Host-Pathogen Interaction (Immunology)
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Lecture: 14
Cells of Immune System-Transgenesis - 2

Hi, so in previous session I have discussed about how to create the knockout mice and whatever I have discussed this is for making a single Gene knockout mice. So, this is a very interesting how you will make the; if you want to knock out two genes how you will go for that. So, the answer is not very difficult first you need to make a single knockout mice for each gene and after that you can achieve very simply the just by Crossing.

You need not to knock out both Gene in one embryonic stem cell. If you will do most likely that embryonic stem cell will be will be severely damaged and it is you cannot get the embryonic stem cell which has both gene allele will be disrupted. So, the most easy and convenient ways that you can create a single knockout mice for each gene; for example Gene one and Gene 2 and then you can do the mating and then by that you can get the double knockout mice.

It is in a very simpler term if you which you have studied very well in maybe class 12th or tenth standard or maybe in graduation it is a simple dihybrid cross. So, by dihybrid cross you can achieve these double knockout mice. So, I am not going to talk about the mendelian law and monohybrid, dihybrid or trihybrid cross. So, it is also may possible to make a tri hybrid also.

You can make triple knockout mice but it takes a huge effort you will you need to do a huge screening it is not generally it is not. So, much recommended generally we use mainly single knockout mice in general we use single knockout mice rarely we use the double knockout mice. And if you are interested what is the use of double knockout mice then maybe during discussion I can explain if you want otherwise no need to go for that double knockout mice experiment.

Now I will take you further in the transgenesis and now I will explain you the; how to create the conditional knockout mice. What are the prerequisite for making conditional knockout mice. And first or very simple question why you make the conditional knockout mice. So, I I think in previous session I have I have explained to you. So, if the gene which you have ~~chose~~chosen maybe it is involved in some development process.

And if it is involved in development process then this and since you have disrupted this Gene then the embryo will not develop and there will be a death of embryo, in utro death of embryo. So, in that scenario and if you want to study that Gene in particular cell type then you need to make a conditional knockout mice. And how we achieve in; there is a some molecular techniques and I will explain you in this session.

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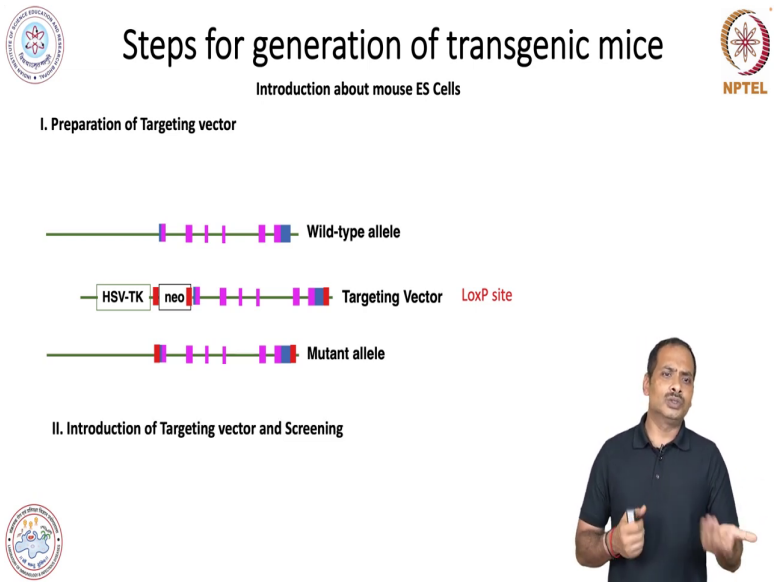

So, this is a; I have already introduced about the embryonic stem cell. So, no need to explain here I am again coming with some example the same gene here you can see this is a this is a gene for that IPS or ~~Mouse~~ MAVS Gene and these are the 5 Prime UTR~~utr~~ and the which is represented in blue colour and the pink colour is the exons and you can see there is a three prime UTR~~utr~~. So, here the task is I believe it is at least two times more difficult.

And overall if I see making of conditional knockout mice's at least maybe three times or four times difficult. So, I will I you will understand this soon. So, the ~~here~~ this is a genomic arrangement of the gene IPS and here we need to make a targeting Vector in this fashion. Now you have probably you understood about that ~~yesterday~~ TK you understood about the neomycin but here you can see there is a some more red colour boxes.

So, what is this red colour boxes. So, this red colour boxes are Lox P site I will explain you what is Lox P site and but before that you should understand in this is a very short stretch of nucleic acid I if I remember I think it is a 34 base pair. So, this you need to incorporate this 34 base pair in the genome then you can achieve the conditional knockout mice. Then you will achieve I will say 50 percent of a conditional knockout mice.

So, what is Lox P site now I have a one additional slide for Lox P sitesde. So, when you incorporate this Lox P site then there is a some enzyme which we call it as a Cre enzyme. So, this Cre enzyme will basically chew off in between two Lox P site Qchew of the DNA. So, in that way there will be a deletion of that Gene. So, this deletion will be achieved when the Cre enzyme will come in action.

Now you might have a simple question from where this Cre enzyme and what is Cre enzyme, so, this Cre enzyme from where it will come. So, I will explain you in a later slide. So, once you make this kind of targeting vector then you again you will put it inside the cell embryonic stem cell and finally you will achieve this kind of mutant allele for that Gene. Here you can see that there are only two Lox P sites and it is far more difficult why I was saying.

Because when you are making the knockout mice and when you are cloning the a fragment of DNA if you remember the previous session if you do not please relook that previous session then at that time when you were cloning this big chunk of DNA in which will facilitate the recombination that when you are cloning then it is far more easier, why because when you are cloning that fragment you do not care about the sequence.

You know that how you clone the gene you basically do the PCR amplification and please remember PCR amplification is error prone method. So, that is a; even if some mutation is incorporated it is not bad it is rather good that the gene will be much more disrupted not only the five Prime utrUTR and few exons but also it will disrupt if there is a some mutation in the exons or wherever then that will disrupt much more efficiently right.

But here you cannot afford to incorporateate the mutations. If you will incorporate mutation then that will be not good here my aim is not to disrupt any sequence genomic sequence and

in that circumstances you have to clone a big chunk of DNA say 10 kilobase pair 11 kilobase pair or maybe 12 kilobase pair which is very very difficult if you have any experience for doing experiment, cloning more than one kilo base pair is very difficult.

And here it is the size of DNA is a huge right 10, 11, 12, like that it is a very difficult and there is a no I am not very confident that in one shot you will be able to amplify such a big DNA fragment even if you amplify such a big DNA fragment then that will incorporate lot of mutation because PCR is a error prone method there is no proof reading. So, this is a far more difficult making a targeting Vector for conditional knockout mice is itself is a big challenge.

And even if you made it then there is a second level of challenge. So, once you incorporate this targeting vector inside the embryonic stem cells then there will be another level of challenge that you need to remove this neo cassette~~eacet~~ or neo gene ~~Gene~~ segment. And how you will achieve? You basically you transfer or you introduce a plasmid which is expressing a Cre enzyme. So, when in this scenario you just look at the targeting Vector in this scenario if you put and consider that this HSV-TK is removed.

So, rest of screening will be same as you have learned for knockout mice HSV-TK will be removed and this whole chunk of DNA will be in the genome but there will be a neomycin and you need to remove this neomycin and when you will incorporate the Cre enzyme in the cell you basically you will introduce a plasmid which will express the Cre enzyme. This is a simple way or this is the only way I will say.

So, when you will introduce the Cre enzyme then there will be several possibility one possibility is only neomycin cassette will be ~~achiev~~chewed off. Another possibility is between the five Prime end of neomycin and the three prime end of or in between this 5~~Phi~~ Prime end of neomycin and three sorry three prime end of neomycin and five Prime end of this Gene will be chewed off this is also a possibility.

Or another possibility is the whole thing will be chewed~~achieved~~ off. So, all these are the multi-level complication and it is not very easy to get out from this you have to keep on trying and then you have to have this kind of situation the mutant allele where only Lox P sites are there which is five Prime which is Upstream to five Prime of this ~~utr~~UTR and there will be a Lox P site which is downstream to three prime ~~utr~~UTR of this Gene.

Once you have this thing then you have achieved but please remember again when you will do all these things all manipulation and all these complicated experiment your embryonic stem cell may differentiate and then you can never achieve the you can never get this kind of mice. You can have the cell but cell will not become a mice. So, these are the different levels of challenges which I am explaining.

So, once you once you successfully of course people got this conditional knockout mice it is not a new thing it is a it is a quite old thing people do made a conditional knockout mice but it is extremely challenging extremely challenging in both financial and labour intensive and all kinds of things. So, once you get this kind of mice you will of course do script screening and all those things.

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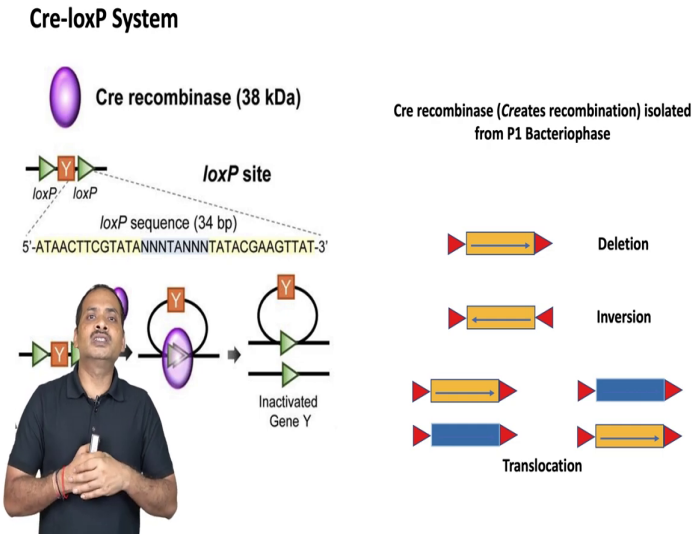

And finally you will get the mice with Lox P site which you want to delete and when you will do this thing then this mice will survive. During development there is no change in the everything will goes as per plan as per plan of the cell in a very normal way. Now you want to delete this Gene in say particular cell type or in particular organ then over there you need to make a Cre knockout a knock in mice Cre knock in mice which is again another set of creation of transgenic mice and it is again very very difficult.

You have to for example you want to knock out this Gene in lung cells or for example you want to delete this Gene in B cells. So, what you have to do you have to find out the gene which is selectively expressing in that particular cell type that is for example B cells. So,

there are some molecule which is ex specifically expressing in that cell. So, under that promoter you have to put the Cre gene.

So, once you have this Cre mice for example B cell or macrophage or dendritic cells whatever you have to find out the unique Gene which is exclusively expressing in that particular cell type. Once you have that kind of Gene then you have to make a you have to introduce the Cre gene and then you have to create this Cre mice. After creating this Cre mice you will mecate them.

Please remember you have to have both allele should be mutated the Lox P side should be present in both allele I will I will show you. Let me explain what is this Cre and Lox P thing. So, this is a this is a Cre and Cre is a basically a recombinasence which is very small protein 38 kilo Dalton and this is a lox P site it is a 34 base pair yeah and you can see that there is a palindromic sequence and then there is a intervening sequences.

So, basically this side this kind of sequence you need to incorporate into the genome and from where this Lox, Cre and Lox space system was discovered it is a basically derived from the P1 bacteriophage, you know bacteriophage which the viruses which infect the bacteria. So, from there this is derived and this is a very beautiful system this is a really very good discovery. S¶

so, using this system you can do a lot of genome manipulation very easily for example if you put this Lox P site. If you put in this orientation for example you can consider this five Prime and three prime end of Lox P site. So, if you put it in this orientation then the intervening sequence will be achieved off but if you change the orientation then the gene will the DNA fragment will be flipped by Cre enzyme.

So, you can do all those manipulations and if you have this kind of situation if you put this four Lox P site in this orientation then there will be a translocation of the intervening DNA sequences. So, this is a very very beautiful system I will say. So, once you once you have these Lox p Cre mice for that particular Gene then you can mate it with Cere mice.

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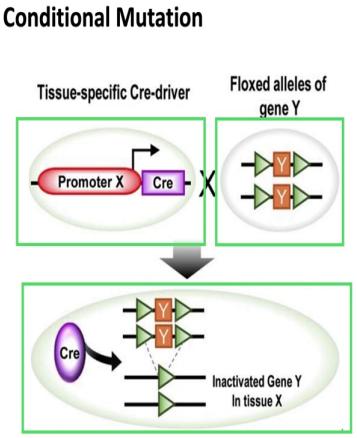

Here there I have a very good slide which can explain how you achieve. So, this is a tissue specific tray I have told you the gene which is specifically expressing in particular tissues or cells. So, you have to incorporate Cre here you can see and there is a under that promoter you have the Cre and Cre Gene and that will make protein and this is a floxed allele for Gene y for example the gene which I have explained you may be IPS1 or MAVS.

So, you have this kind of situation and then when it will when. So, Cre is expressing for example in macrophages and over there is a Lox p site both allele will having the Lox P site. So, in those cells the that that targeted Gene will be deleted and once this Gene is deleted then you can you can you can achieve the knockout in that particular cell type you can do it a beautiful experiment using this kind of mice.

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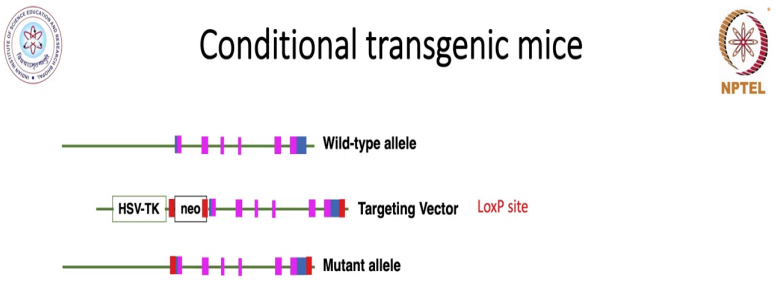

So, this is the concept for creation of a conditional knockout mice eventually you will have this kind of thing and then you will cross with Cre mice and then you can create the conditional knockout mice. So, this is a very complicated system and I think the biologist not only immunologist they should know these techniques and it is very useful not only for studying Immunology for any biological sciences using animal model particularly the mice model.

So, with this I will stop these transgenic mice. I have explained you the conventional knockout mice how to create the conditional knockout mice what is the uses of those sorry conventional knockout mice and conditional knockout mice. And it is very important biological reagent in order to prove any phenomena or concept under physiological condition. thank you, thank you very much.