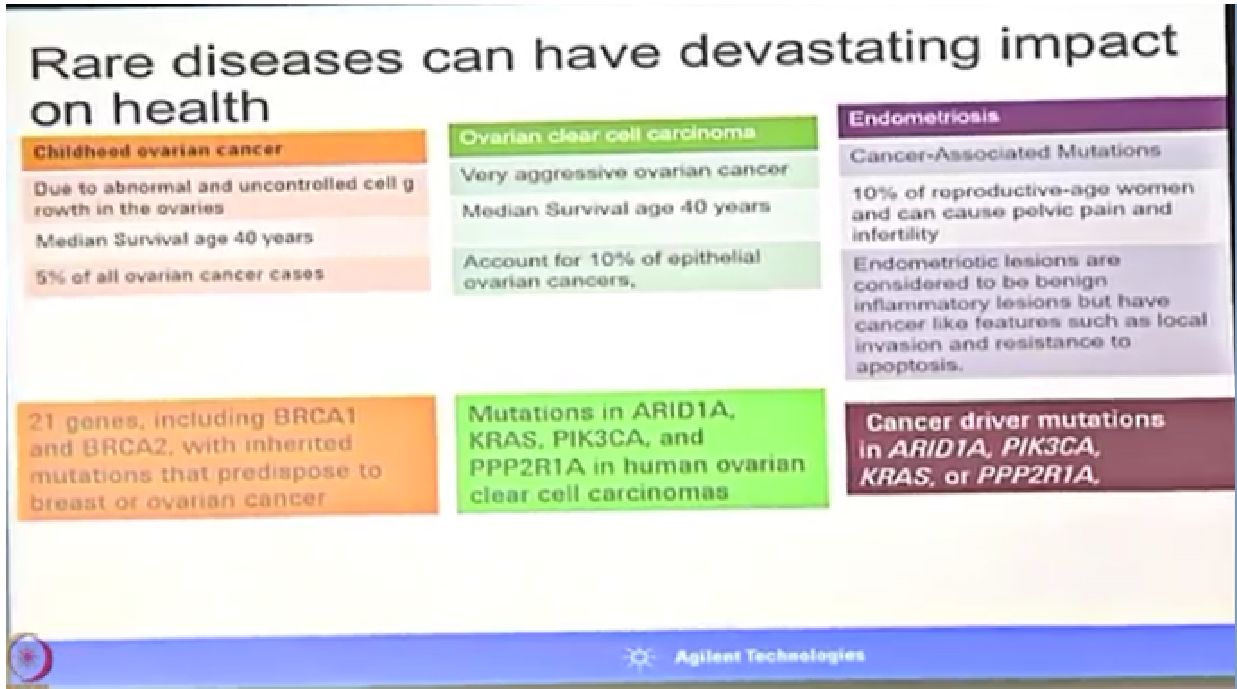
So I'm going to talk about some of the rare disease which is existing worldwide, this worldwide, so let's come in the first cystic fibrosis, this one, so this is the disease basically a based excessive mucus is deposit on lung, or pancreas, which called the respiratory failure, and

inability into digestion part, and the median survival rate for this disease is 40 years, right, and the worldwide is a 70,000 patients are known worldwide, then leukodystrophy this one, and this is again the progressive disease affect the brain and spinal cord and the nerves system, and this is basically, children's basically affected of with this disease and 5 to 10 year especially children's and around 60,000 children's are affected worldwide.

Retinitis Pigmentosa, this is another disease and it is affected, it's got blindness and it basically the survival is like 40 year, and around 100 thousand or 1.5 million worldwide patient are known for this disease, so what's the cause of this?
(Refer Slide Time: 05:56)

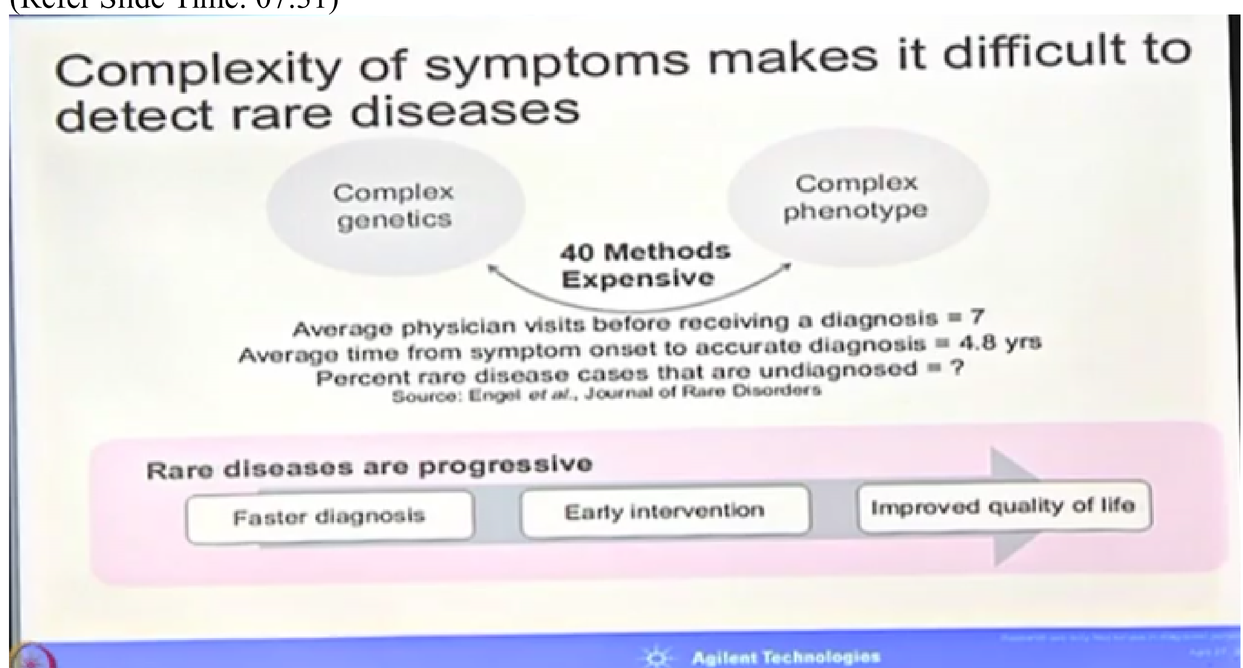


What is the cause of this? The cause of this basically a sometime it is affected by the single gene or sometime it is affected by the single gene or sometime is affected by the multiple gene, right, so if it is like the first one cystic fibrosis is only the one gene that is CFTR their sublime nutritional CFTR and then this transporter, transporter is basically disturbed and that caused the cystic fibrosis, so in this cystic fibrosis only one gene got affected, right, but if you see these two the multiple genes are affected, so it's very difficult to identify when the multiple genes are affected, so here like 30 genes are affected here, and in this disease it's 77, right, so problem is that, this cause that damage neuron and here in the Retinitis Pigmentosa it is cone cell and the rods cells are basically disturbed, right, so these are the disease and they are multiple genes are basically involved in there,
(Refer Slide Time: 07:00)



some more which is included in the ICMR project basically, so this is childhood ovarian cancer, ovarian clear cell carcinoma, endometriosis, and they are multiple genes basically involved in this disease also. So this is also incorporated in ICMR registry, you can go in there website you can basically look what are the rare diseases.

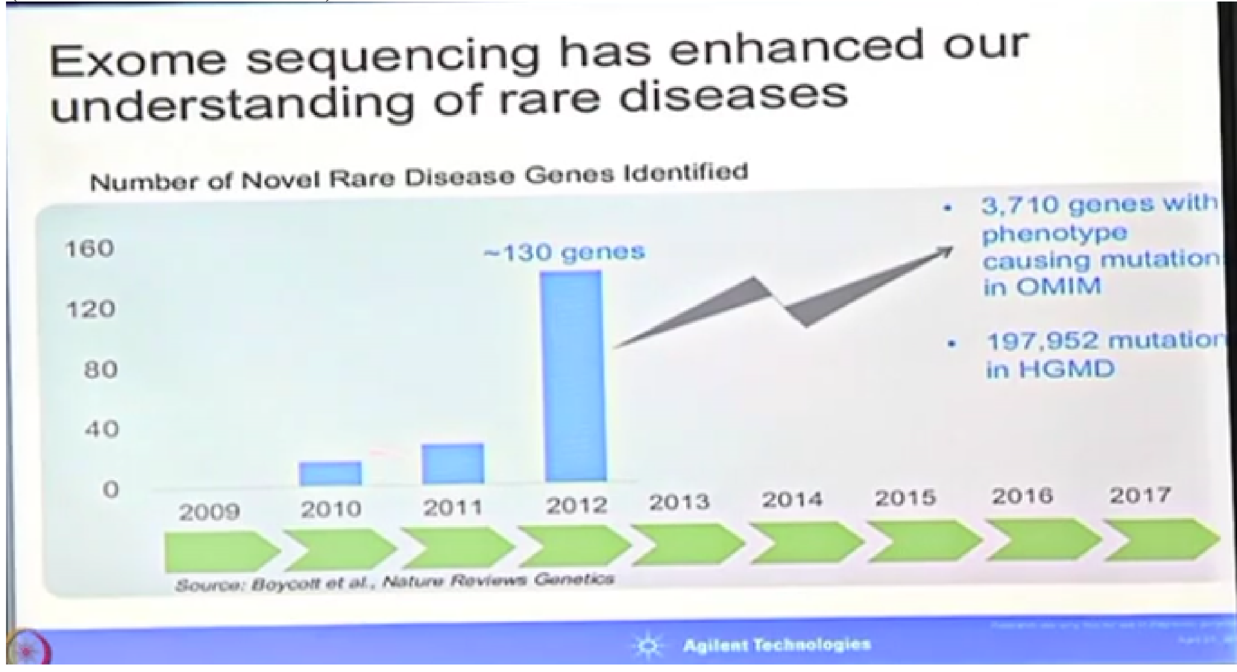
(Refer Slide Time: 07:31)



The challenge is that diagnostic, how to diagnose this disease, right, so if you go the regular process of 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’s like multiple gene they are affected, right, and basically they take at least 7 year and 40 method to use for the diagnosis, so there is a challenge, it is pretty expensive it take time, but for the treatment purpose if you have early intimation of this disease you know the cause of early, very early then the very early diagnosis then you can do early intimation and then improve the quality of the life, so that is, this is the challenge but if it take lot of like 8 year to diagnose only it would be difficult, right, so that’s why it is very important to diagnosed the disease in very early stage.

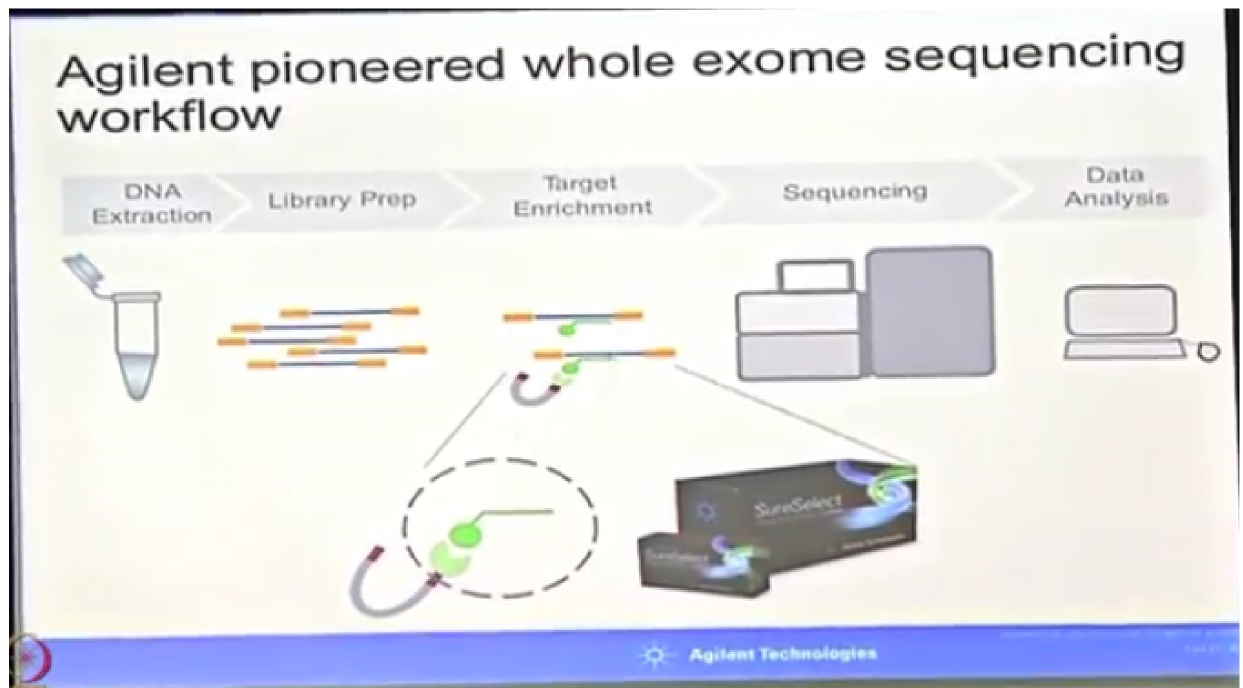
(Refer Slide Time: 08:53)



So right now if it is, if you see this rare disease now 2012 when the exome panel is just started it was only 130 gene, now 2017 it’s more than 200k mutations are known for that rare disease, basically this is because of the more advancement of the exome panels.

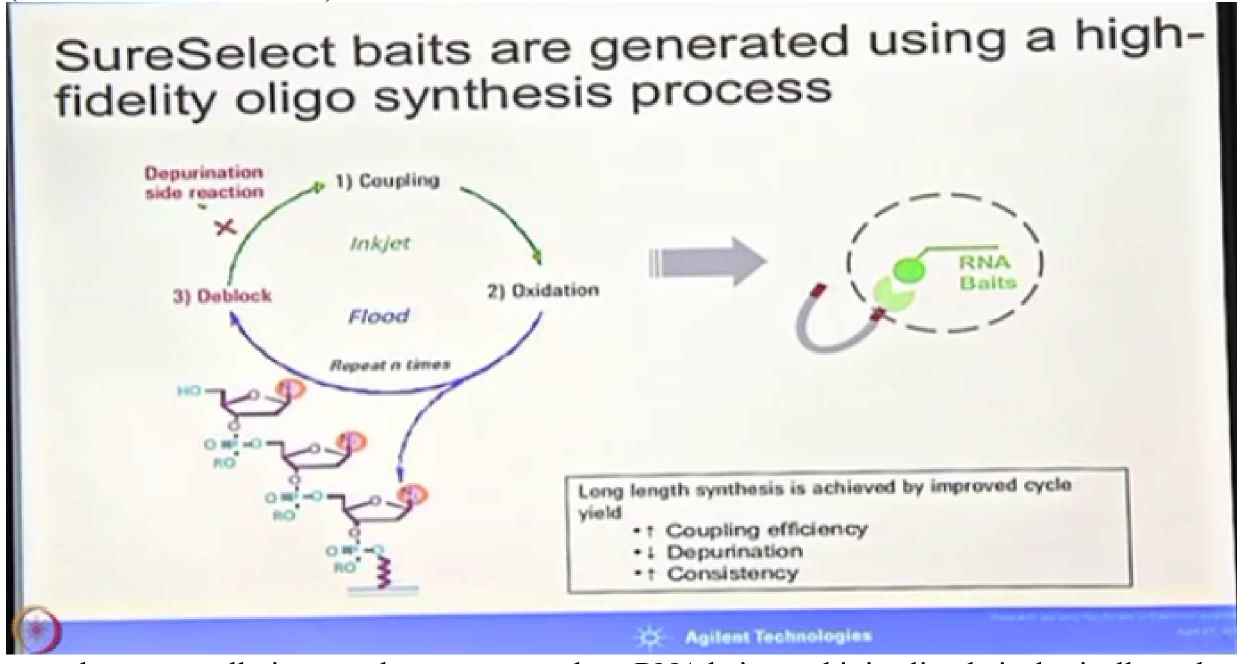
So you need to extend that DNA from the patient, right, and then go for library preparation, library preparation and then target enrichment,

(Refer Slide Time: 09:26)

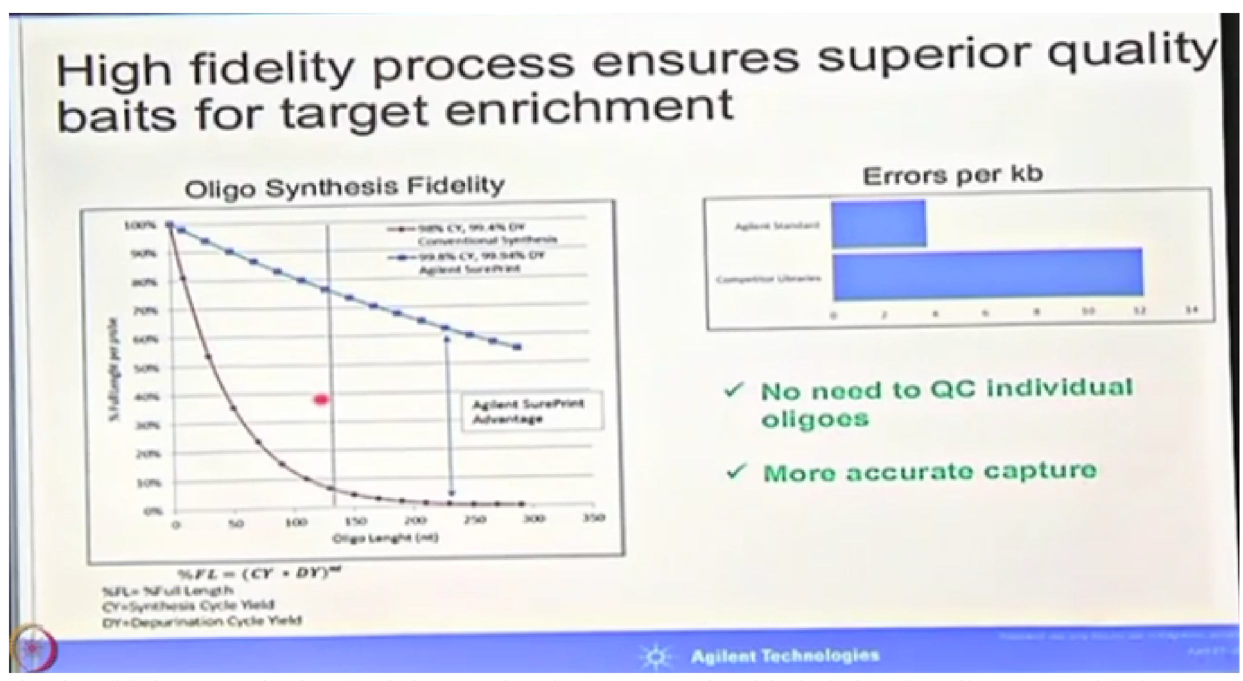


after seeing the library specifically perfectly fine, you can sequence and go for data analysis, so this kind of one work flow basically you can use our exome panel for the diagnosis of the rare disease.

So the challenges is always there, but I would talk about the why the Agilent exome panel is
(Refer Slide Time: 09:55)



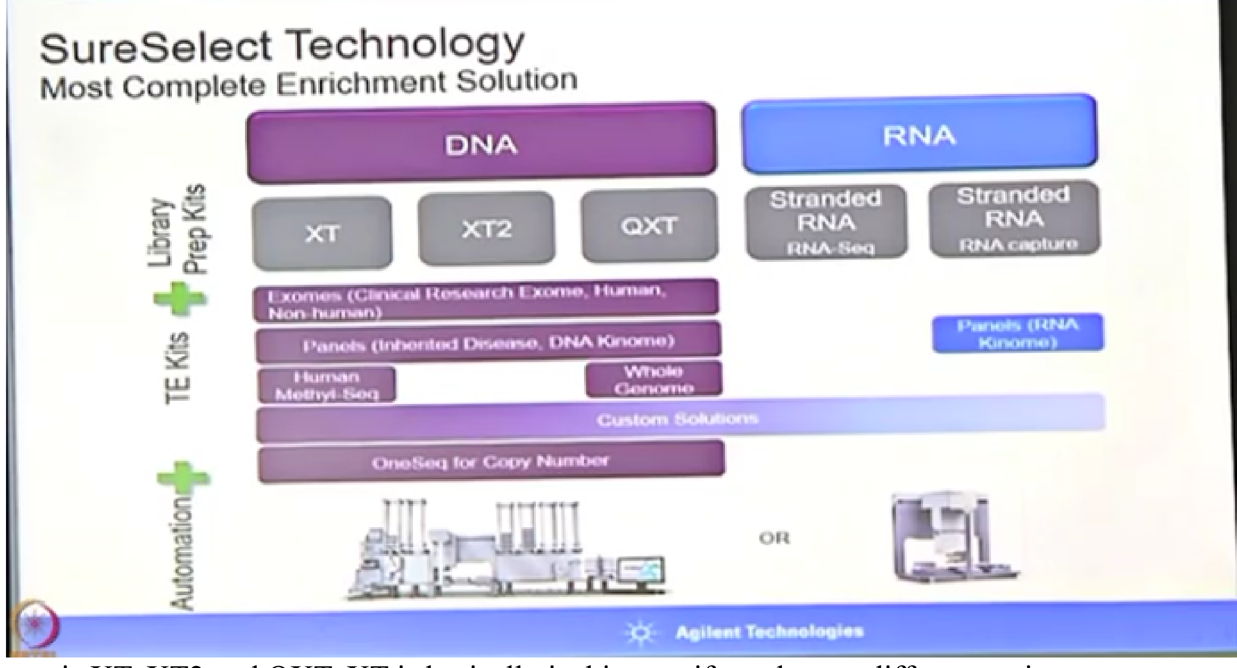
more better actually in sense because we make a RNA baits, and it is oligo baits basically and these are, because we make the RNA bait they have the better RNA DNA habitation and this are the high fidelity
(Refer Slide Time: 10:18)



basis which we make by the inject technology, we make this bait by the oligoes, so this is our high fidelity basis, if you see the error rate in the preps basically is very low in Agilent, it's like one or two error basically in 1KB, but others have lots of, so we have the high fidelity preps basically which are biotinylated, it is used for the making the libraries.

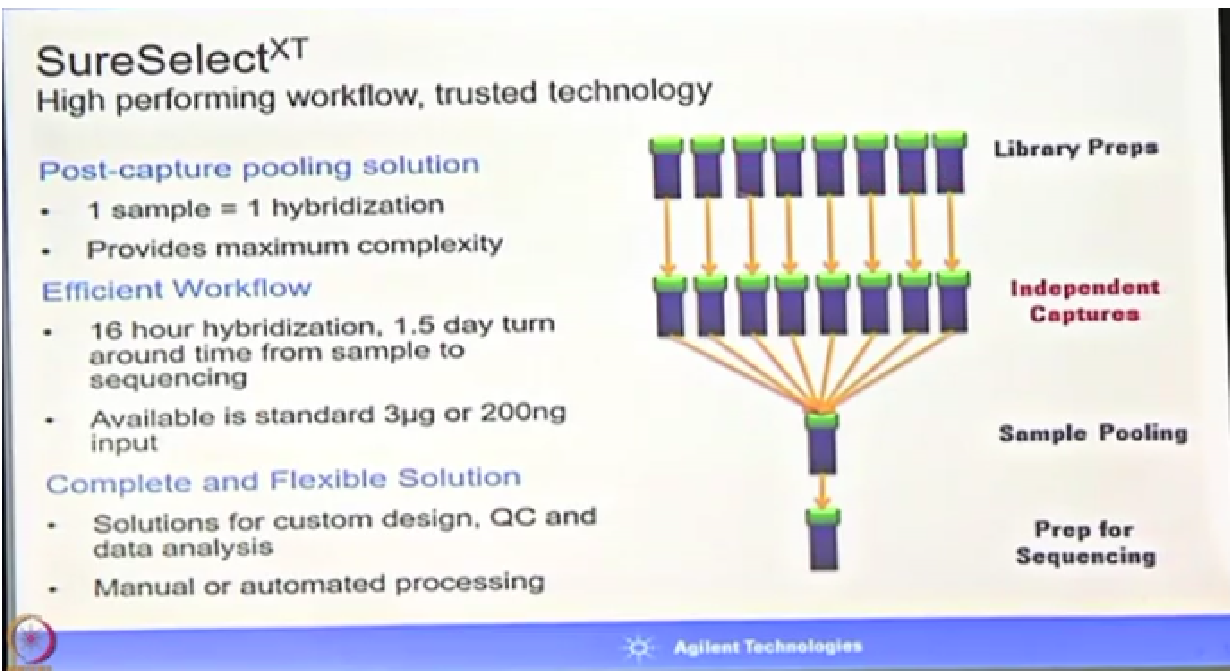
So this is the different ways you can make the exome libraries, so starting middle always with the genomic DNA, right, we have 3 different ways to make the libraries for the exome sequencing,

(Refer Slide Time: 10:58)



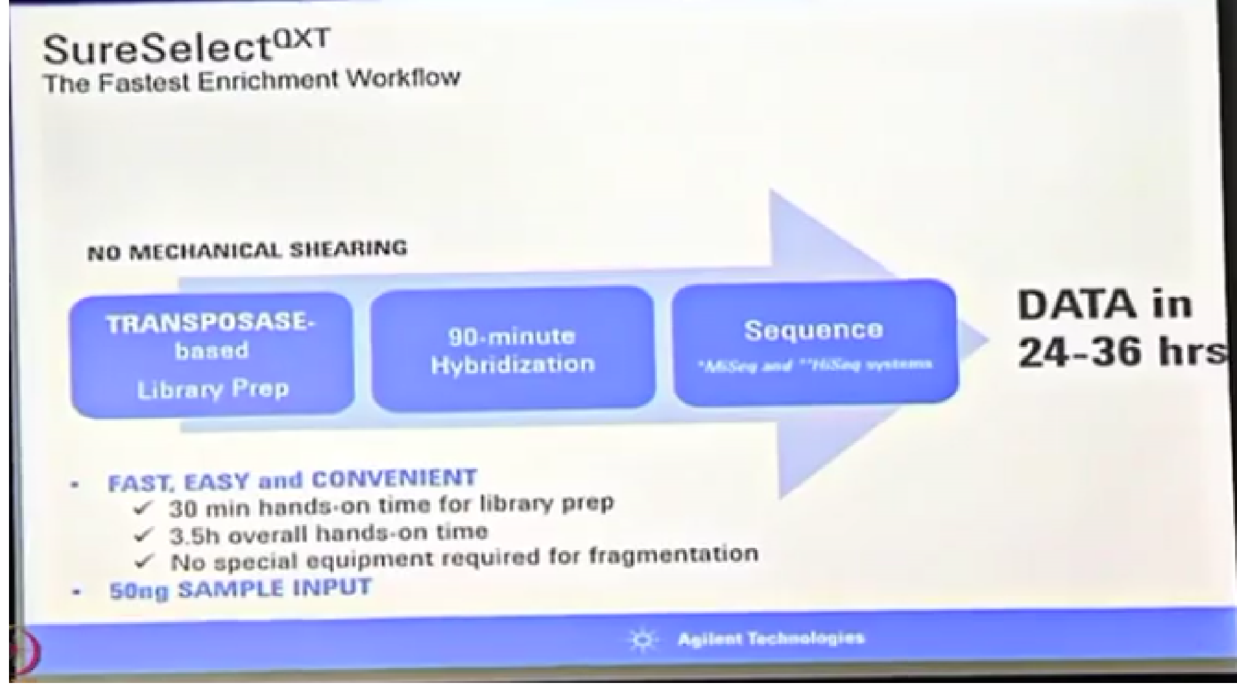
one is XT, XT2 and QXT, XT is basically is this one, if you have a different patients,

(Refer Slide Time: 11:08)



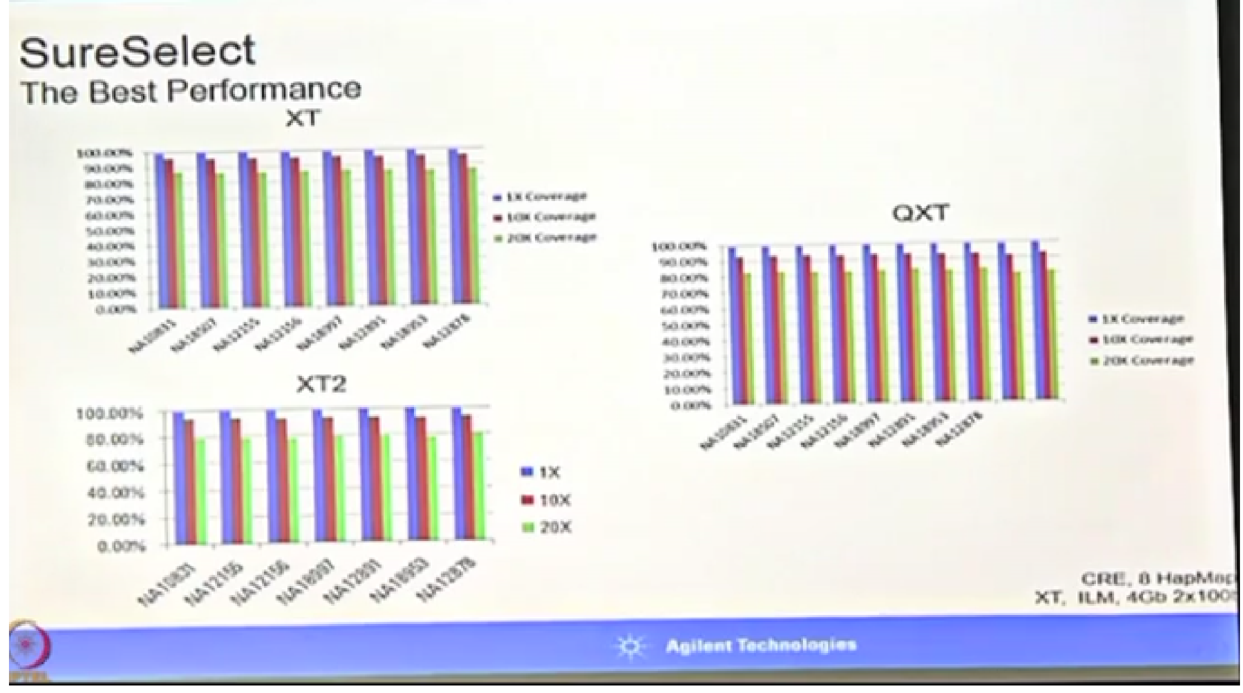
8 patients right you can make a individual library from each patients independent capture and you can pool while sequencing, right, when you go for sequencing you can pool this sample and go for one sequencing run, so that's the XT preparation of the exome preparation.

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 follow by the capture and go for sequencing, so you can compare between the patients also that is XT2.
(Refer Slide Time: 11:49)

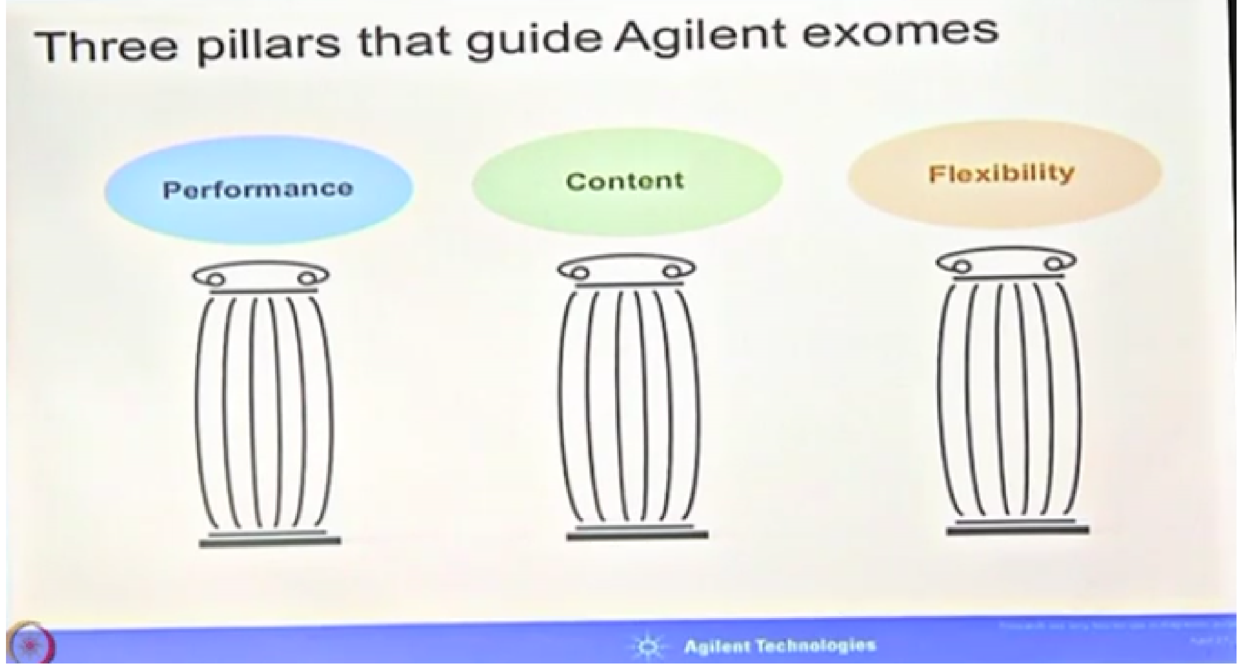


And another is hybridization is based on the geometric sharing so you can use transposes enzyme for the, to make the library basically, transposes enzyme than hybridization followed by the sequencing, this is the fastest way you can make the libraries for the exome sequencing.

Okay so performance for all these mattered to make the exome library are is pretty good,
(Refer Slide Time: 12:10)

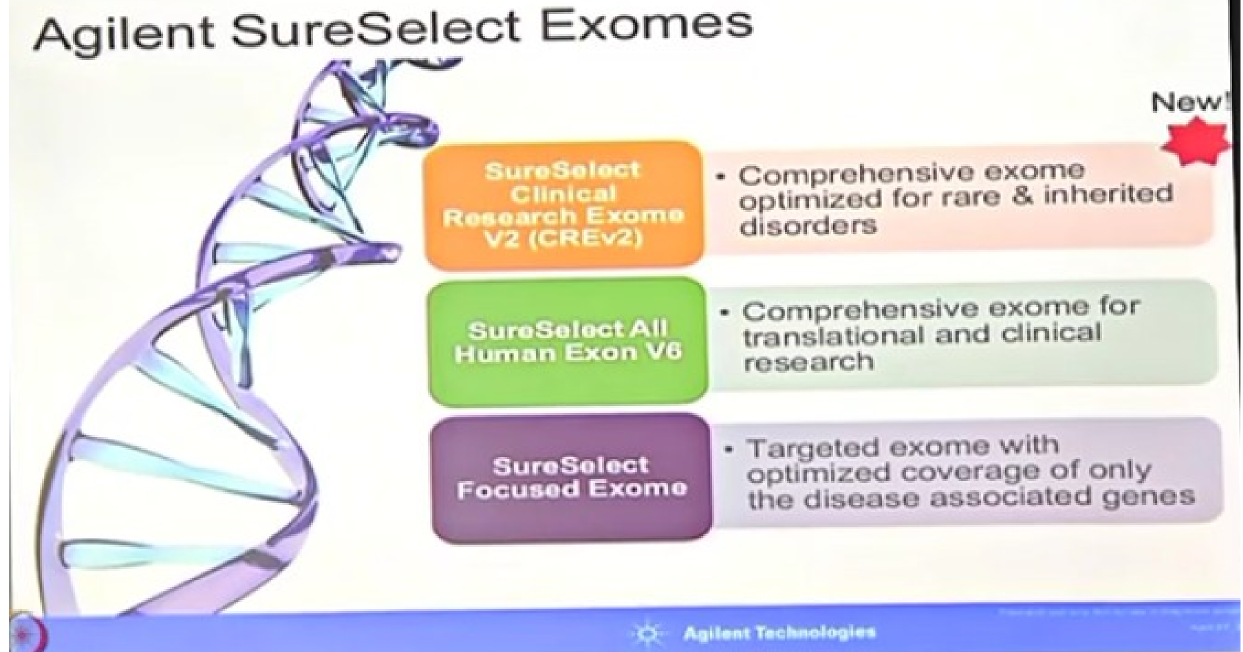


and they get very good coverage more than 95% coverage all the methods.
(Refer Slide Time: 12:18)



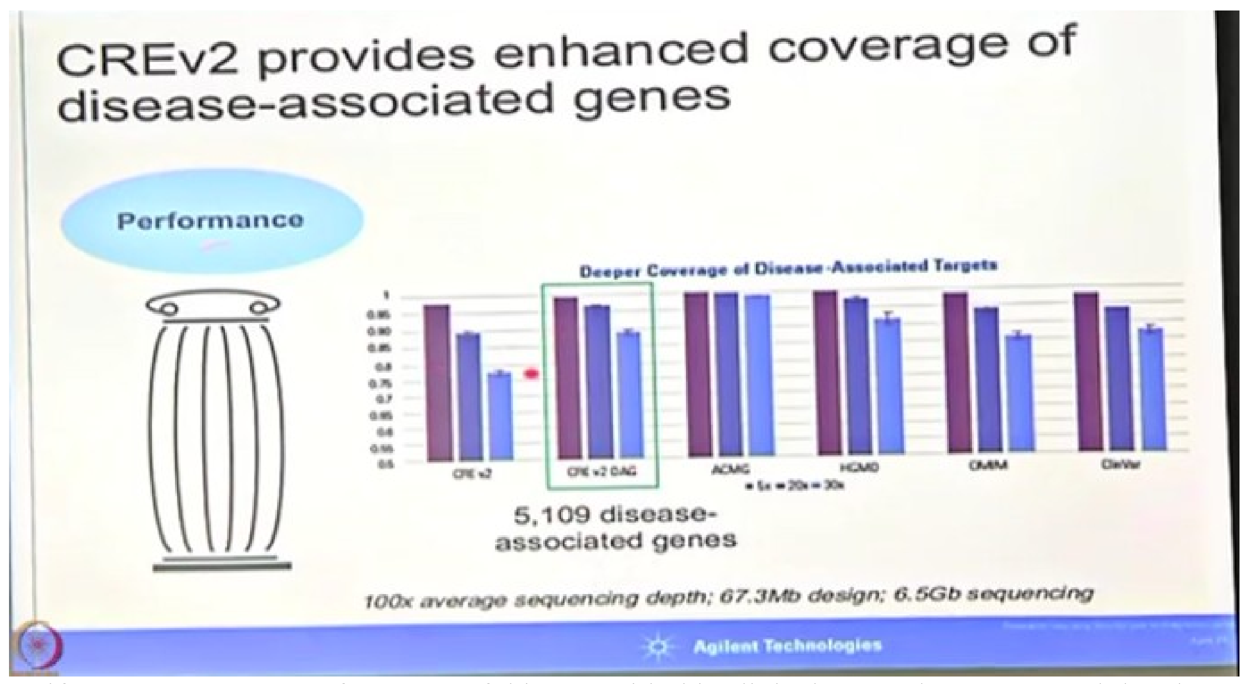
This is the three pillars basically Agilent works on basically, performance of the exome library contains in the flexibility, you work from decades to improve the performance content and the flexibility of the kits basically.

So if you see most of the rare disease basically now is studied by this panel is called Clinical Research Exome Panel,
(Refer Slide Time: 12:43)



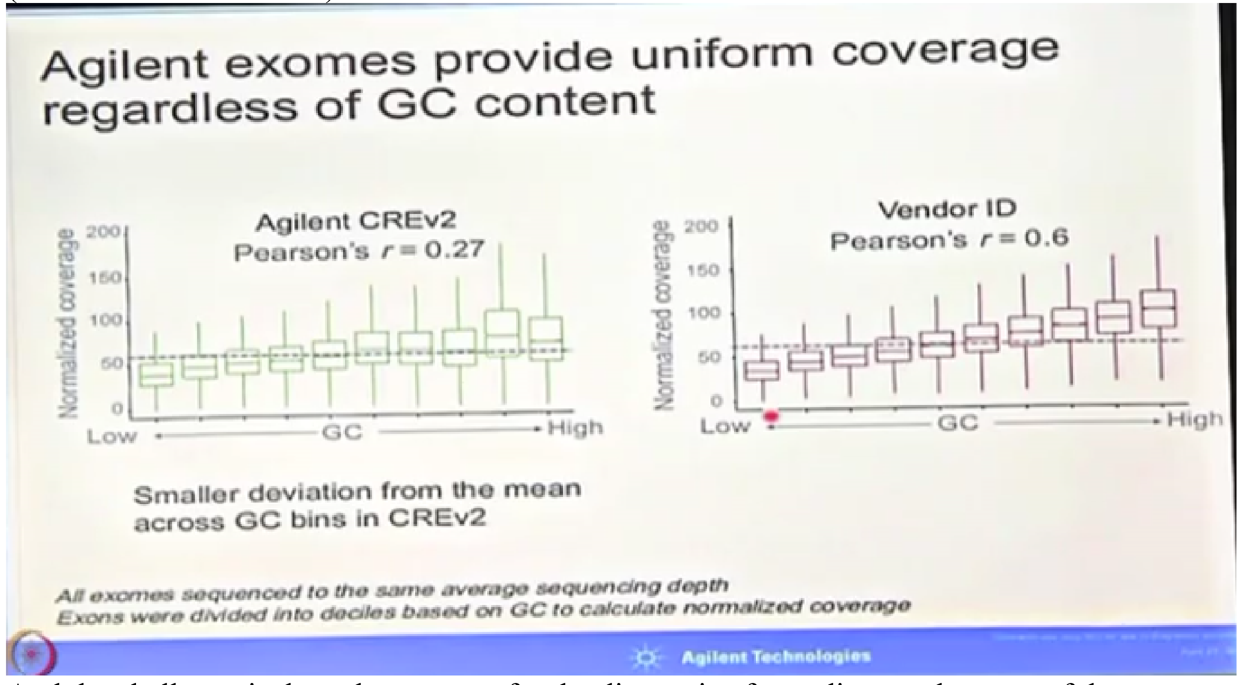
and this exome panel contains all the exome reason and also the panels all of the intronic part of the reason, which basically situated with the inherited disorder, we have the latest exome panel V7, this is mention V6, but we have now V7 and that basically cover all the translational and the clinical research panel, it cover whole exome.

Another we have the very small panel the Focused Exome, basically is covered a disease citizen, but most of the rare disease basically which I talked before it is basically used for the clinical research panel,
(Refer Slide Time: 13:30)



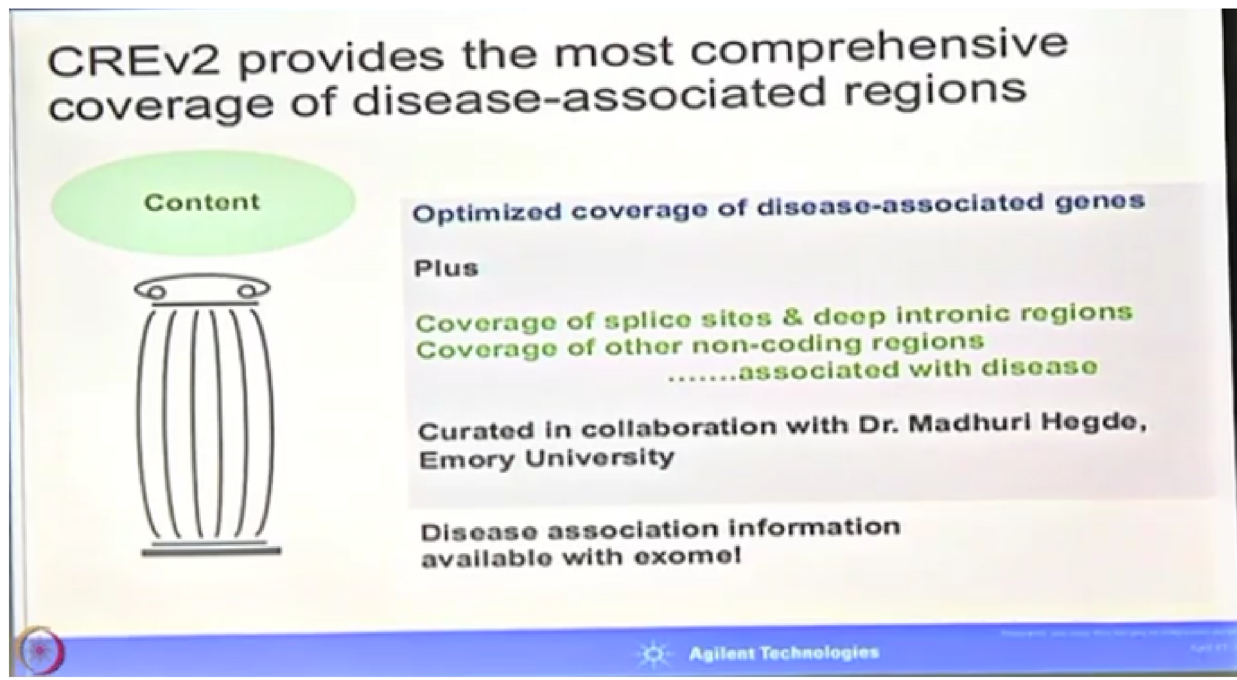
so if you see contents performance of this one with this clinical research exome panel they have like 5,000 gene is basically it deeply covered with the disease associated gene with the clinical exome.

(Refer Slide Time: 13:46)



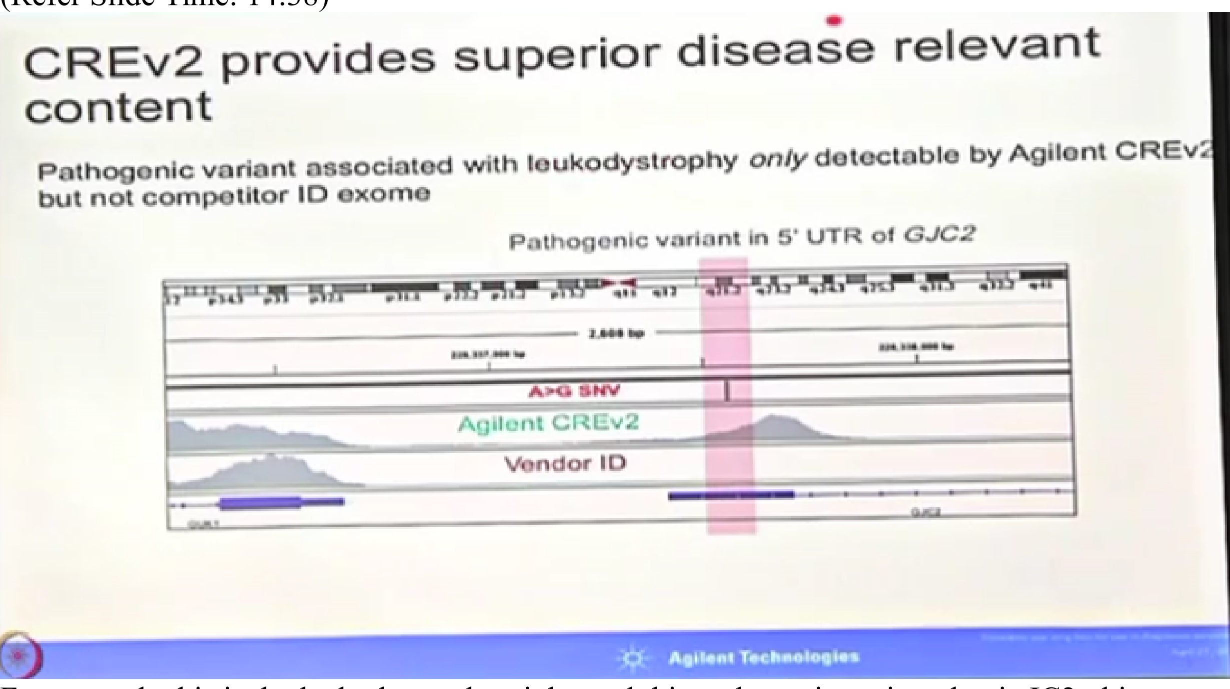
And the challenge is that when you go for the diagnosis of rare disease, the most of the mutations are present in the GC that region, to make the library for the GC is that, is always challenge so but with our clinical exome panel the performance in the GC regions is very good, is very uniform preparation of the library when you go for the GC preparation, so content basically, what's the content on the, for the preps basically,

(Refer Slide Time: 14:21)



it is basically a design by the doctor Madhuri Hegde from Emory University, and they make the basically the preps which is covered all the associated disease exome and intronic part which covers maximum rare disease parts.

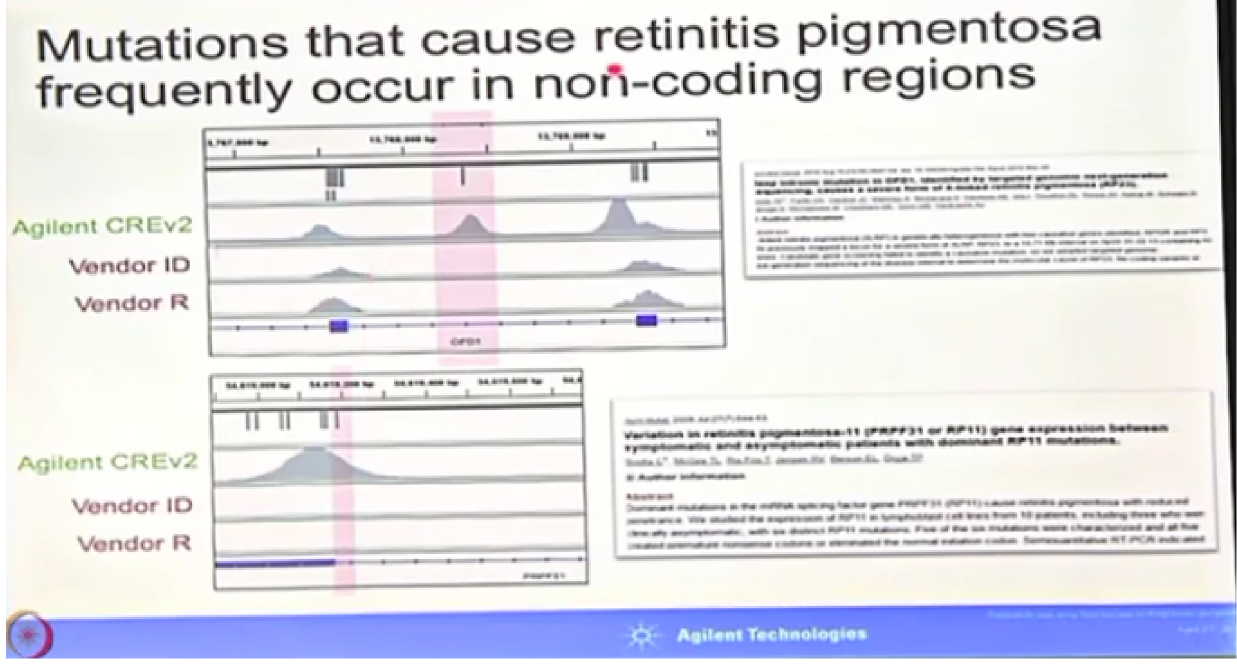
(Refer Slide Time: 14:38)



For example this is the leukodystrophy, right, and this pathogenic variant that is JC2, this gene basically if you see compare, because this is the GC enrich region and if you compare with the other vendor you don't see any coverage, there is no coverage for this gene and if you see the clinical example we cover this part also to able to detect the 5 UTR variants with this disease,

so in this disease this is the kind of pathogenic variant and you can easily detect by the clinical exome panel.

(Refer Slide Time: 15:28)



Another retinitis pigmentosa, if you see to other vendors these CFD1 gene is not covered in this part, because this intronic part, here exonic part, intronic part are not cover, right, but if we see our one is fairly covered that part, that means this detection of that mutation is very easy on that, then if you see this region again is well covered by this, these all the non-coding region, these all well covered with the, our clinical research exome.

And most of the pathogenic variant basically and for rare disease is present on noncoding regions, so it is fairly cover with the clinical research exome panel, so if you see overall in the clinical panel we cover all the clean variants, pathogenic regions and it cover mostly like 98% regions are covered,

(Refer Slide Time: 16:26)

CREv2 provides more disease-associated regions

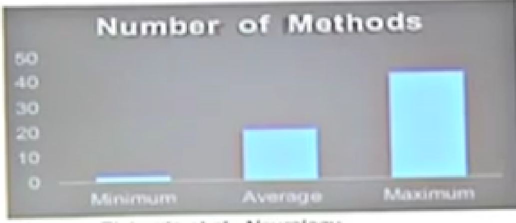
	ClinVar Pathogenic/Likely Pathogenic Leukodystrophy Variants covered	ClinVar Pathogenic/Likely Pathogenic Retinitis Pigmentosa Variants covered
Agilent CREv2	98.1%	95.3%
Competitor ID	90%	87.9%
Competitor R	90.7%	94.6%

Agilent Technologies

and other specifically has low coverage,
(Refer Slide Time: 16:30)

Accelerate the detection of disease-causing mutations with CREv2

Average time to detect leukodystrophy = 8 yrs



Source: Richards et al., Neurology

Comprehensive coverage of disease associated regions means
- ✓ Fewer method iterations
- ✓ Lower cost
- ✓ Faster detection

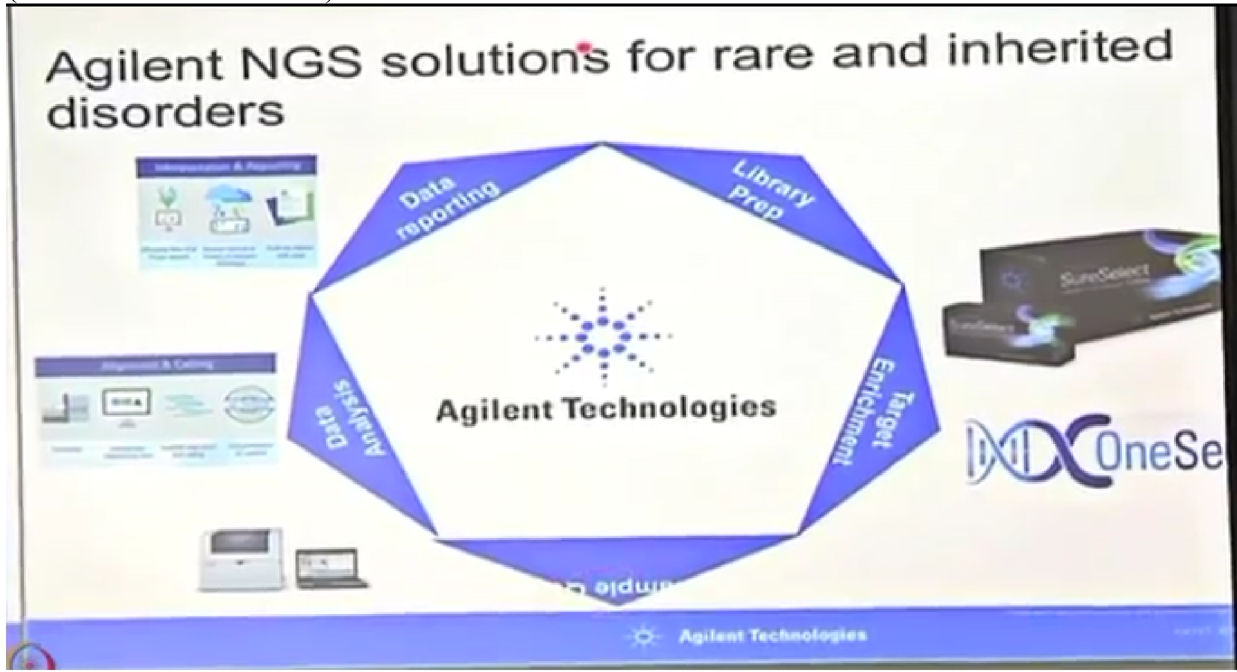
Agilent Technologies

so if you think about when the doctor is going to diagnose this leukodystrophy it take 8 years, right by the normal method, and average test basically they do around 20, 30 test to do, to diagnose for this one, it take 8 years, right, and the cost is goes up like $20,000 right, but when you do once simple test our clinical exome panel is easily identify this mutation and that is basically for a leukodystrophy and it is very cost effective, right, and…So it is just going to cost like 15,000 rupees, right, so one test cost like 15,000 rupees for exome panel.

Unidentified Speaker: _17:22_

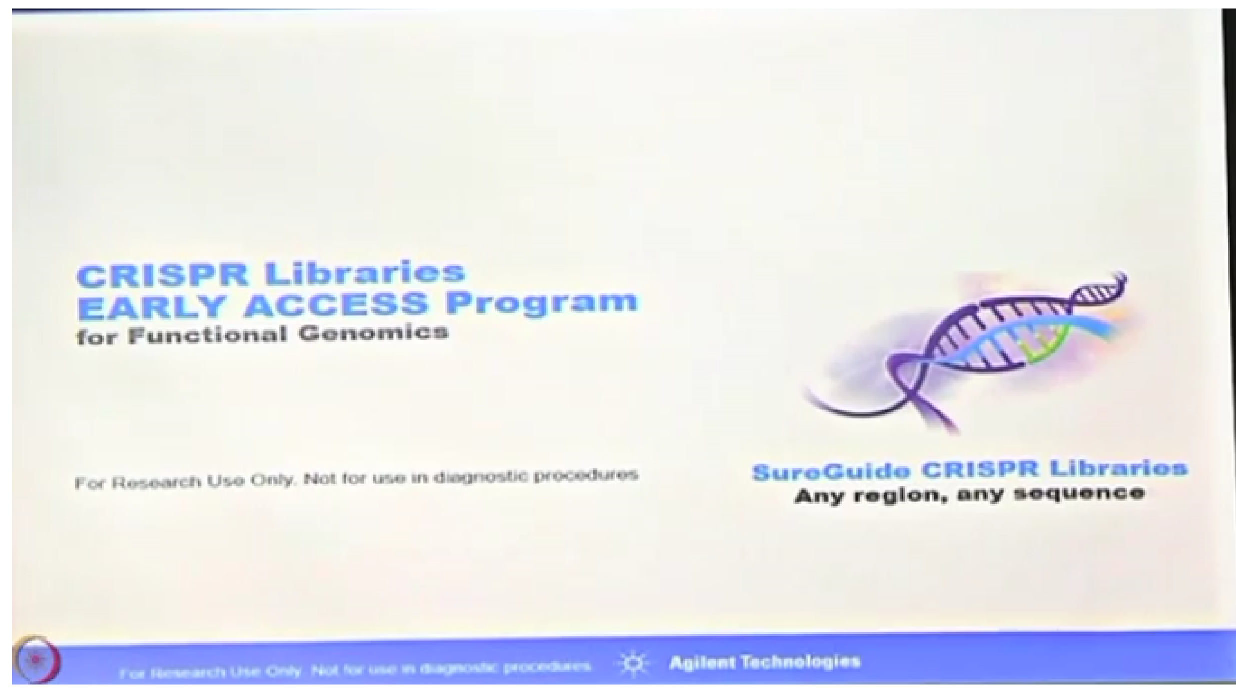
Dr. Mukesh Jaiswal: Yeah, because it covers, the intronic part, exome part definitely it is going to detect that thing, but detection rate is faster, right, and it take less time, so doctor start their intervention much earlier, with that like suppose this is our content, we doesn't make this content vacuum, we do the research and we make the preps for cover all those region, right, but sometime when you did some, you 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 a panel according to your requirement also, suppose any gene and it is not a basically 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 and incorporate in your panel, so that's our flexibility, so we work on three parts performance and the contents always optimize year by year and then flexibility if you want add more, right.

So it's very simple workflow that I told its start from the library preparation, than we make the targeted panel by the probes right,
(Refer Slide Time: 18:52)



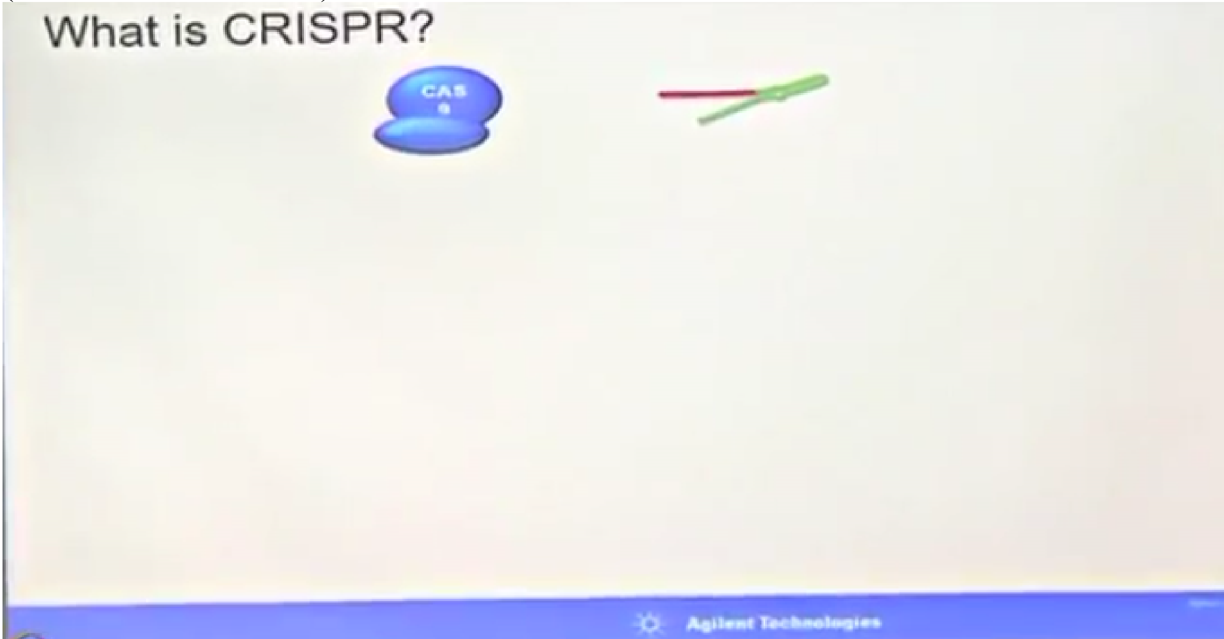
and then data analysis says and reporting, whole workflow basically takes 3 to 4 days and it's easy to identify the kind of challenges for a diagnosis of rare disease right.

(Refer Slide Time: 19:10)



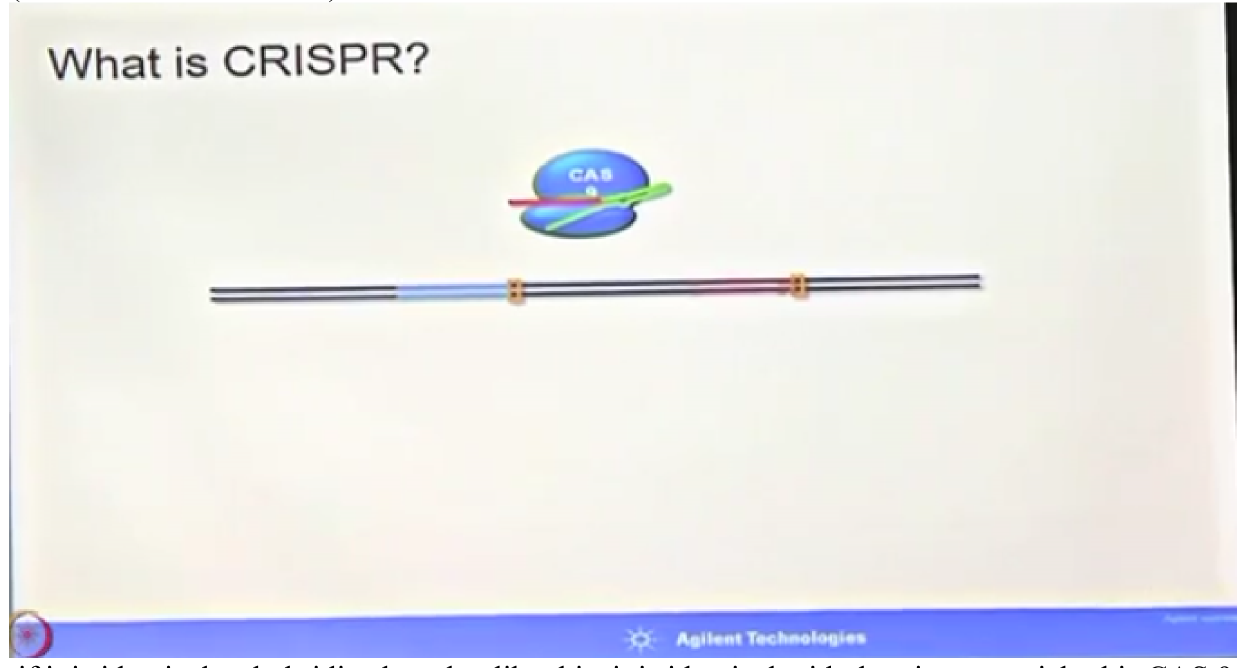
So now this part is kind of over like if you get a some kind of mutations right in any disease, not only in rare disease of course cancer, right, and but it is multiple mutation and you want to solve this problem for a treatment purpose, so we have a tumus called as the Crisper Cas where basically you can do the gene editing, right and to solve, to fix that gene for the treatment purpose but this is very early, we launch some libraries for the treatment but it's very early stage, let's start with that, what is the Crisper Cas?

(Refer Slide Time: 20:11)



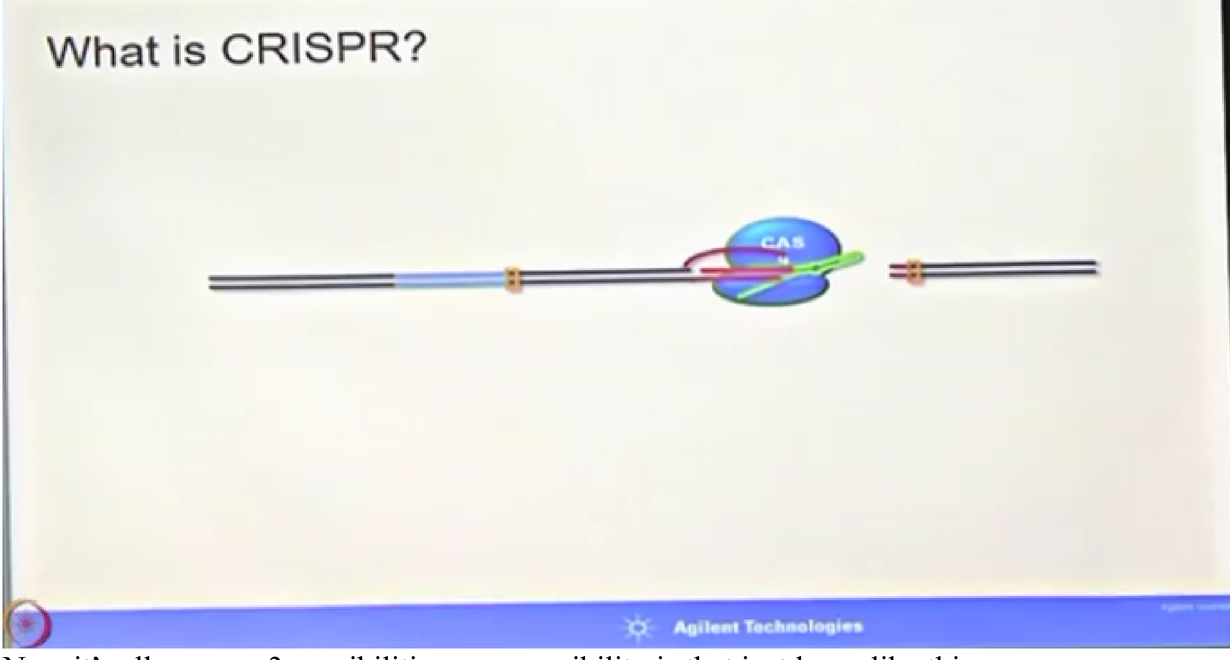
This allow you basically to allow the mutation to correct maybe, or generating, so it is based on the guide RNA, right, so this is the guide RNA, this one, and this is the site of recognition, and this is the CAS 9 enzyme, when this become active they attach and it goes to the PAM site like this,

(Refer Slide Time: 20:24)



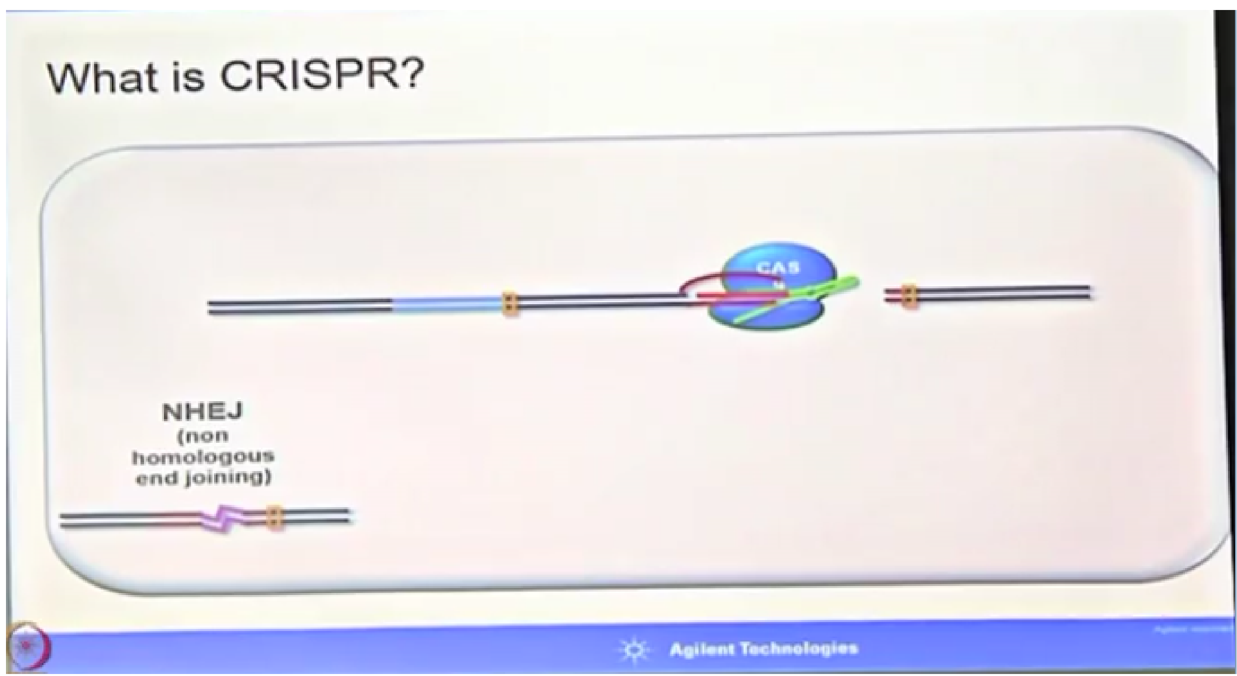
if it is identical or hybridized on that like this, it is identical with that, it gets a nick, this CAS 9 enzyme when this guide around to this specific to that it create a nick.

(Refer Slide Time: 20:38)



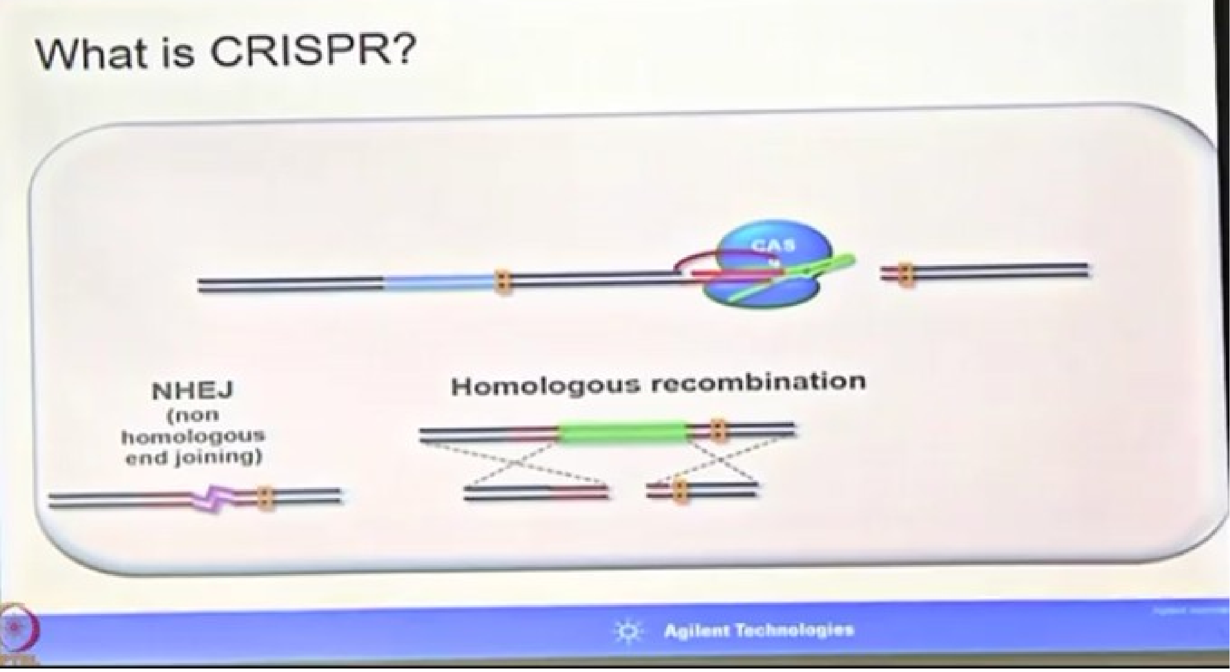
Now it's allows you 3 possibilities, one possibility is that just leave like this

(Refer Slide Time: 20:54)



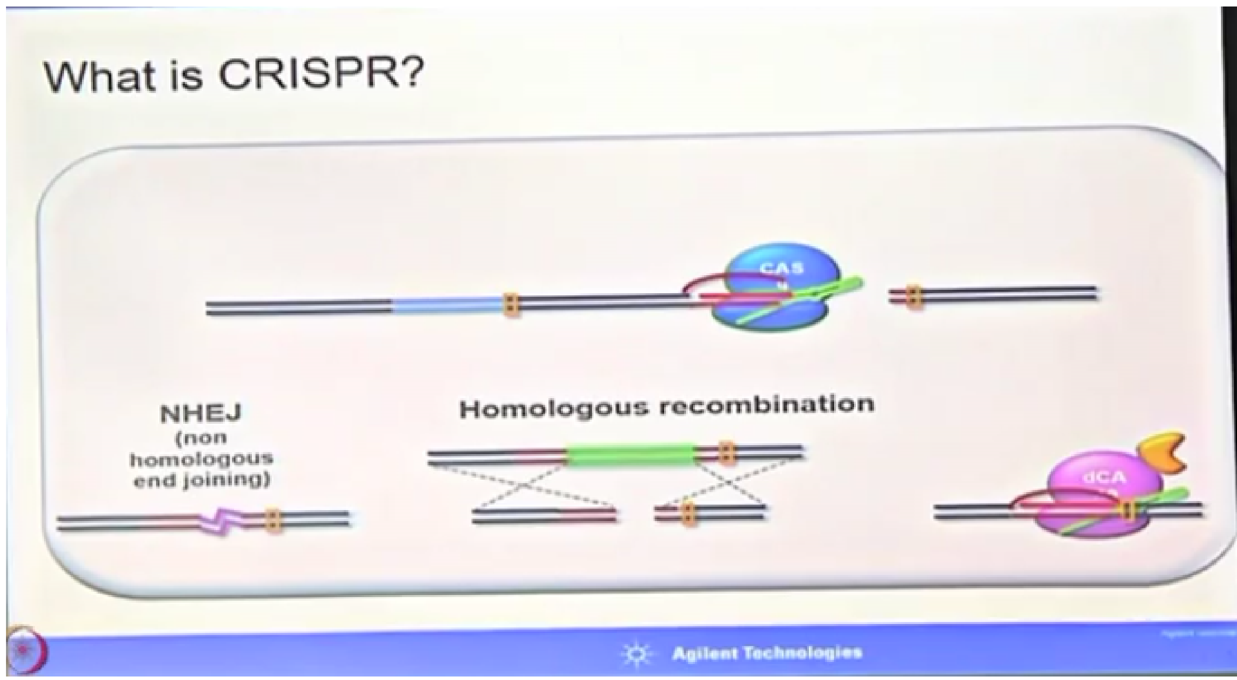
and body our cell system basically go for non-homologous end joining and cause the knockout of that gene, right, here is the knockout.

(Refer Slide Time: 21:07)



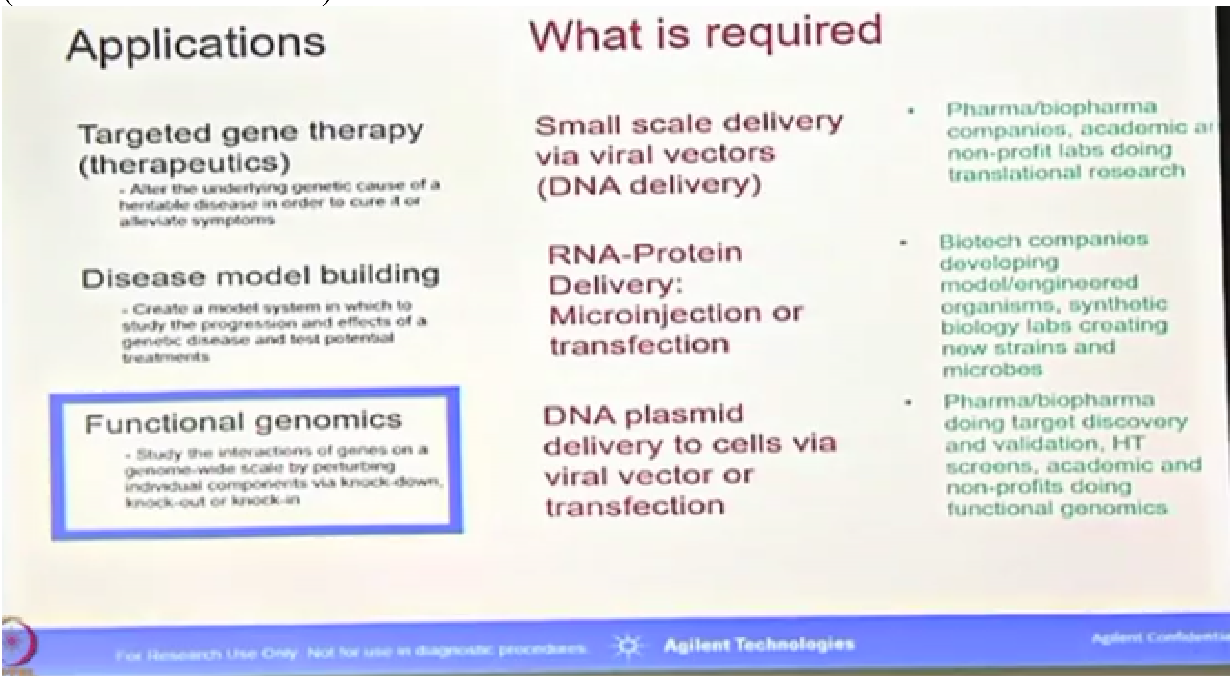
Second is the homologous recombination, if we want to do the gene editing and you some have homologous sequence you can incorporate this homologous sequence in that, right.

(Refer Slide Time: 21:20)



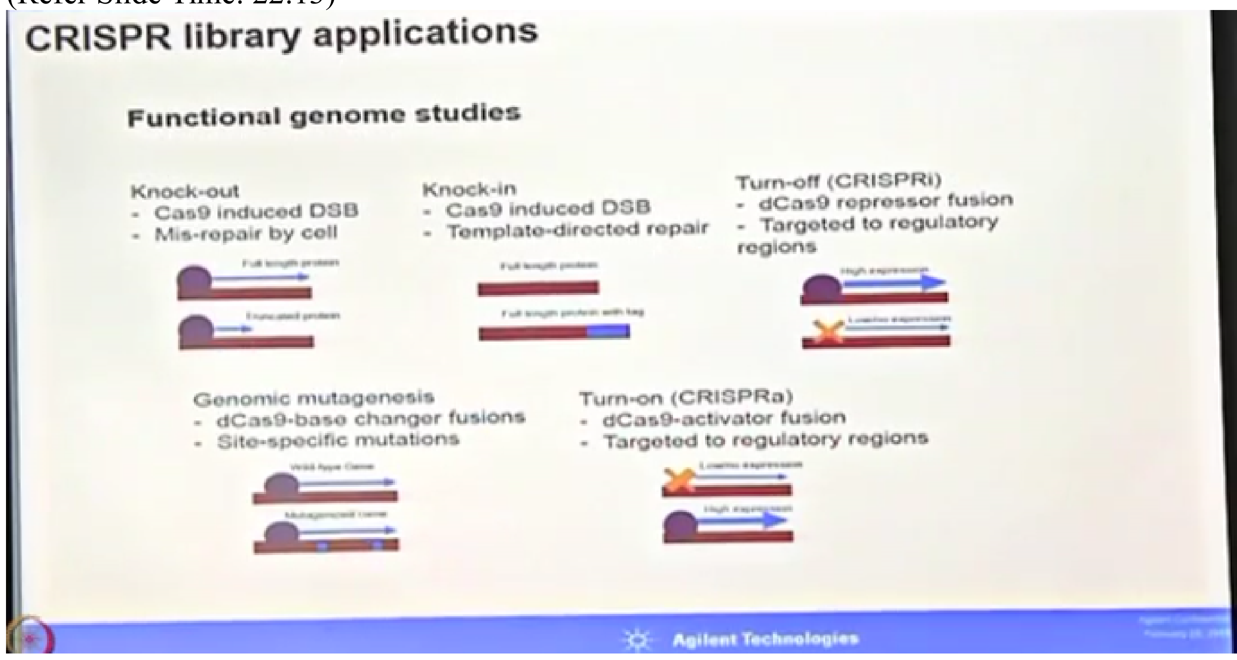
Third part this is the deactivated CAS 9 enzyme, this yellow colour part it might be activator or deactivator depending upon the activator it can induced the gene expression or reduce the gene expression, so it allows the whatever mutations basically you got from the exome panel right, you can basically try to correct or you can do the gene editing for the treatment purpose, so that's whole story exome panel and what is the follow up, so we are working on the functional genomics

(Refer Slide Time: 21:55)



where basically we can try to edit this genes in a large scale like, and it might be any knockdown, knockout and knock in, anything, and try to edit the genes to solve the problems.

(Refer Slide Time: 22:13)



So if you study the functional genomics, so very first prospective is knockout, right, if it is knockout means guide any breaks that one it means that truncated protein, right, so your protein is not going to work, this truncated protein. Knock in means is basically it going to add some tag on that, on the protein. Turn-off means if it is high expression, this is the repressor fusion if it is turn-off means the lower expression of the gene, this is the genomic mutagenesis, here basically it's a side specific mutation can create by the Crisper 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 SNP's you got right, you can correct that part, so this allows you to side specific changes with the CAS 9, and if you want to do some genes are basically lowly express in sub disease you can basically induced the expression to the higher level, right, so it can induced the gene expression, so you can do multiple function by the CAS 9, you can induced the gene, you can represent the gene expression or you can do the side specific changes in the gene, right, that's allow you 5 different possibilities you do.

(Refer Slide Time: 23:49)

SureGuide gRNA Synthesis Kits

- Synthesize and purify gRNAs in under 2 hours
- Extensively validated synthetic system, troubleshoot your application not our reagents
- Enzymes and reagents are optimized for use in conjunction with SureGuide Cas9 Nuclease

Agilent Technologies

So now the question is that suppose you are like in cystic fibrosis right you were on single gene and there maybe one or two mutations, you got with the exome panel, right, and now 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 RNS, not more than that, right, so for that one you can make the guide RNS in your lab, so suppose this is the target basically, you are going to make a guide RNA for that one only, so this is the target panel, target you want, just add the T7 promoter on that, right, if you add the T7 promoter on that and go for,

(Refer Slide Time: 24:37)

In-vitro Transcription

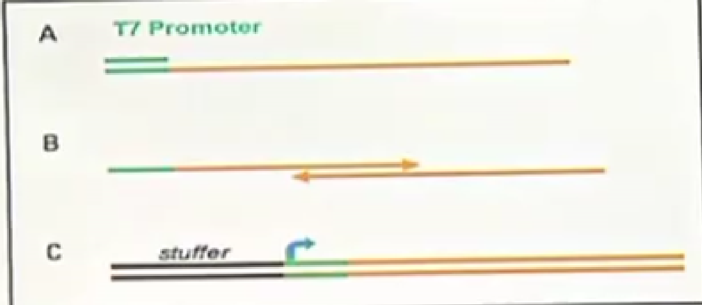
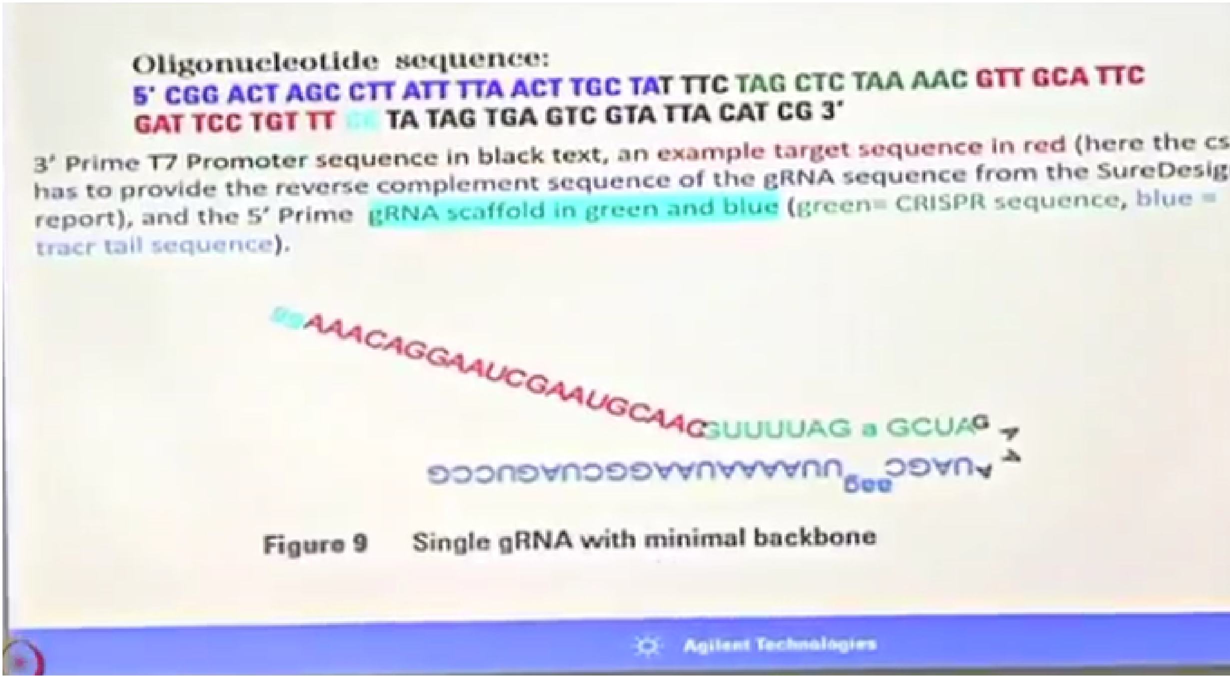


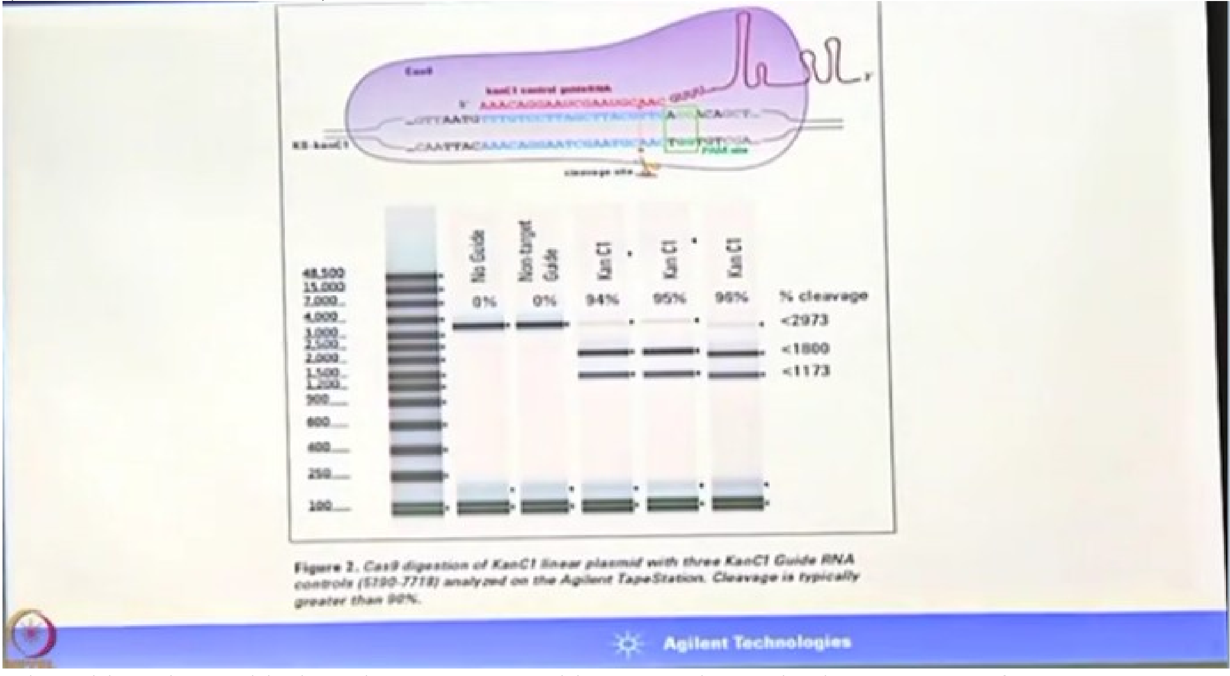
Figure 2 DNA Template options. A) Long, single-stranded oligonucleotide template. B) Two partially overlapping oligonucleotides; requires fill-in with DNA polymerase. C) Synthetic double stranded gene fragments; may require 5' stuffer sequence to meet length requirement.

Agilent Technologies



so the kits basically what they have? They have T7 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 complementary to the target, this is going to be reversed complimented to the target.

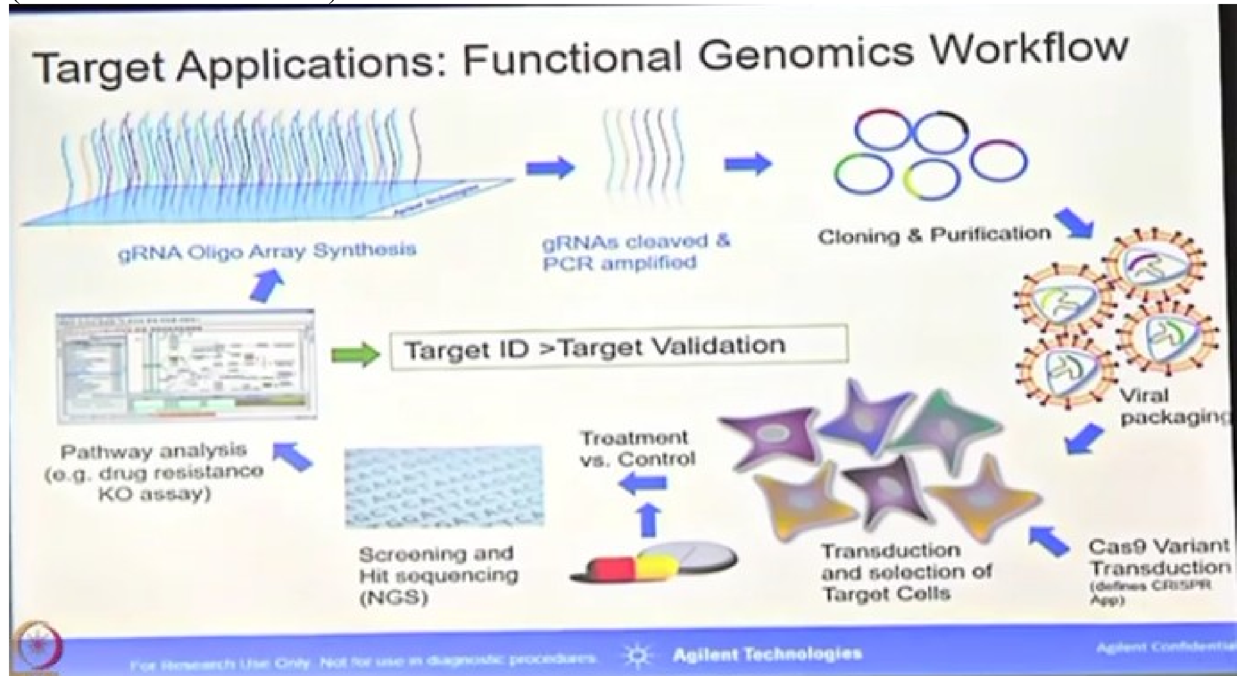
So this is the kind of guide RNA, this is the red part basically it's very specific to the target, to the part where you are interested for, and this is the backbones, minimum backbone, and this, (Refer Slide Time: 25:20)



when this going to bind on the target gene this targeted part, in the presence of CAS 9 enzyme this gene basically cleaved in two part, if you verify this one in the presence of CAS 9 this gene

basically give off, right, so this allow 5 different possibility again, basically it is going to, maximum time it is going to knockdown 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 on your lab also, right, but mostly it's not a case, it's not a case, there are multiple genes are going to,
(Refer Slide Time: 26:00)



is involved for any complex exome gigantic right, like retinitis pigmentosa 77 gene is involve, and mutations is like more than 1000 mutations are basically involved there right.

So what we do? We make guide RNA for that panel, right, and this is totally custom thing, this we don't make, we use to make some catalog but it 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 packed on the viral particle, now it's ready to transfect in the cell system that much guide RNA basically which is for that mutation, right, it going to ready for transduction and you can use for the three particular opportunity for this, right. So this is the…

Unidentified Speaker: So the target sets that actually _27:03_

Dr. Mukesh Jaiswal: Yes, it's good, so it's a viral particle, these are cells basically to transfect directly into there. Transfect with this guide RNA they already have a CAS 9 enzyme, so it create the, means the CAS 9 is there right, so it's going to cleave that part of the gene or is depend on what? What do you design basically?

Unidentified Speaker: So there is no _27:30_

Dr. Mukesh Jaiswal: Yeah.

Unidentified Speaker: _27:33_

Dr. Mukesh Jaiswal: So see I told you this is in very, this is very early stage, again to do this thing you need to do lots of screening, lots of NGO's work to identify it's going to work or not, so this is just a thought and guide RNA is, you can synthesize for you but again the protocols and how it's going to translate to the actually patient is in very early stage, it is like 10, 20 gene you can make in your lab, but it is more than 1000 gene, in high scale than we can make. Means it's for the high scale, not, otherwise you can just make in a lab, your any target add T7…

Unidentified Speaker: _28:20_ you these are the specific target?

Dr. Mukesh Jaiswal: We have specific target, just add the T7 promoter on that, use T7 primer and do in-vitro transcription, that's inside the guide RNA, so it's a 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 yeah, I would tell you this is not easy, this is very, very hard, right, it's very hard because if you see, this is going to be like thousands of the guide RNA, and going to transfect in the cells, right, now you are going to get 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, these 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 contest Agilent is happy to collaborate with the peoples who are interested for to make a guide RNA's right, and we already had some guide RNA's of different disease like if you have the cancer panels, you already have the,

(Refer Slide Time: 29:46)

Human CRISPRa-v2

Sublibrary	Genes	Number of sgRNAs (controls)	
		5 sgRNAs/gene	10 sgRNAs/gene
Kinases, Phosphatases, Drug Targets	2320	13,000 (250)	26,000 (500)
Cancer and Apoptosis	2921	16,335 (280)	32,670 (560)
Stress and Proteostasis	3093	16,905 (290)	33,810 (580)
Mitochondria, Trafficking, Motility	2220	12,285 (250)	24,570 (500)
Gene Expression	2288	12,695 (250)	25,390 (500)
Membrane Proteins	2405	13,145 (250)	26,200 (500)
Unassigned	3968	20,145 (325)	40,290 (650)
Genome scale	18,915	104,540 (1895)	209,080 (3790)

Mouse CRISPRa-v2

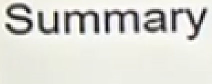
Sublibrary	Genes	Number of sgRNAs (controls)	
		5 sgRNAs/gene	10 sgRNAs/gene
Kinases, Phosphatases, Drug Targets	2268	12,340 (250)	24,680 (500)
Cancer and Apoptosis	2856	15,475 (280)	30,950 (560)
Stress and Proteostasis	2796	14,950 (290)	29,900 (580)
Mitochondria, Trafficking, Motility	2099	11,290 (250)	22,580 (500)
Gene Expression	1915	10,330 (250)	20,660 (500)
Membrane Proteins	2104	11,225 (250)	22,450 (500)
Unassigned	5001	31,405 (800)	62,900 (1200)
Genome scale	19,909	107,105 (2170)	214,210 (4340)

Agilent Technologies

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 catalog that panel, you can use that one but again the, how it's going to work and how it's going to screen, it is a little difficult task.

(Refer Slide Time: 30:18)

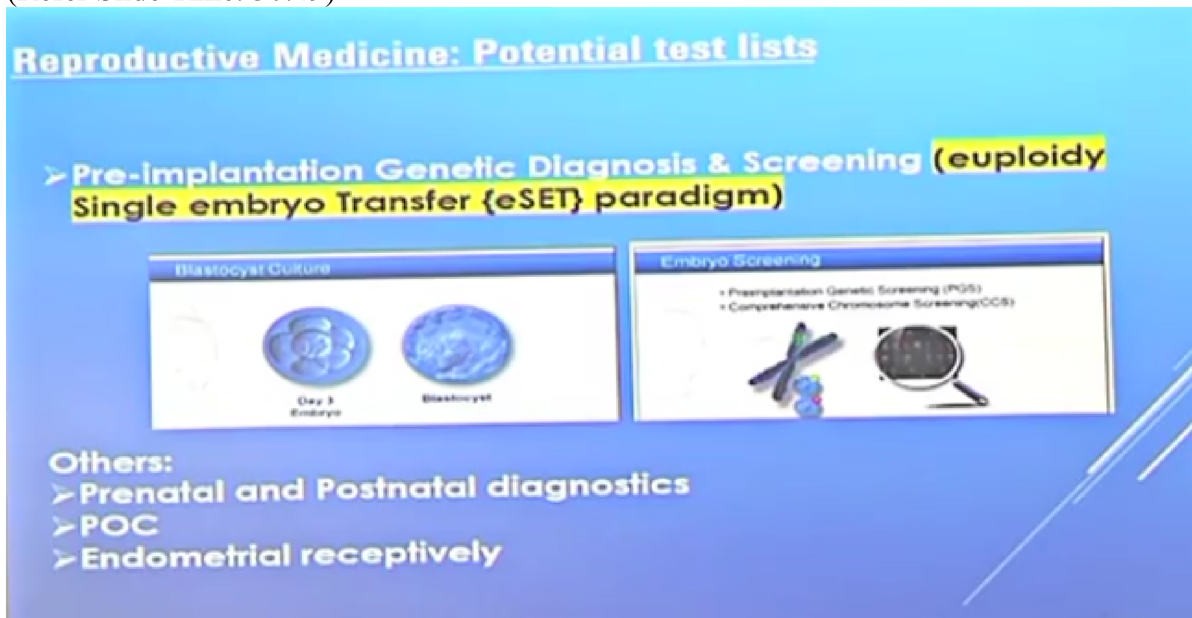


Microarray and NGS Applications helps discovery of causation of diseases

Crisper/Cas9 can be best solution of Drug validation and personalized genomics

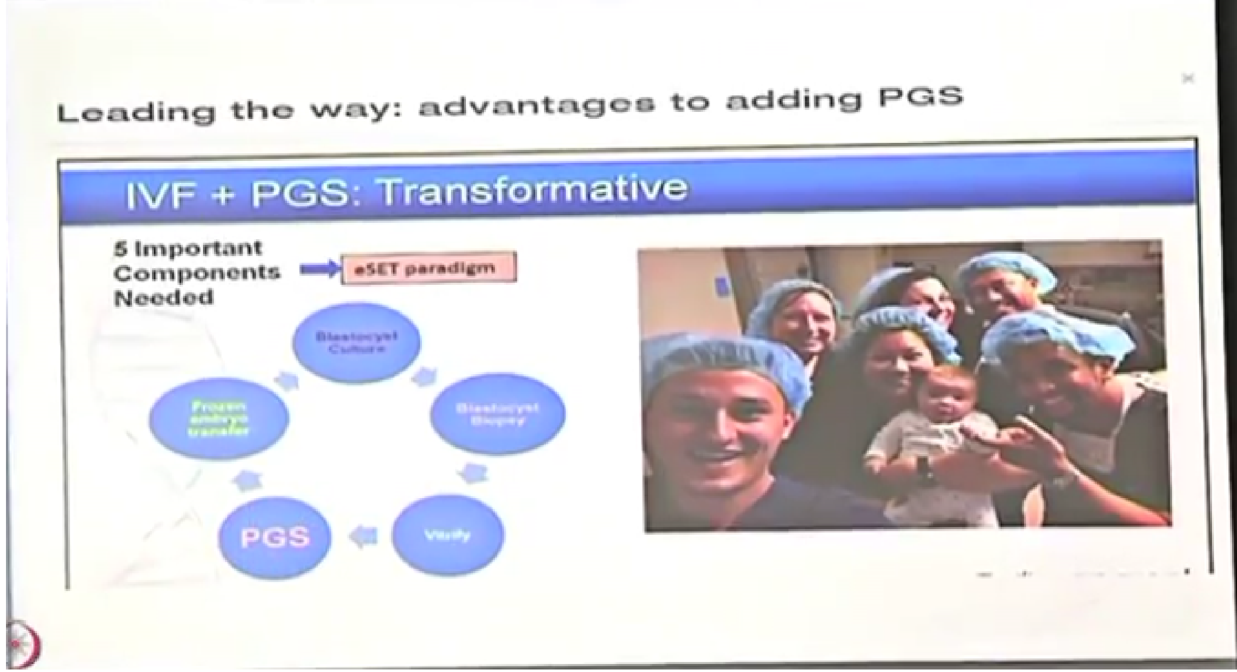
So in summary means you can use the NGS application to identify the causation, what is the cause of that, and I gave the little brief idea how the Crisper Cas can be best solution for the drug validation and personalized genomics, so this is something you can use the NGS application to identify the problems and Crisper can might be used for the treatment part, right, so now I'm covering of 5 slide from the IVF segment,

(Refer Slide Time: 30:49)

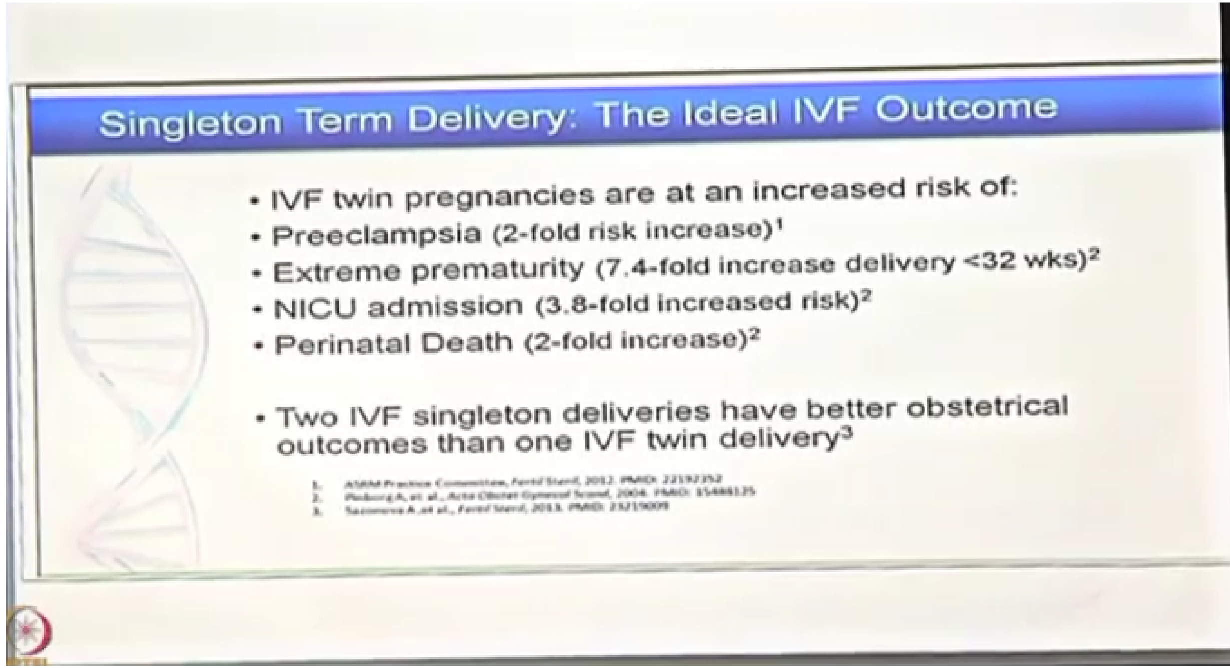


so in reproductive medicine the most challenging part in the IVF is that aneuploidy embryos, whenever couples go for the IVF and they do the in-vitro fertilization and 70% are the embryos are basically aneuploidy, right.

And so what doctor do, actually in India what doctor do, they look the good looking embryos, identify it and basically implant like 3 to 4 embryo, sometime 2, sometime 3, depends, so challenge is that when doctor do this thing, if the good looking aneuploidy not going to implant, so yeah I was our cycle is fail, if it is 2 embryos are good, so both going to implant, it's going to give twins, if it is 3 good embryo it going towards 3 kits right, so it's a challenge you get either 1, 2 or 3, there is no control on that, right, so we are discussing with the IVF clinic and working on the single embryo transfect paradigm, means check the embryos they are good enough, your euploidy embryo not aneuploidy, and only one single euploidy embryo is basically, go for the further IVF cycle and for the implantation, so we are discussing this thing, this thing is embryo transplant, doctors basically do they do the blastocyst culture, and they don't do, they do a biopsy and frozen of the embryo, but they skip this part PGS, pre-implant and genetic in India.
(Refer Slide Time: 32:42)

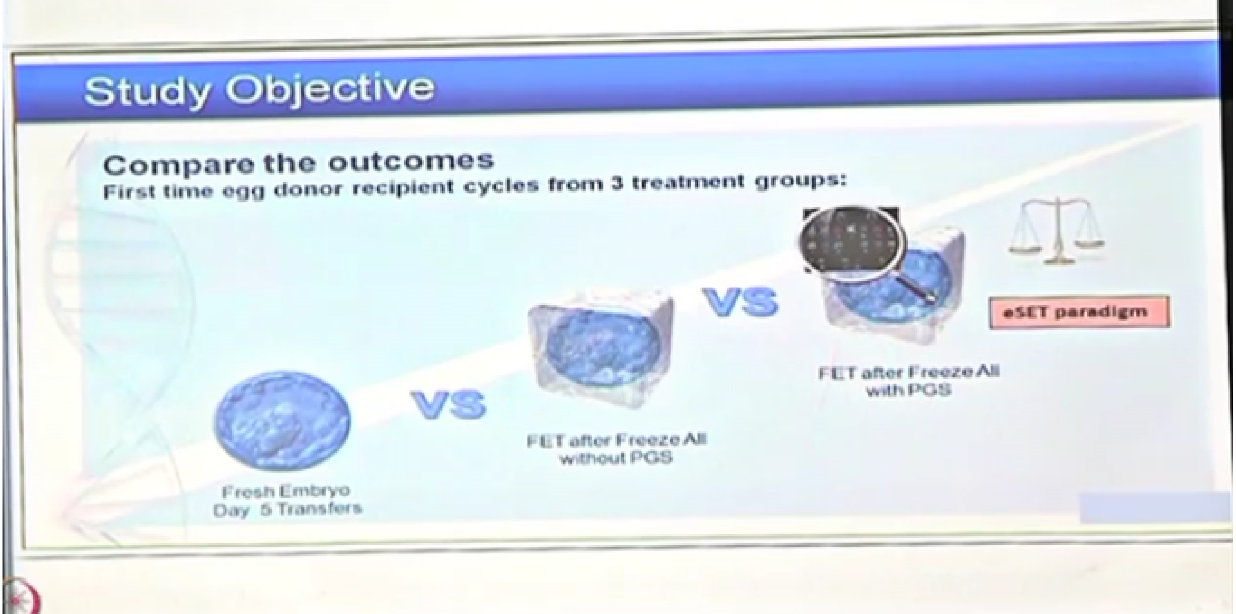


And after frozen these embryo taken and go for the IVO, so they use 3 to 4 embryos and directly to the plant, what we are talking to doctor? Do the PGS, identify the good embryos, once good embryo an implant process for the implantation, so the couples going to get only one kit, right, so when we talk about the problems,
(Refer Slide Time: 33:13)



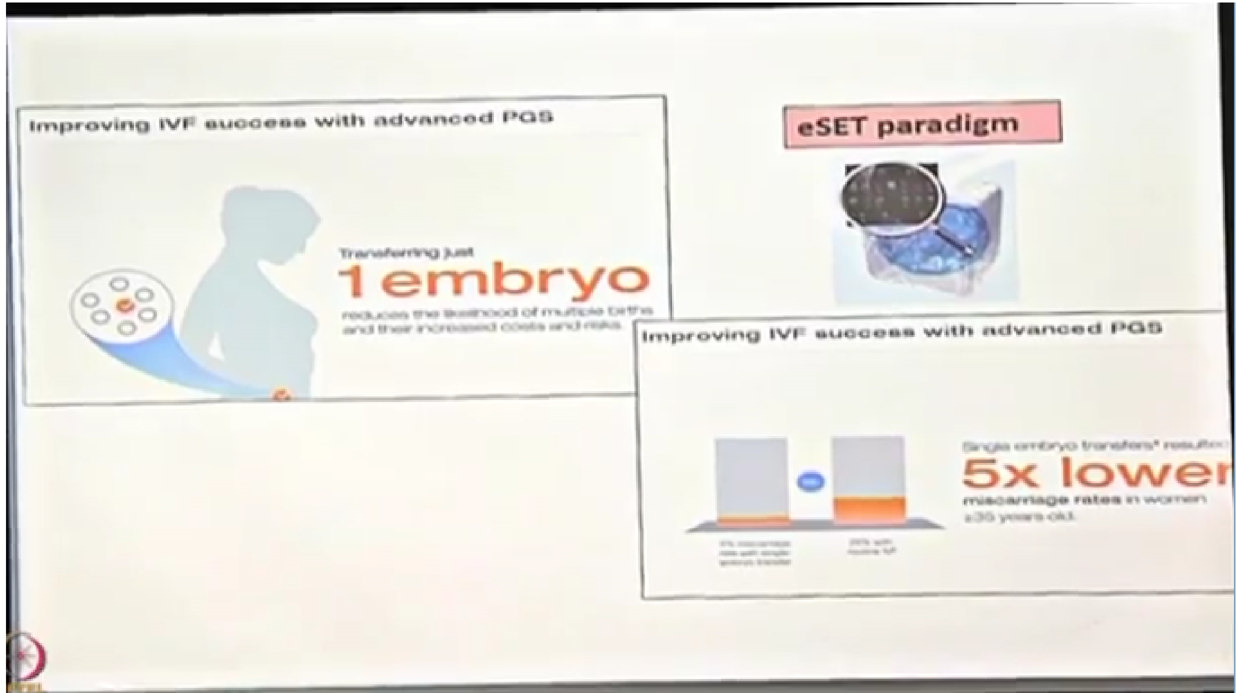
if the pregnancy goes with the 2 twins, there is a lots of problem with the preterm labour, preeclampsia, and this high rate of the perinatal death in the twins right, so that's why we talk to doctor, go for this test, means you can go for frozen with that do the PGS,

(Refer Slide Time: 33:36)



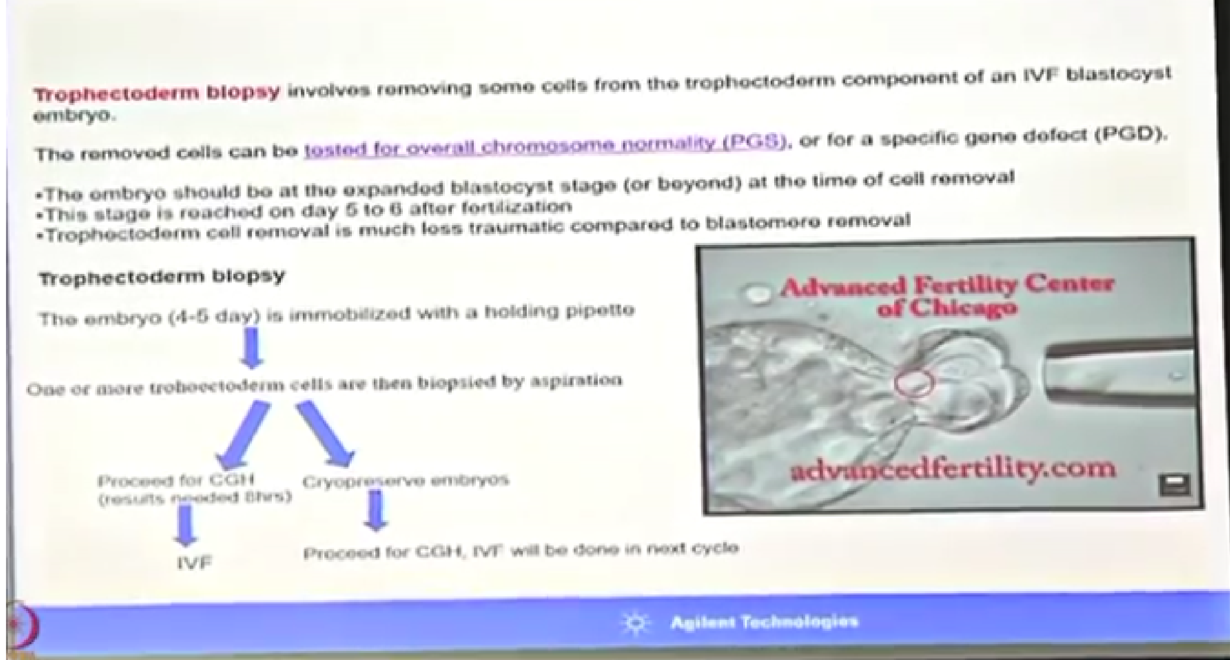
PGS means you just go for the screening of all 24 chromosome, identify all the chromosome are good enough, identify the euploid embryo and then go for the IVO, so how basically they do? By transferring the one embryo basically it also

(Refer Slide Time: 33:52)



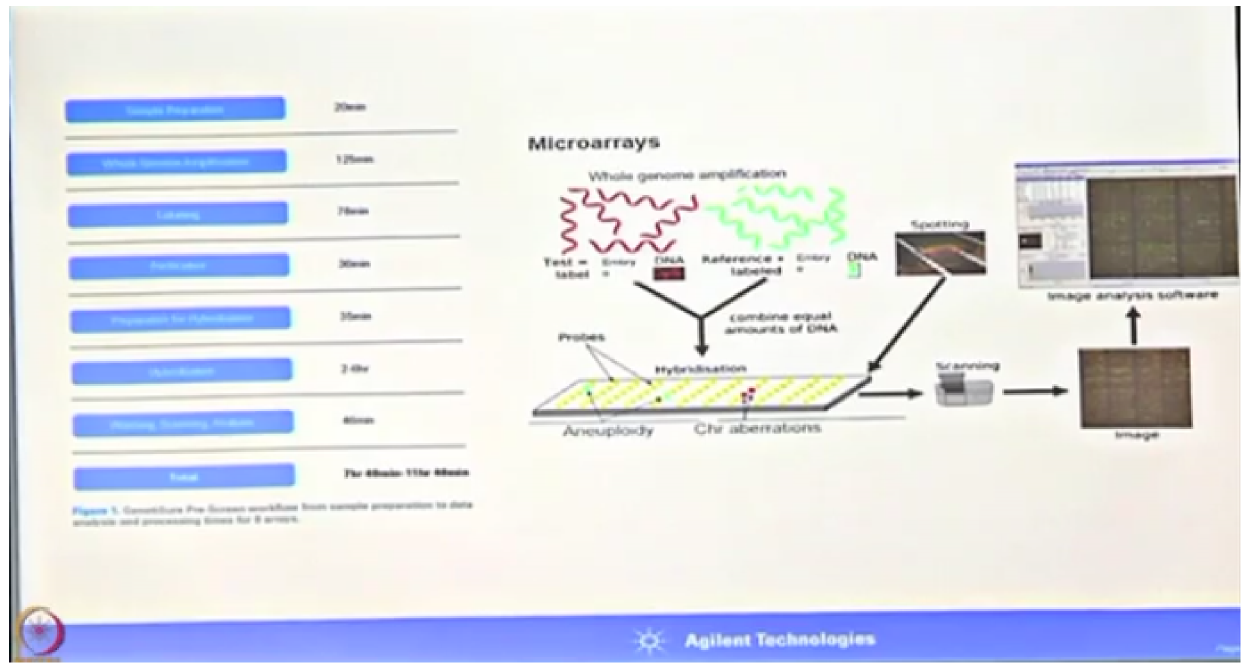
is cost effective or the couples also, is 5 time less mean basically to grow the kits right.

(Refer Slide Time: 34:00)



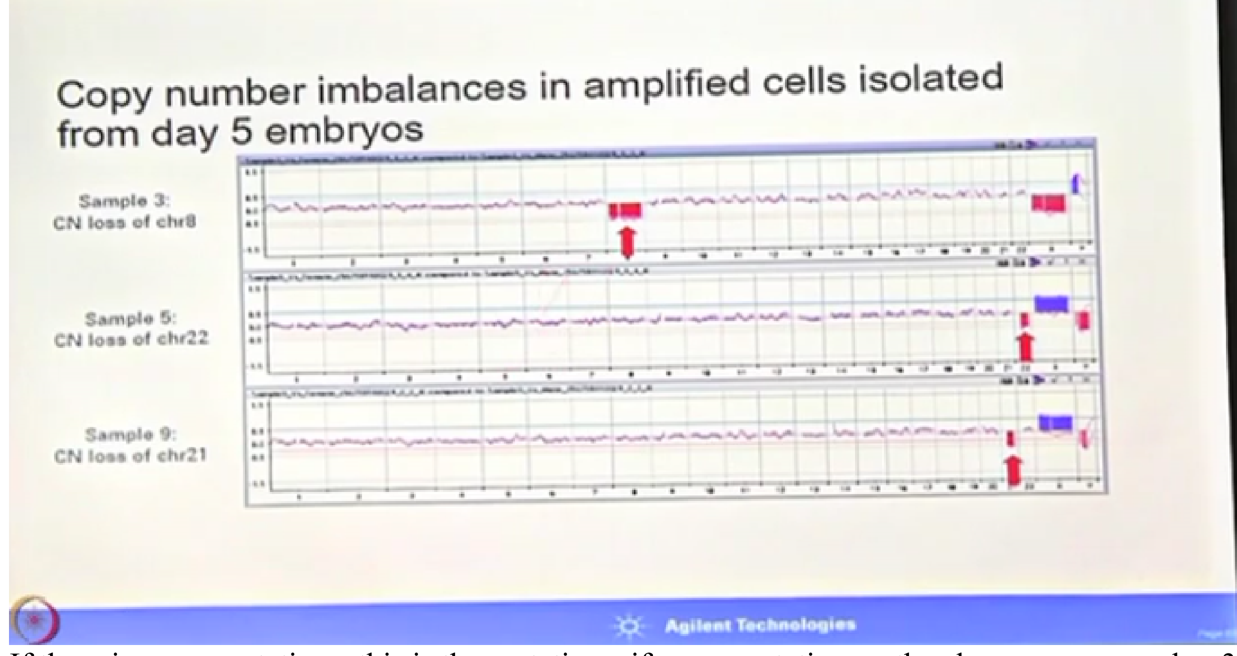
So how they do? This test is basically done by the biopsy of the embryo, so this is the embryo, and this is the blastocyst and they take the one cell of the embryo, only one cell of the embryo, so this is one cell they collected, so when this biopsy is done,

(Refer Slide Time: 34:34)



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 label with this DNA with the sai 3 and sai 5 type, right, and hybridized on a microarray slide and go for the analysis, right.

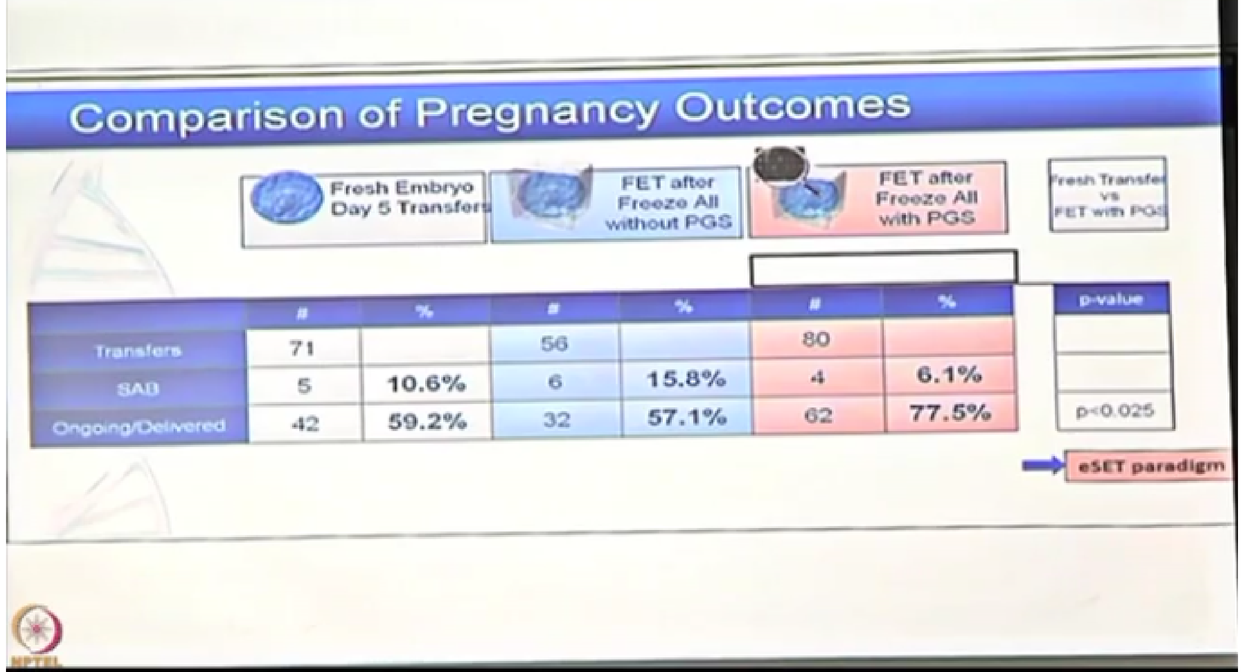
(Refer Slide Time: 35:05)



If there is some mutations, this is the mutations, if some mutation on the chromosome number 3 or chromosome number 22 or deletion at chromosome number 21, it shows that this embryos are aneuploidy and do not process for that 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, when they don't do any test, the success rate going to be 80%

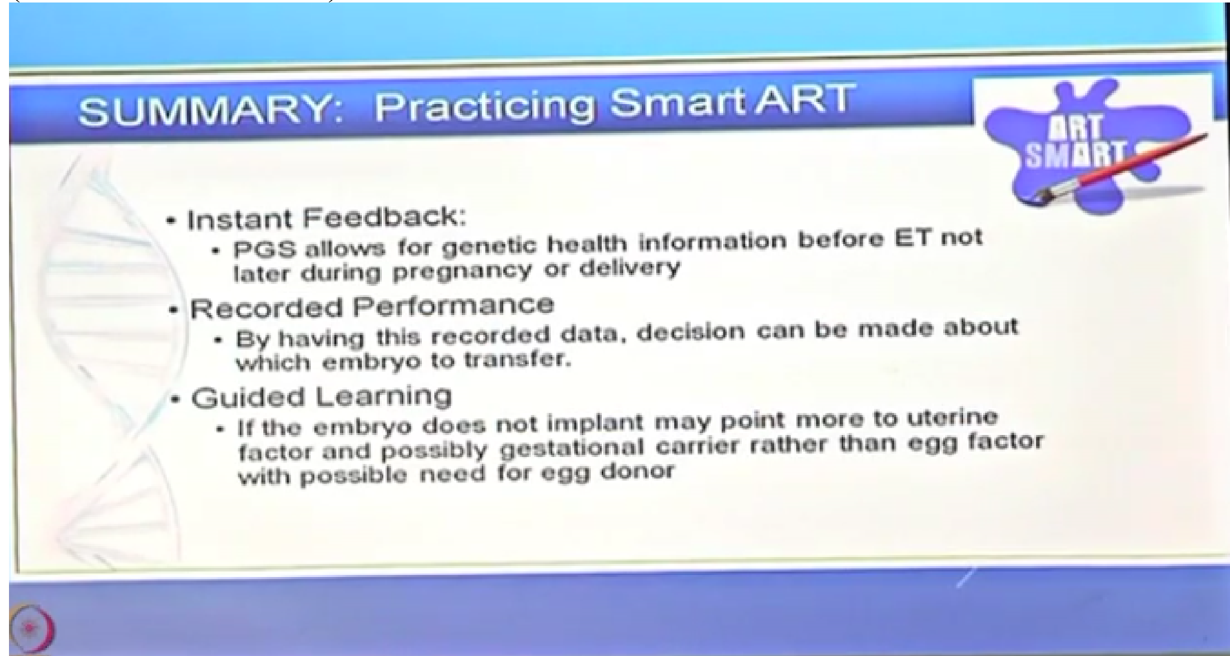
with this single embryo transfer paradigm, right, because they identify the all the chromosome based that where is the problem,

(Refer Slide Time: 35:50)



if there is a deletion don't process for the IVF.

(Refer Slide Time: 35:54)



So this is the smart ART, right, before doing process for the aneuploidy just do the PGS, validate this embryo or good enough and then go for the IVF cycle, right. Thank you.

(Refer Slide Time: 36:24)

Points to Ponder

- Basic principle of CRISPR/Cas9 technique of gene editing.
- Modifications of CRISPR/Cas9 and its applications.
- Limitations of IVF and its solutions by Smart ART technology.

MOOC-NPTEL IIT Bombay

Sanjeeva Srivastava: Alright, so I'm 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 diseases, and how the results can be used to choose the right treatment, I'm sure this is 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 Crisper Cas technology which is one of the must talked about gene elodie 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 assays coming to the clinics for the patient care, so your understanding and your knowledge about these applications and these novel technology platforms and definitely going to be very useful, and I must say there is 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'll give you one instance, one example The Cancer Genome Atlas TCGA is one of the good resource for looking at the you know patients cancer data available, and while they publish that word couple of years ago in science and HR series of paper published, but what is more important when they made data publicly available for thousands of patients genome data, then the meta data analysis from that data many people have looked at various specific type of questions, what is the impact of giving gene since patients survival for example, or looking at a specific pathways, and you know maybe hundreds of paper 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, if you are interested you can just go download these data, 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 if it is possible just by looking at these data for addressing certain questions.

So I hope some of these exposures 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'll thank you to stop this lecture, but we will have more exciting things to continue, in the next lectures as well when we talk about the new resource for you which is Human Pathology Atlas. Thank you.
(Refer Slide Time: 39:21)

Next lecture....

The Human Pathology Atlas: A Pathology Atlas of Human Transcriptome - I

MOOC-NPTEL IIT Bombay

Prof. Sridhar Iyer

NPTEL Principal Investigator
&
Head CDEEP, IIT Bombay

Tushar R. Deshpande
Sr. Project Technical Assistant

Amin B. Shaikh
Sr. Project Technical Assistant

Vijay A. Kedare
Project Technical Assistant

Ravi. D Paswan
Project Attendant

Teaching Assistants

Apoorva Venkatesh

Shalini Aggarwal

Nikita Gahoi

Bharati Sakpal
Project Manager

Bharati Sarang
Project Research Associate

Nisha Thakur	**Vinayak Raut**
Sr. Project Technical Assistant	**Project Assistant**

Copyright NPTEL CDEEP, IIT Bombay