Functional Genomics
Professor S Ganesh
Department of Biological Sciences & Bioengineering
Indian Institute of Technology Kanpur
Lecture No 04
Forward vs. Reverse Genetics

Yeah welcome back to this course functional genomics, this is the fourth lecture for week one and in the previous lecture you have seen the evolution of the field of genomics and how what kind of impact that it could have so we are going to continue discussing on genomics we are going to look into the approaches people use used in the past and currently being used for the understanding of genome and how it functions.

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Let us look at this slide here what is shown is, is an abnormal condition a condition where in we can see that the two digits or fused to other. This condition is called as Syndactyly the fused, this is a kind of a partial condition because it is not completely fused so this region is anyway separated but you could have conditions for example here like in this baby's hand all the you know three digits all fused together again this is an abnormal condition. It happens because of some genetic defects. So how do you really find the gene that results in or defects in that gene results in this kind of condition.

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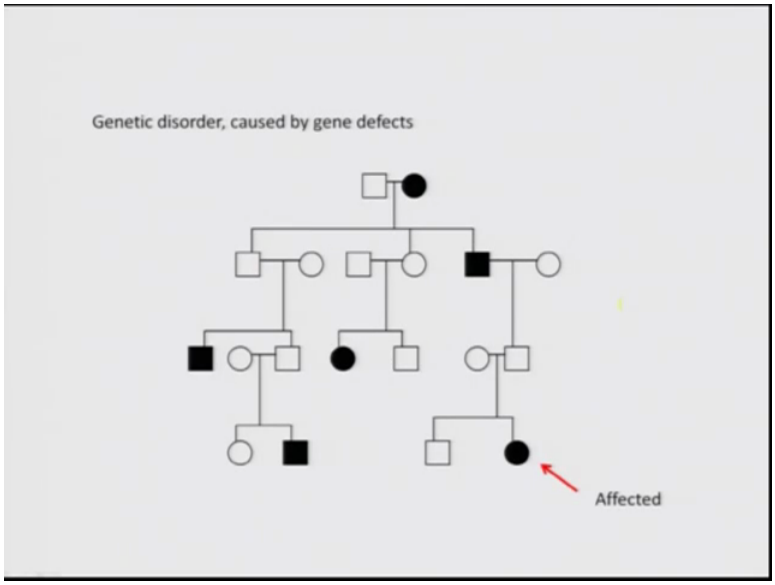

This is something that you know people from the human they look into the pedigree and look at how this phenotype or abnormalities run through the family and try to come up with certain models and that results in that identification of the gene. So depending on what kind of organism you use you know the approach varies for if the human we start with this kind of pedigree this is a female, this the male. This is line indicate they are married and they have got three kids, two sons and one daughter. One of them is affected having again Syndactyly henis generation next to next you find a person is affected here also affected and so on right. So this is the mode by which you understand that it is something has to do with genetics because runs in the family and so on right.

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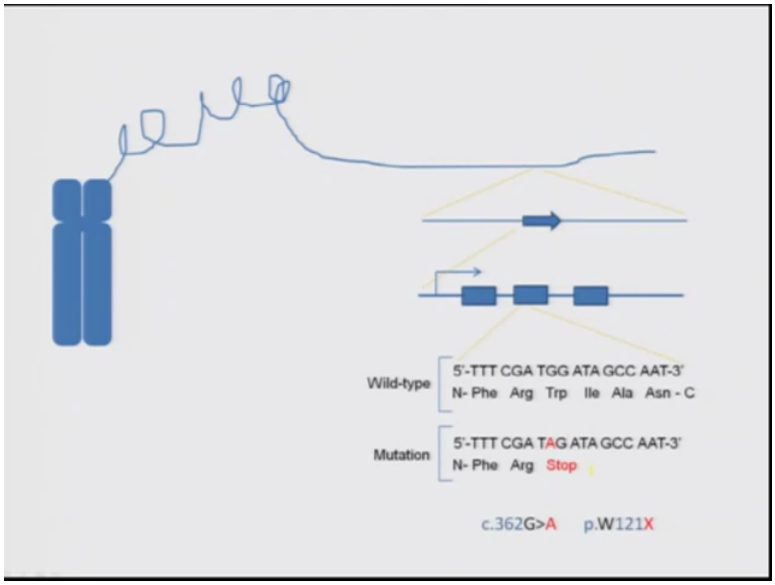

So how do you really find the gene so obviously gene is located on the chromosome on the DNA and one has to go and look at it right so how it is normally done. So this let us assume this is a gene that causes the disease and it causes the disease because it has some mutations variations in the DNA. Let us see a normal gene has these sequences and they code for this particular protein but in this individuals you have a gene whose sequence has been altered like what is shown here for example the G is converted to A as a result instead of the amino acid you have a stop code on the peptide no longer be made is a truncated peptide and it cannot make the protein. So as a result one might have it right. Just giving an example not necessary that this is the imitation that causes the condition that you have seen.

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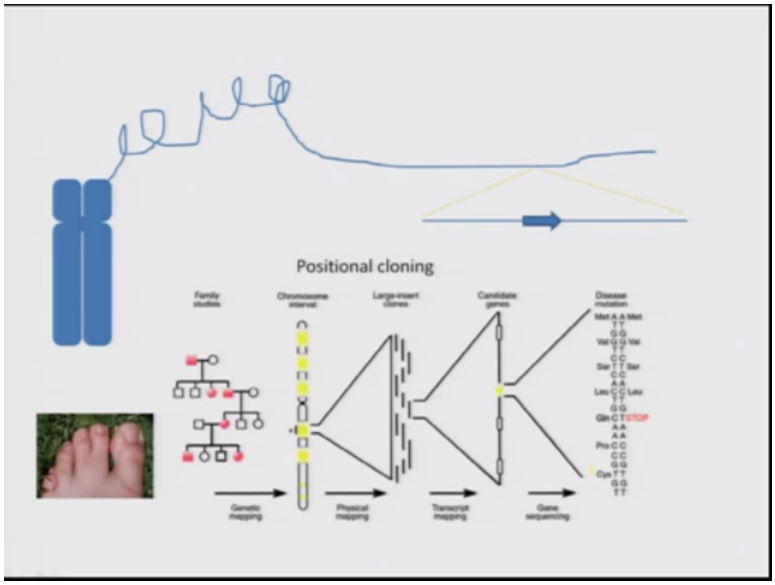

So how people go about looking at it so they do what is called as pedigree analysis and the use the DNA markers located on various chromosomes. These markers are repeats and with the repeats you know which repeats (())(03:44) often present in the affected and not in the unaffected where able to locate a possible reason of the chromosome which could harbour the gene and using you know the genomic technologies you would be able to identify a given gene to be the gene that caused the disease because now we have sequence and we found there is a sequence change and that is present in all affected therefore that is likely to be the gene that causes the disease.

This is the way you go about looking for the gene in the humans and this is called as positional cloning. So this is approach is called as from phenotype to genotype, so you looked at individual that have some variation and go and look at what is the gene that costing that phenotype right, that is called as phenotype to genotype.

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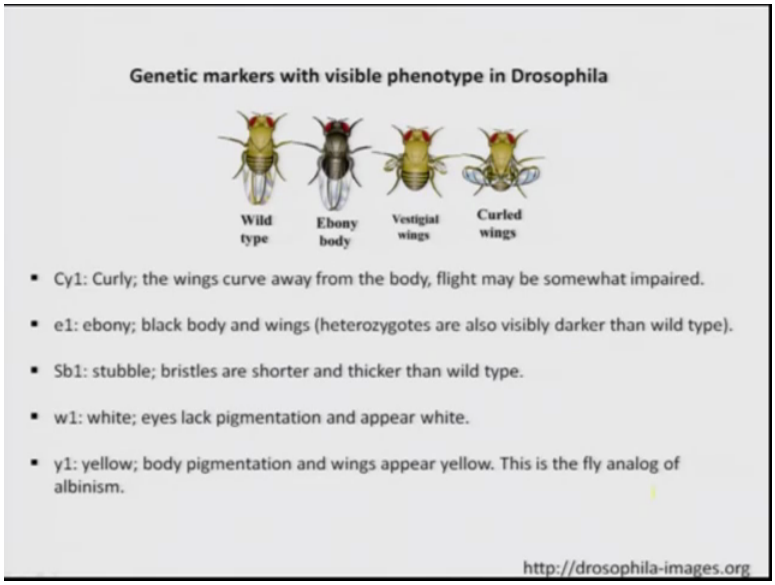

And very similar approach they are being used classical genetics in for example in in Drosophila this is wild-type meaning a normal insect fly but it could have variety of genes which gives distinct phenotypes for example you know you have much darker body you know body of the fly called ebony. Vestigial you can see that wing is much smaller or could be called wings. The wing became you know distorted and so on so this is again a phenotype and for example Morgan used this phenotype to link to map it on the chromosome and say that indeed there is something on the chromosome that gives this phenotype ok. So there are markers which help us to identify that.

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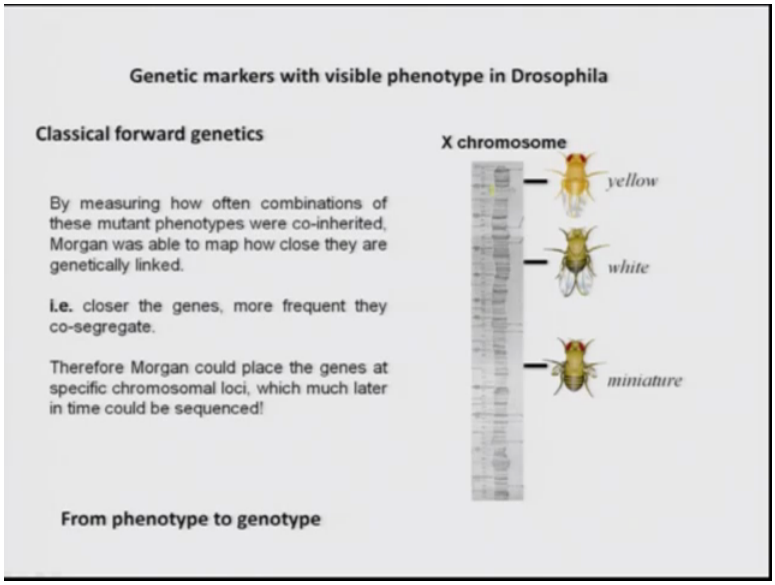

So this is how we are really looked into and decipher for example what is show over here is an ex chromosome of Drosophila and is able to map that the genes that gives the yellow body colour is located here that gives white eye colour is located here, that gives a phenotype of a small wing called as miniature is located here is able to do that. He did it by what is called as linkage analysis meaning how often a phenotype is segregated together when you cross you now having different you know phenotype and that gives you if the two genes are linked together then present close to each other than more likely that the phenotype is seen together right.

And that led to mapping genes on the chromosome the first time you know that for this he has been awarded noble price and again this uses the same approach that is the phenotype to genotype just like what you have seen in human that is possible much later but in fly able to do much much before. But the current approach for the majority of genes to understand what is a function off course you know whatever Mendel has done now people have sequenced now people have identified the genes and they are able to tell exactly why a defect in the gene can result in this phenotype but what people are trying to do now is to do the reverse ok.

I have this gene but I do not know what is the function of the gene so make some changes in the DNA of that particular you know representing that gene and see what is a phenotype. So this is called as reverse genetics because you start with the genotype and ask what would be the

phenotype so just to distinguish these two, this is the classical forward genetics which is phenotype to genotype.

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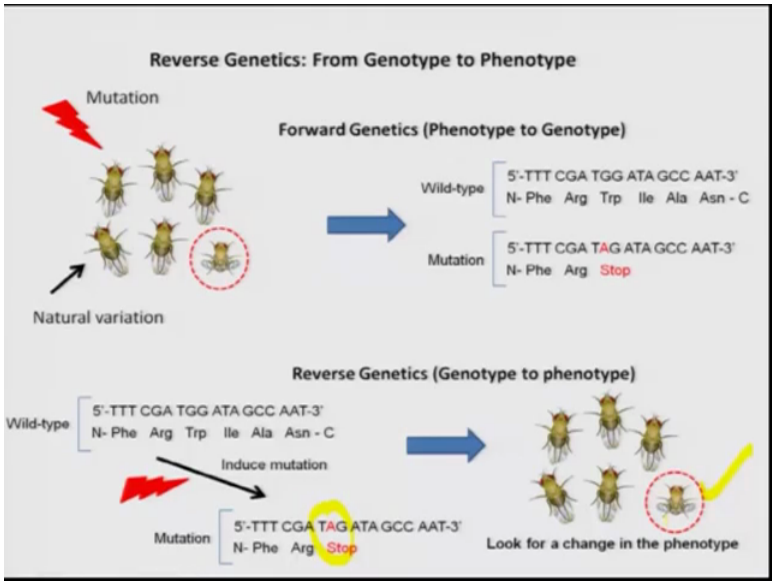

So you either expose the fly to certain mutationted which chemical or whatever is randomly produced submitation or it could be natural variation resulting in a given phenotype. And then you go on look at you know as compared to this fly how different they are and identify the genotype that unique to this fly which we call as a mutant. In the reverse genetics we do not really bother about what would be the phenotype. You take a gene that you want to study and induce imitation for example I have changed it.

Therefore now it has become a (())(07:44) peptide if it is transcribed translated and then look at you know all the fly that has got this mutation as to what is the phenotype and I may find for example as in when I create this mutation the fly becomes this phenotype . So I can now link this phenotype with this gene or easily right, so this is called as a reverse genetics.

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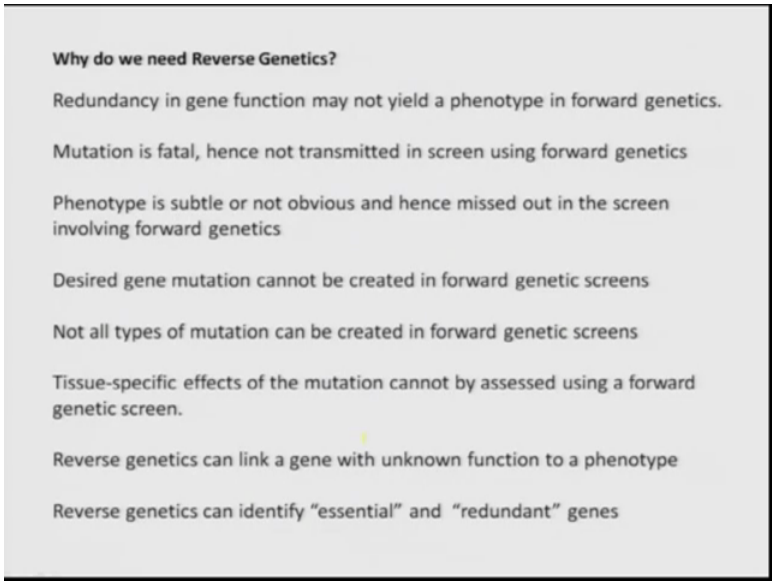

So why do you need reverse genetics because this is how it led to the new field of genomics (() (08:12) because there are redundancy engine function because when you have a forward genetics it does similar things why do you need reverse genetics at times for the lab you animals or models like this. There could be the same phenotype is given by two different genes, so even if you mutate one the other one is giving that phenotype so now you will be able to get that phenotype in a in a forward genetics. In reverse genetics I can bring in one mutation again I can bring in another mutation so you know that is possible in case of reverse genetics.

Sometimes the mutation could be fatal meaning that mutation may not come to the population so you will miss out. You know in a classical genetics crane whereas in reverse genetics I know that whenever I make this the fly dies that I know it is a lethal phenotype which otherwise you would miss out. The phenotype could be very subtle we cannot see that. So in a when you do a forward genetics crane you may not minutely check each animal because you do not know which one carries a mutation, so miss out here obvious non-obvious uhh phenotype but when you do create a mutation you know that this fly has got for example a mutation.

So you can more closely observe that fly and find a difference which tells connects the genotype with the phenotype. Now in forward genetics you have no control which gene you are mutating but here we can you know mutate the gene that you want that is possible and in classical genetics you cannot say which kind of mutation you want to create for example I want to create what is

called as a (())(09:53) instead of x amino acid I want to bring in y amino acid in a given region of a protein and see what kind of effect you have that is possible in reverse genetics.

Tissue-specific effect, for example what is the effect of this gene or this mutation in hot or in nervous system, so you do not affect the other but only affect a nervous system that is possible in reverse genetics not possible in forward genetics and then you know another important thing is the reverse genetics can link a gene with unknown function you do not know what is that and with a phenotype.

So you understand the function of the gene and finally you can classify what are the genes that are essential for the survival what are the genes that are redundant in survival when you do a reverse genetics because you know you create mutation they do not die, you create mutation they are normal which you would have missed out if you do a you know forward genetics.

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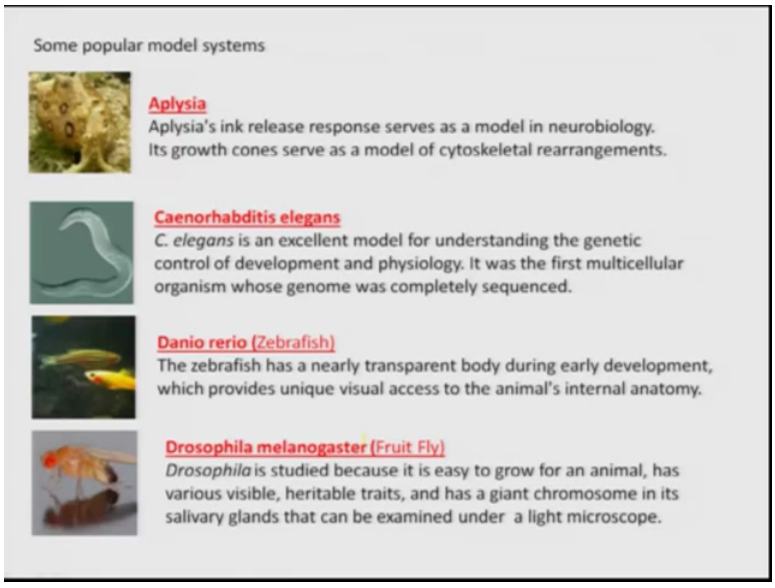

So there are the genetics reverse genetics is doable only in model system not doable in humans, not doable in many other larger animals which take long time to breed. For example there are many model system depending on what kind of analysis you do with nervous system, it could be Aplysia genetics development, reproduction, C. elegans, zebra fish development you can understand off course Drosophila and so on.

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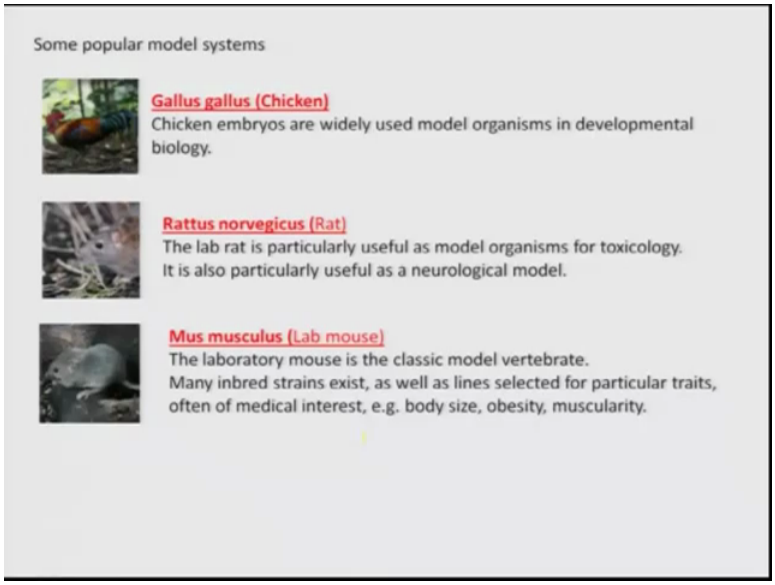

There are many models when it comes to more closer to the human it is the rat and mouse people use for understanding the gene function and their physiology. So now people have done a large number of such mutations and these are data bases that are available for example you have a data bases called fly base which gives you what gene what phenotype. For example you go and you know you select this particular tab and give that curvy wing it will tell you what are the genes that results in curvy you know wing phenotype and so on.

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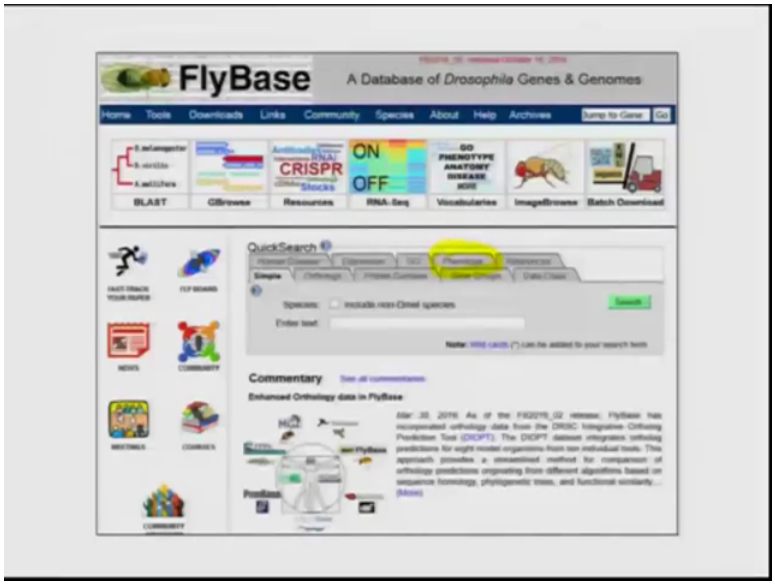

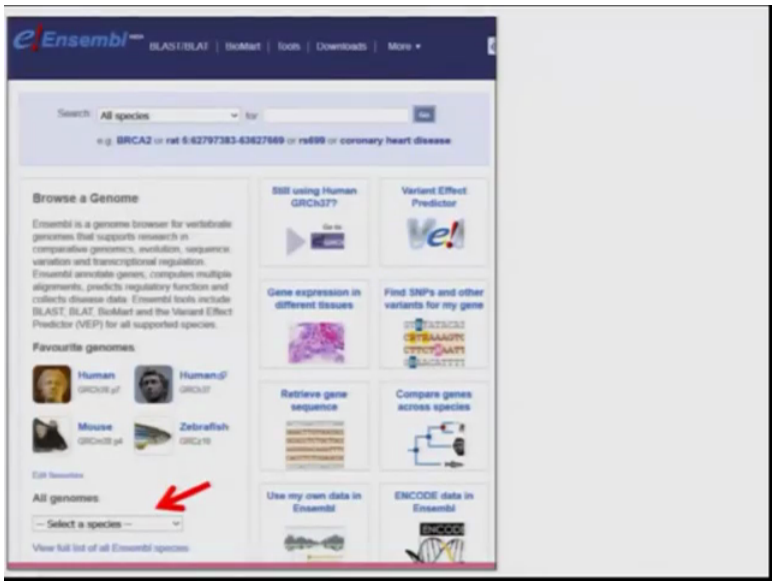

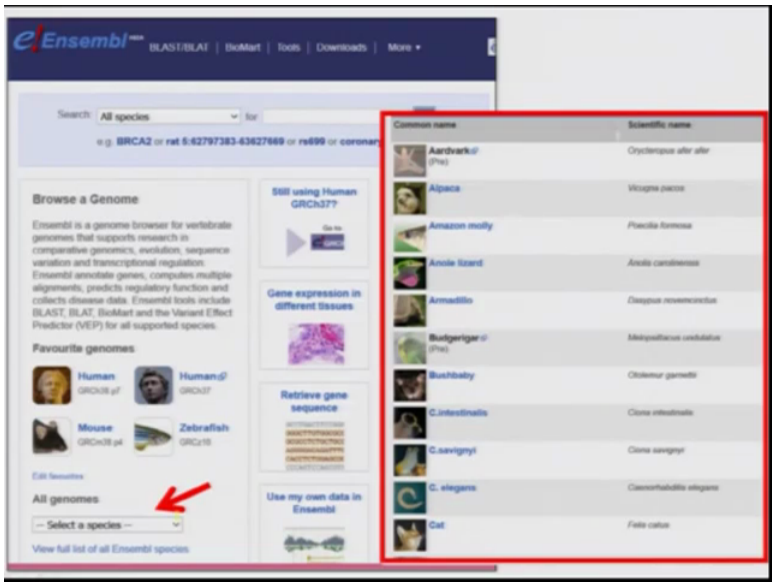

For example more genetic data base are (())(11:59) again you know you go to this European depository, you go and take for example click here what are the species you can select there are large number of species the genome has been annotated some of the mutation is available mutant phenotype is available.

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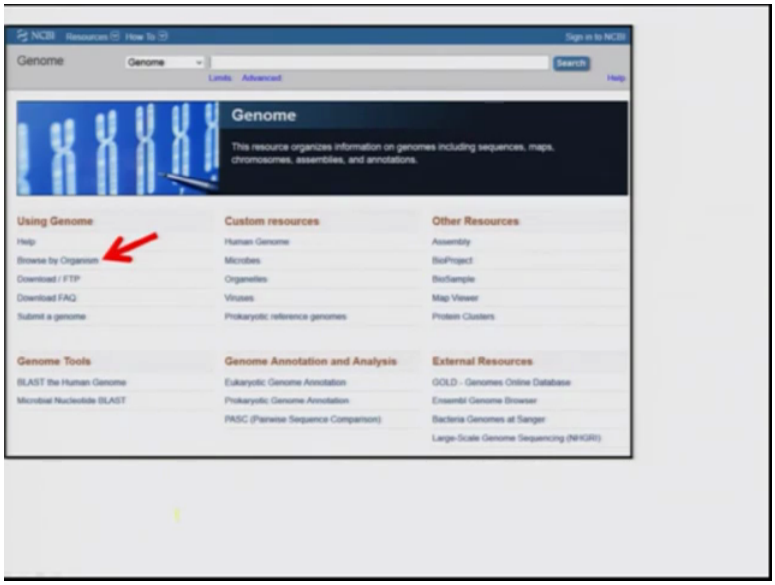

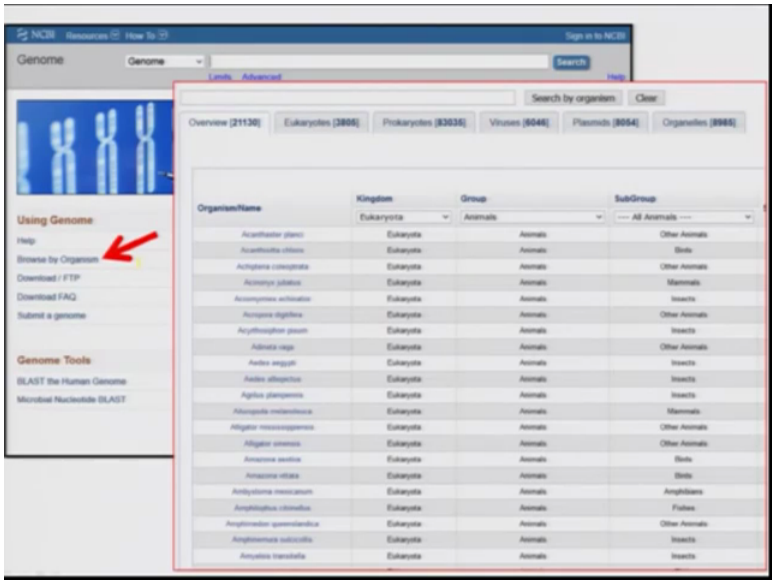

Or you can go to this more popular NCBA national centre for biotechnology information website and you can you know go and browse the organism data base. It gives you all organisms, look at their genome DNA sequence and and so on (())(12:35).

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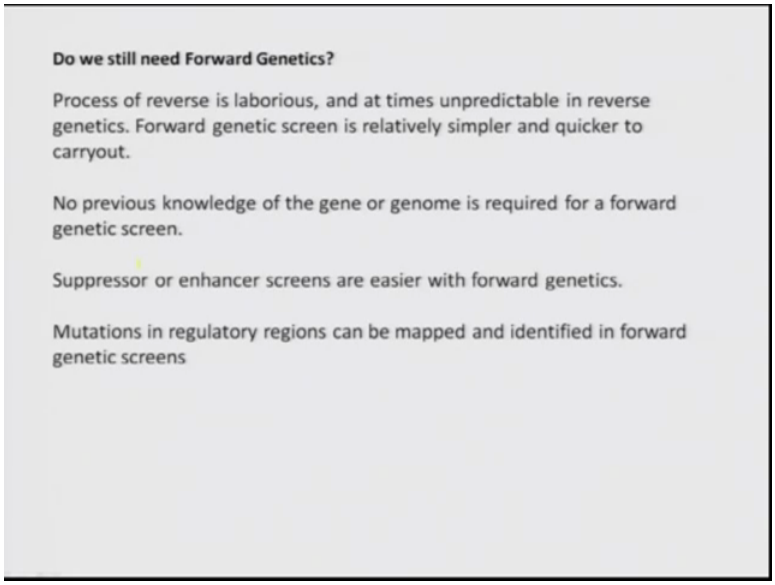

So if you when you have this data base we can go on look at genes and you want to ask a particular question that what is the function of the gene that is what you want to test. So the question is with all this new technology do you really need forward genetics, so I can mutate anything so why do you need forward genetics the classical genetics is it redundant now in today's life. The answer is no it still has its relevance the process are reverse genetics where you create a mutation and ask questions is laborious them consuming right and at times unpredictable you know what would be the outcome you do not know right.

So the forward genetics gives you simpler tool and you can quickly do that and still it has relevant and here you do not need to when you do you know a forward genetics screen where you expose animals to certain chemical which cause some defects on where you do not know where. It does not really require the knowledge of the gene or genome because I can take a new species whose genome has not been studied still I can do. So that is still big big plus for that, the other thing is important you know if you have a mutant background again I can subject that mutant trying to another round of you know random genesis using classical genetics. I may come up with a suppressor or enhancer for example a second mutation may reverse the phenotype to normalcy or it can make it worse.

These are all you know still possible using forward genetics so still you know these are relevant and likewise mutation and regulator region can be mapped. So you have a region of stream of a

gene. Now it is mutated now the gene becomes non-functional alright and finally when you look into then you can find the regulatory regions that important which would have been very difficult if you go for a reverse genetics because reverse genetics more often the coding sequence.

You do not know the regulatory sequences so this approach still gives you the understanding so therefore it is extremely important even today to continue the conventional studies but you know top of with the reverse genetics approach you can handle and understand and you answer important questions.

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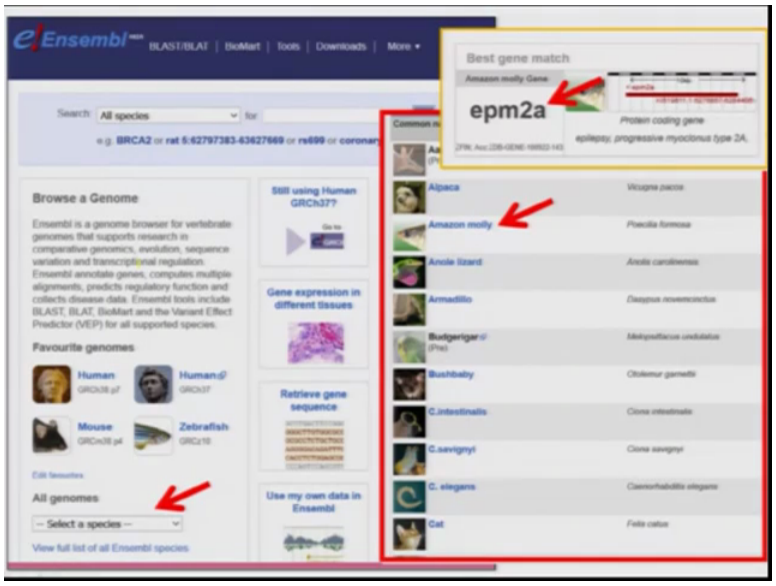

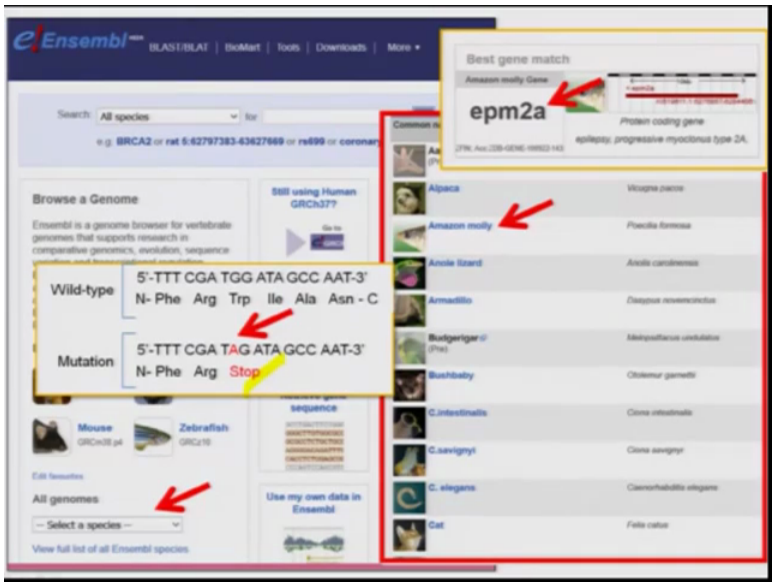

Let us see how we can use this for example you have go to ensemble and to select for example all genome you go to Amazon (())(15:00) is the fish and ask a question ok. You know do I have a gene of my interest in this animal I say epm2a it says yes it is there it is present in this region all this details it is given. The sequence is given where is expressed is given now I can go and want to create a mutation like this right, convert the gene having a mutation and make the mutation in the fish let us say and ask what phenotype it will have right.

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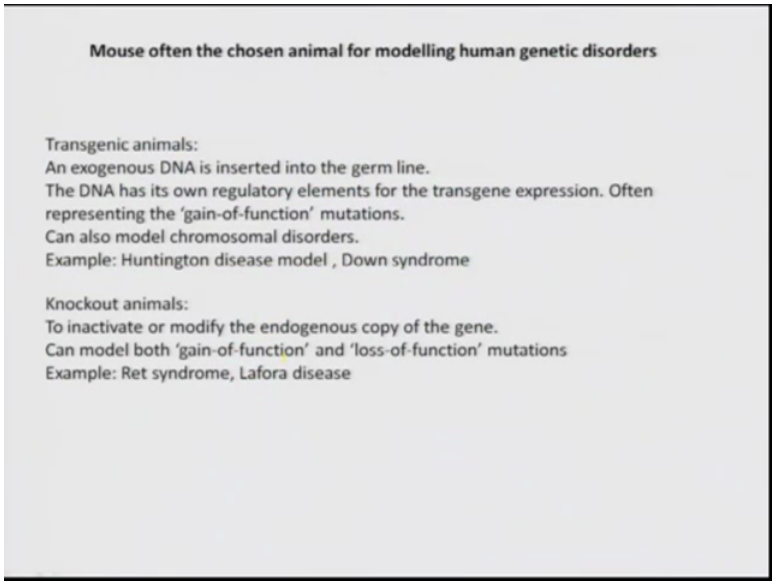

How do you go about doing it ok, so you know you need to model right so there are various ways people do let us not get into that?

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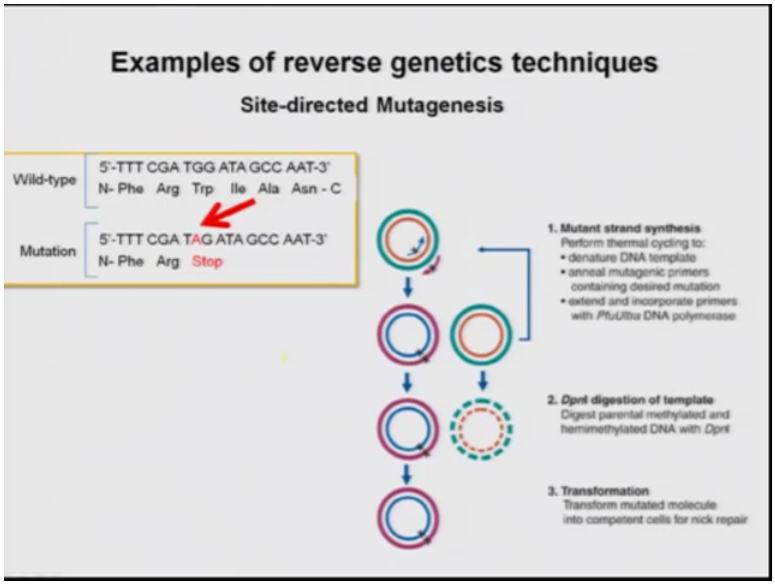

So let us first ask question as to how you can create a mutation in a DNA. Let us say I want to change the base G and make it to A therefore this codon becomes a stop codon. So this is called a site directed mutagenesis. So how do you do this?

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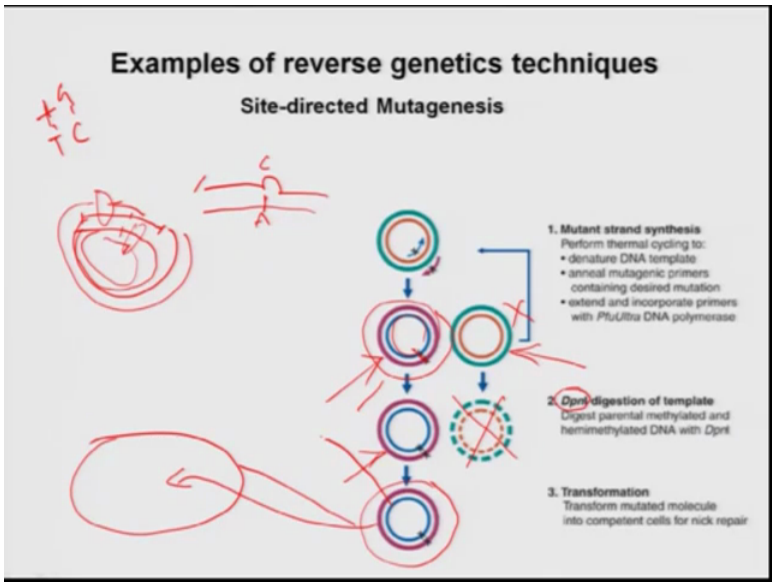

So the request it is that you should need to have the gene cloned in a plasmid. plasmid is a in a circle DNA that harbours a gene sequence so you have plasmid in which a particular gene sequence from here to here is you know cloned and you have it here double stranded. What you

do now, so let us say that you are sequence is here this is something that is for example A and T you want to make it to GNC this is complimentary sequence what I do. I make alligo nuclear types which you otherwise call as primers which spans that region and such that the new sequence that I made has got the desired you know changes.

So it goes (())(16:47) to all the sequence accept this sequence because now it has got A here so I introduced for example C therefore it will not pair, so likewise on either side I made it that is what shown here. This region has got the change and then off course you allow the DNA polymers is going to copy the whole section likewise from here it is going to copy all the section and you are going to make you know a two new DNA strands which are primed by the primer having desired mutation and that would have everything identical accept that you have created a mutation here right.

Now you have this new DNA strand also the original DNA strand that Plasmid had right the wild-type one. So how do you select this and remove this, so if you extract the plasma DNA from equalai normally the equalai you know add methyl group to certain bases of the DNA and there are restriction enzymes which will cut the DNA if the DNA is mutilated. So if I use for example an enzyme called DPN1, which would look at the DNA which had got the methyl base it will you know degrade it will cut here and there so the plasmid no longer functional.

Whereas the DNA that I made using the primary in a test tube will not have a methyl group so they survive, it does not gets digested so now if I use this DNA and put it inside an E. coli bacterium. Now this would get in there because it has every other elements like the original plasmid. It can replicate it can survive on antibiotic medium because resistance gene now it grows and if it grows I know that it has got a plasmid in which I have introduced the particular residue being mutated.

Now you know I had a wild-type gene right of the fish that I wanted to work on and I have created a mutation that I found in the human, now I want to put it inside the animal and ask the question now if I replace the normal gene with this mutant version what would happen to the animal right.

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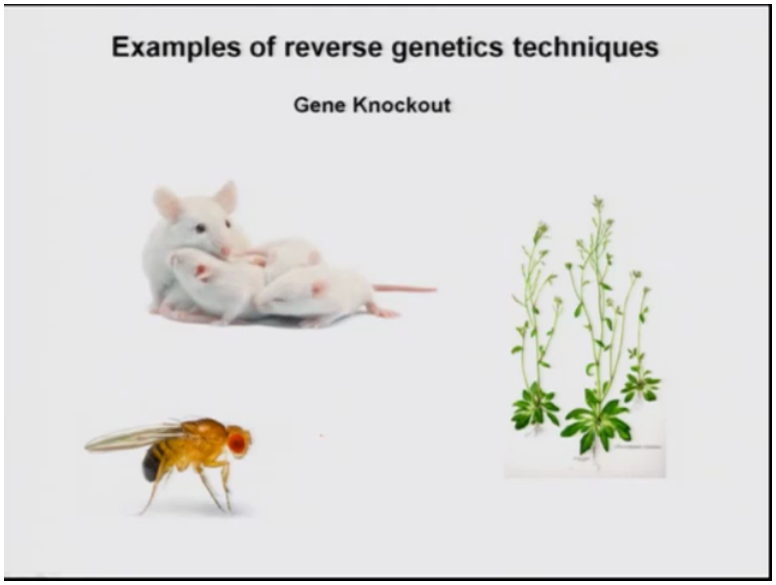

So that is the process by which people do, so that is the process people do and I want to discuss one such approach called gene output ok. So you can remove a gene from the genome of every cell and ask the question as to what happens to that animal right. So let us this approach is done you know you can use it in mouse or fly or for example plant. This is successfully carried out may be there is a difference in the technique the approach but it is doable. You can remove the gene from the organism either in all the cell or in a particular group of cells that is doable.

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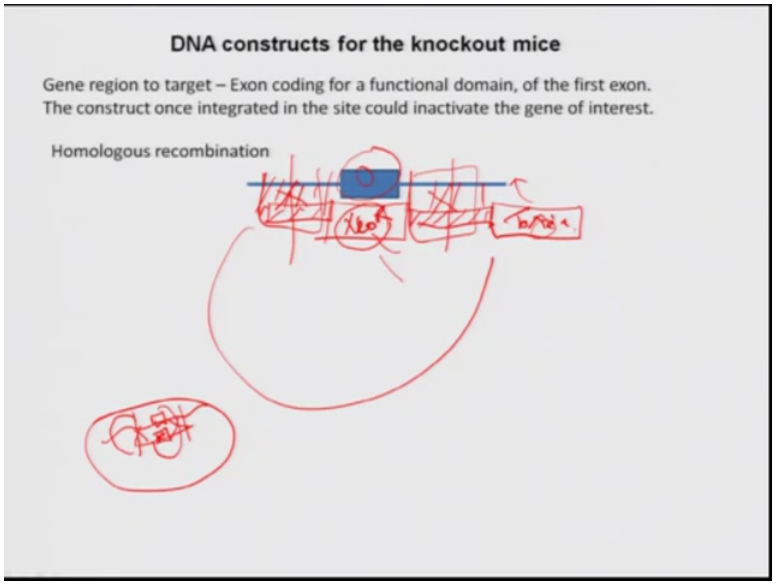

Let us look at how we can create what is called as knockout mice or you delete here region of the genes therefore the gene becomes non-functional using you know this approach in in species for example mouse so what you need is you identify a region that you would like to delete or mutate right and normally this is taken as the first exon of a gene. So you delete that or a region of an exon a coding region that gives functional domine for the protein.

Let us say this is the region we selected and that is there in your plasmid now what you do is that a flanking this particular coding region is selected from the genomic library of that particular organism and then what you do is you have taken flanking regions and then instead of this important region you put for example a small segment of a foreign DNA which codes for example a neomycin resistance protein right will talk about why it is.

So this segment carries now a neomycin resistance coding sequence and you know so once you have this what will have is that when you make this construct right, so you have a construct in which this segment is identical to the gene here and here but this region which you want to delete and mutate it is removed but in that region you have placed a foreign DNA having a small segment of neomycin.

Now what do you expect that to happen, so if this gets into a cell ok, a mammalian cell for example a mouse cell so now you have the chromosomal DNA in which this exon is here so that this DNA would go. This is your neomycin resistance gene but this two regions are identical. You expect a process called homologous recombination in which you know there is cut in the DNA here and here and this segment gets in and that segment comes out we will explain it in the next slide.

And then how do you select this cells now you can grow this cells in the presence of an antibiotic called a neomycin and cells that do not have these proteins coded by the neomycin resistance gene would die. In other words cells that survive in a medium containing neomycin or those cells in which you know your DNA is integrated in the genome and it is coding for the neomycin resistance gene.

So this can happen in two different ways one if it gets in exactly the same place replacing this exon as we wanted or it can get integrated elsewhere and still course for the neomycin resistance gene. So how do you distinguish these two population of cells, so what people do is that at the

other side of this is DNA they code for some toxins. They put a DNA that course for some toxins, so this made the cell is killed however if the integration is happening because of the homologous recombination meaning.

You the homologous sequences or identified and the DNA cut happens here and there it exchanges this toxin region will not get into the genome as a result you know this cell would not have this toxin coding sequence inside the genome so it will not make the protein so it will live and it also live because it as now the neomycin resistance coding sequence. So it codes for the proteins therefore it lives in a medium containing neomycin resistance gene.

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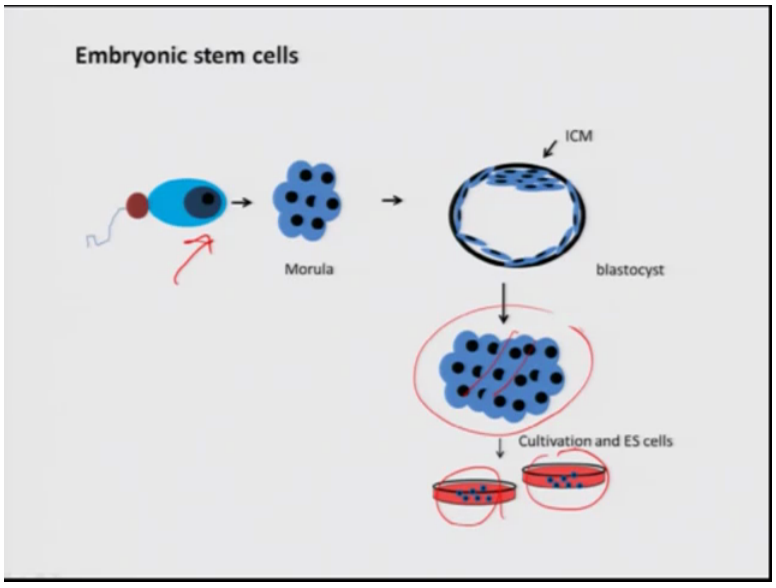

So this is how we are able to replace a small segment of genome in a cell, in a group of cell and then you identify these cells right. So what are these cells so these are the cells that are called as Embryonic stem cells so these are basically you know derived from a blastocysts you know this is sperm and egg that fused and then you have this blastocysts then you can disassemble this blastocysts and you have these cells these are called as Embryonic stem cells because they have the capacity to differentiate into any part of your body. They have the stamina that is what they called.

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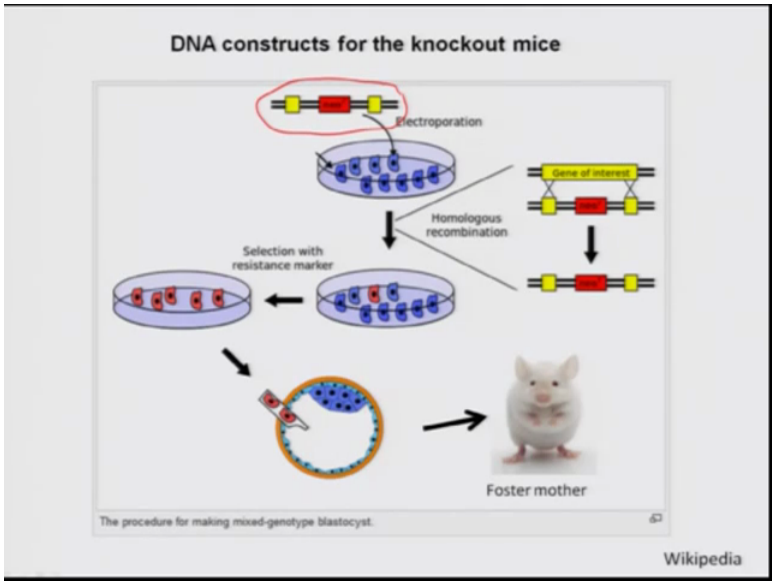

Now what you do is that the construct that you have created that we discussed a little later little before. You can introduce into the cells that are shown in the dish like this and then in some cells you know you would have the integration as I said for example you have the homologous sequence, they recombine, so when the recombination takes place the neomycin genes gets into the genome and therefore when you played them in a plate containing the neomycin antibiotic only this cell in which this integration took place would survive so you have all the cell that in which you know this change this region of the gene disrupted.

So what you do after that we take these cells inject into a blastocysts this is an Embryo about to differentiate so you put in there there is a cavity here. You can take about 10 to 12 cells and then you take this Embryo put into what is called as the foster mother. Mother who accepts Embryo of other origins for in female other female.

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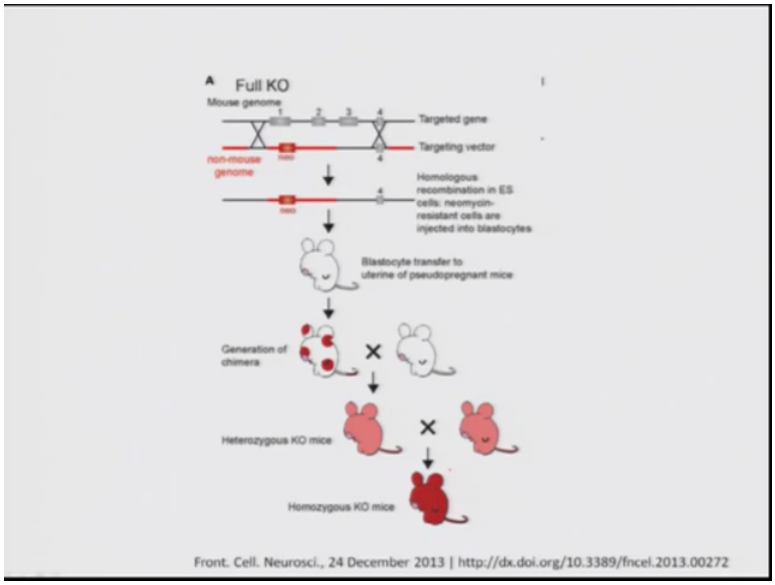

And then you have you know the chimera bonding so that is what is shown here. So for example the recombination happens here and here the neomycin gone there and you mutated the gene, this is the blastocysts mother in which the blastocysts is put inside and then the Embryo develops into an animal and and what you can find is normally the people used the Embryonic stem cells having different from different stems that have different coat colour.

For example the Embryo is from a female which is white coat colour but the Embryonic stem cells could be from a species or a mouse friend that has got say brown or black coat colour so whichever segment of the skin derived from the Embryonic stem cell that we integrated inside would have that coat colour. So therefore I know that this is the chimera having some part of tissue derived from the Embryonic stem cells so it could so happen you know the gem cells you know some of them would be derived from the Embryonic stem cells and if you cross them with for example another male or female having a white colour and you look into the coat colour.

Any colour any new born animal having say brown colour or dark darker colour likely to have derived from a gambit from this Embryonic stem cell because it is not white then I know this is 50 percent chance that it would have (())(26:26) is removed or mutated and you cross this two and you will get an animal which is likely to have the knocked out gene right. So then you look at what is the phenotype of this animal and this is how you create you know dark coat animals but at times it could have a problem that that any knock out the gene the animal may die.

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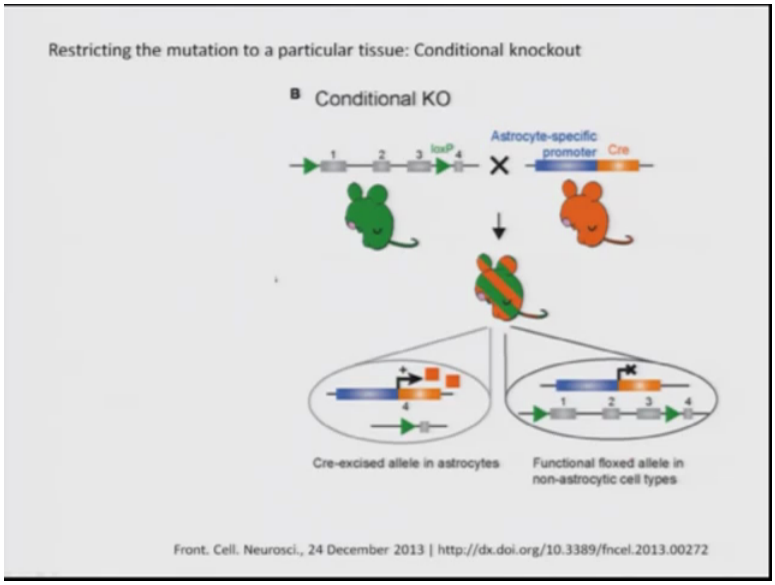

So you do what is called as a conditional knockout wherein you restrict the mutation to only certain tissues that is something that we will discuss in the next class yeah.