Functional Genomics
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Lecture No 19
Lab Session: 2

Student 1: We are in the facility where we house our mice theses mice are kept under may kept under control and regulated temperature and dramatic conditions and also the we are given the filtered air using hepa filter.

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So these machines this machine controls the temperature, humidity as well as it provides a hepo filtered air to this gate system where these mice are kept. So we have different types of mice. We have wild type mice which is a normal mice while the normal as they do not have any kind of relations or insertion or any kind of mutations while we have knockout mice in which a gene of interest is deliberately deleted or mutated and hence the gene of interest is not expressed throughout the lifetime of the mouse while we also have conditional knockout mice and usual gene of interest is specifically deleted specifically not expressed during the course of time.

Any particular course of time in which we do a in which we study the functional (())(01:05) of particular genes. So we also use these animals to model human diseases like suppose because of the immunization in a gene there is any disease which happens in humans, so we will delete that

gene specifically in these mice models so that we can mimic the human disease in these mice models.

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For example here we have a mice model of epilepsy.

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Student 2: Now in addition to the reverse genetics approach as I can now explain as that we have mice model which are good examples of forward genetic approach. In addition to reverse genetic

approach in which a gene is deliberately related. We have mice models which are examlpes of a forward genetics approach. In forward genetics approach we have a mutation in the animal which can be seen through the phenotype but we do not know what gene mutation or which gene is mutated that is leading to this phenotype.

For that various screenings are done to pin point the gene responsible for that phenotype once the gene responsible is know we can have detailed understanding of the function of that gene by various other experiments and if that phenotype of the mutation is related to some of the human disease the mice can be used as the model for getting detailed understanding of that disease.

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For example here we can see these two mice both of them are born to the same mother and are of the same age but you can appreciate the difference in their sizes, so this one is the obese mice and has very high glucose blood glucose levels as compared to the other mice. So this mice, this obese mice can be used as the model for diabetes and can be used to detailed understanding of the detail diabetes as well as to develop the drugs targeted against diabetes.

Student 3: Hi everyone so this is our quarantine facility, which also has a facility to attend the genome as we had learned in R1 of the lectures, the procedures to edit the genome. So here some people are working, let us look into what they are doing.

Student 3: Hi Akansha

Student 4: Hi

Student 3: What you guys are doing

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Student 4: Actually here we are trying to create transgenic mice which is a process of genome editing here we are trying we are using ETSY which is intracytoplasmic sperm injection, we are trying to inset that construct in the testis of a one month old mice so here is Komal and she is the technique.

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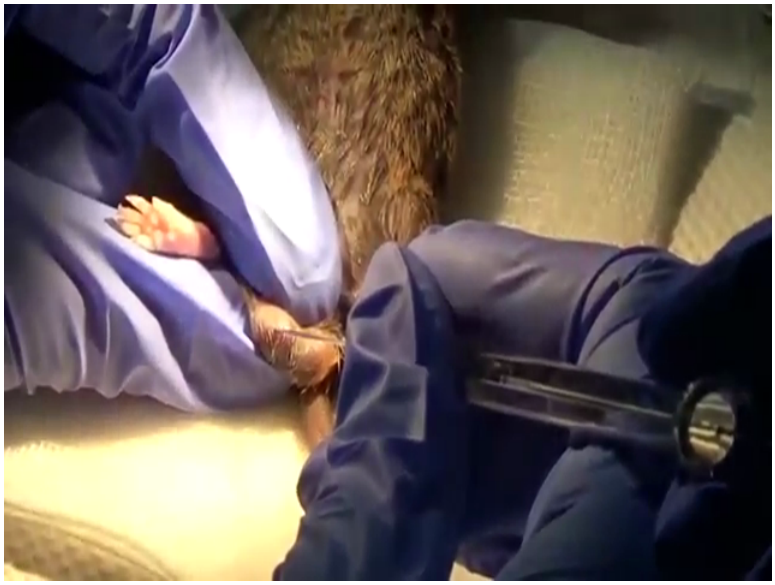

Student 5: So here as Akansha told you we are trying to do testicular trans-genesis. This is mice which is undergoing anaesthesia using isoflow gee so what I am going to do is, this is the Hamilton syringe with which I am going to inject trans-gene into the testis of this mice. Let us see the procedure, so what this syringe is so this is special kind of syringe Hamilton syringe that does not damage the skin and can still enter into the skin and deliver a transgene in directly into the germ cells of male.

The target the germ cells present in the seminiferous tubules, so here here is this Akansha can you please press the syringe when I, ok so can you zoom a bit more to see (())(05:23). So like

this we inject the transgene into the testes followed by electroporation that involves giving four pulses of 60 volts each to both the testis. Four reverse and four forward so we will keep the testes in between electrodes and then we give pulses, four forward pulses and four reverse. Finally we apply this povidone iodine solution to to the testes so that any damage that has happened with syringe should not enter inside and this mice is then kept for 30 days more, so for recovery and then finally after 35 days we set the meeting of this mice with a female and we we expect the pro-gene to be transgenic.

Student 4: So welcome all we will be looking into few model organism which are already there in our department. We will talk to the respective people who are working on this so can we come in, just want to see so you were one mosquito there is the model organism so can you show us some like how do you culture them.

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Student 6: So we have these are the mosquitoes into these cages. We are going to tell you how we cultured the mosquitoes so this is the very first stage so these black dots these are eggs so we put these eggs in the water and within three to four days they hatches into larva into larvae so these are the larvae, so it took around 8 to 9 days for larvae to emerge into very next stage that is pupa. So these very these spherical, the round structures the very small ones these are the pupa.

Student 4: What these black dots are?

Student 6: They are eggs.

Student 4: Ok these are eggs.

Student 6: And so these are around these so larvae are in three stages the first is you are not able to see because they are very small. So these are around second stage larvae and these are third stage larvae, so after this they get converted to pupa as a (())(08:01) then these pupa we keep them in the cages.

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So these are the cages and these containers we have pupa so within 2 to 3 within 2 to 3 days they emerges into mosquitoes so these are the mosquitoes so mosquito feed on sugar so this is a sugar feeder so this is a sugar feeder. So we could 10 per cent it grows, so

Student 4: What is this?

sStudent 6: This is basically the (())(08:34). So after emerging into mosquito within 3 to 4 days they emerges they we are them to mate so by 3 to 4 days they have already mated so then we blood feed them using mouse so these are the mice. So after blood feeding then after 3 days of blood feeding we collect eggs from them and then the cycle continues.

Student 4: So how this model organism has advantages over the others or what kind of questions can we answer using this model as we.

Student 7: So as you all know that mosquitoes are causing lots of diseases like malaria and dengue so our basic question is how call faction is a rule in mosquito attraction to humans so faction, so we basically study how orders which orders are attracted to mosquitoes which are repulsive to mosquitoes and our end goal is, to design some chemical methods or other methods which we can use to repeal or kill mosquito and not so so that they cannot cause these disease first.

Student 4: And as our course is from functional genomics say according with respect to that so is this you do whole genomic gene special analysis to identify language order in DNA causing enhancement or decreasing in a particular gene or such type of studies are also conducted that this model.

Student 7: They are different but we do not do the them right now but probably we will do that in the future.

Student 6: Yeah in earlier we have two specialists so this is a special which we got from malaria institute in Delhi and this is another species liver so they are sequenced. So their whole genome is sequenced so since thank you.

Student 4: So this was Dr. Niteen Gupta’s team from our department and thank you.

Student 8: This a room to maintain drosophila stock and the room temperature is maintained at 18 degrees Celsius so usually drosophila is a cold blooded animal although but the temperature for their you know viable conditions is 18 to 25 degrees Celsius. It will not be below 18 or above 25, so this is one of the flask of drosophila flies that are climbing.

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So this is a plastic bottle. So below is the drosophila food that we have put inside. The food consists of nothing but sugar, corn, flour and then yeast. So and of course to make it as a semi solid medium so we add agar on this so after solidification we put the flies inside and all the other activities like (())(11:40) to the you know formation of larva and to the pupa and the entire life cycle of this fly is in this flask.

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So we maintain lot of genetic lines which are majorly we got from blue maintain stock centre drosophila stock centre in U.S. so and we maintain here depends on our need.

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This is an dissecting microscope so what we have down here is the the adult drosophila flies, so these are the normal wild type flies ok so etherised using diethyle ether in a setup which was just we made indigenous drosophila etheriser so it is nothing but the cartoon and then the perforated

you know plastic on which a funnel was kept and it is nothing but you put a tigor ether 2 to 3 drops like this.

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2 to 3 drops and since it is volatile so through perforated tube it will go inside and put your flies over down, so it will get etherised.

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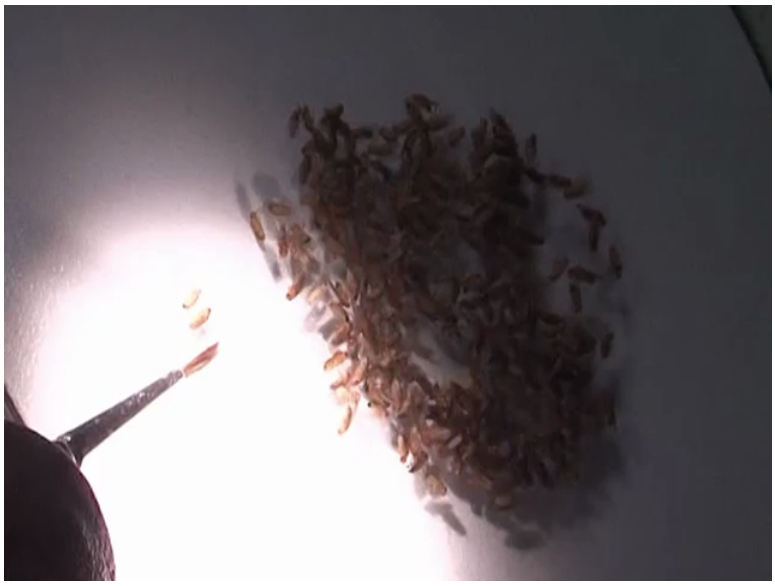

So now these are unassessed flies which is kept here and you can watch these flies under microscope very clearly. This is a female fly usually the female fly is characterised by bigger abdomens and the size specially the overall size would be bigger when compared to a male fly which is on the left hand side. So there are some body markers which we usually used to differentiate male and female and this is a normal wild type flies which has a red eyed (())(13:46) and there are many mutations which we used to generally develop.

Develop and characterised has many genes which are maintained for body parts suppose if we have a mutant for a gene called white the eye colour would become white, this is one of the mutant fly it has mutations specially in wing as well as in eye.

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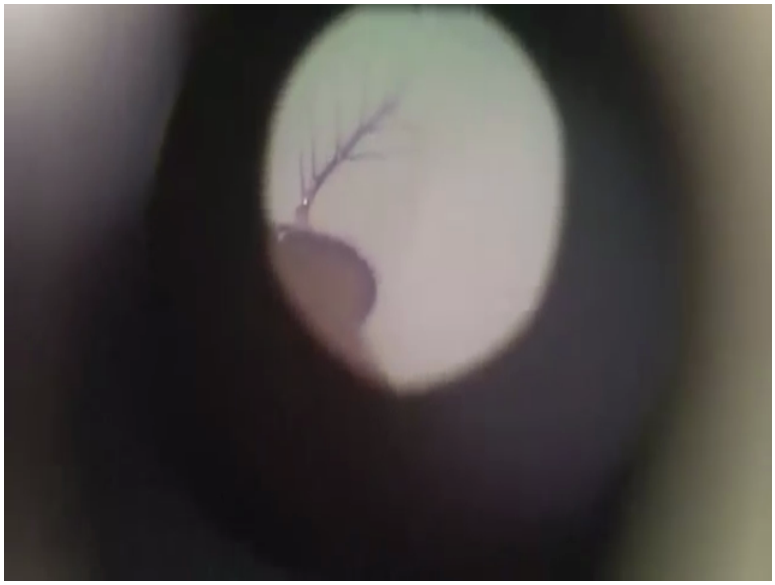

The colour of eye it would be white so antenna and we (())(14:17) I up to. So what we suppose to do is before transfer back into fresh bottle. So this is it, for the next 45 days it will become yes.

Student 4: So do you also make mutants or you...

Student 8: Usually in this lab we do not make mutants normally because all these mutants we you know buy from Glomint stock circle suppose if we want to generate any transgenic stock, India we have a facility in CCT in Bangalore NCDS Bangalore. So there they will develop the transgenic.

Student 4: What are the techniques people use in drosophila too I did that you know?

Student 8: Yeah main technique that we use is the (())(15:16) mutagenesis so that is jumping gene that we call in in biology or the transposons so these transposons are the major majorly used in drosophila transgenic generating transgenic lines. So what we have is we will genetically engineer these transposons so we insert our gene of interest then we give and trace it in a plasmic and give it to you know those facilities. What they used to do is in the embryo stage of drosophila we have a cell group of cells called pole cell, these are germ line cell so they will do a micro injunction of these plasmate into it so they are now this plasmate will integrate into drosophila genome. So now the next generation of flies will carry this transgenic flies. So now we need to sort it out and then we will develop this it’s a major

Student 4: Thank you.

Student 4: So welcome we are in the chicken laboratory where they used chicken as the model organisms and we will know from Thagat how do they edit the genome or how do they insert foreign DNA into the chicken embryo. So I suppose this is the incubator.

Student 9: So this is 37 degree incubator where we keep ice so that they can progress with the stages so when we incubate them they very early stages. So if don not want them they progress through the chicken takes around 21 days to completely develop into full grown embryo so what we do in our lab is we have two labs one working in vein and the other one working on bone. So we use viral DNA and we electroporation the viral DNA into the chicken tissue and because its viral DNA the viral DNA integrated itself into the chicken genome and then we get the necessary phenotype of which of the gene does we have studied or we want to study.

Student 4: Can we open this?

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Student 9: This is the incubator you have different students having different plates and as you can see there is window, so what we do is we do is we open a small window and then you manipulate the embryos inside and then we seal it up again with cello tape and then we again put it back for the rest of the rest of the day according to experiments. If you need it then adult embryo then we will keep it from may be 10-12 days, if you make it early then we can harvest it in within 3 days now we can go to the electroporation disk a student is already performing electroporation where we introduce a DNA with help of voltages so DNA being regularly charged .

So what we do is we inject the DNA into a pouch may (())(18:07) pouch or in brain pouch and then we use electrodes which almost are given a shock and the DNA moves inside the cell and then we have genome ready for re-manipulation with the vital gene. So this is chamber and this is Baba, M.TECH student of (())(18:27) what he is doing is he is using electrodes to put .

Student 10: You want a demonstrate.

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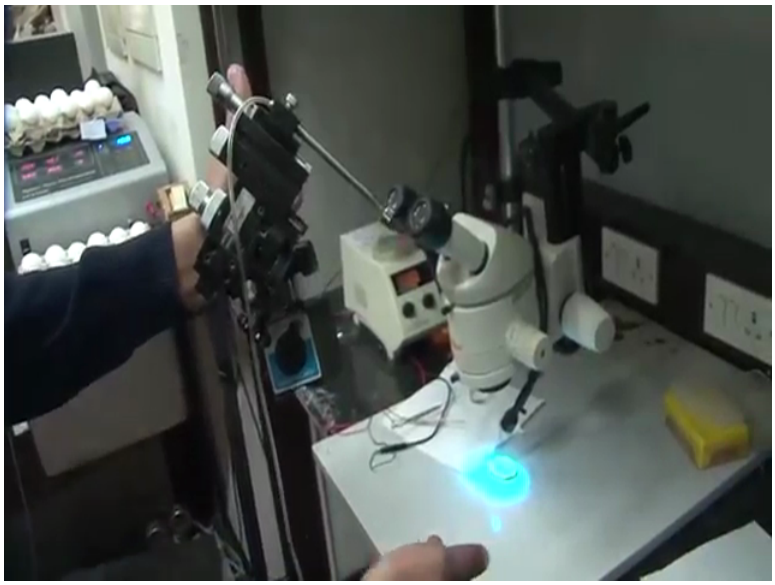

Student 9: So this is a (())(18:46) this is the micro injector through which we introduce (()) (18:53) will be we use to inject the DNA. So you have this magnetic base with which we stick it on to the base metal base. We have the capillary tube in which we have our solidyne solution and we use pneumatic pressure pump, through which we force in the DNA into the embryo so just what we do is at first we load the embryos that is we take out some amount of albumin from the eggs so that the yolk settles down and then what we can do is we can cut one small window (()) (19:35) and we have, you can see the yolk.

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Now this is very early stage embryo as if and you would not be able to see much of the embryo but there is an embryo which is developing very early stage. Stage 10 so it is like almost day one of incubation so it is just a speck of line. What we do is we manipulate everything that we want to do in through the microscope and then we seal back up with the cello tape. So I will just show what we do the first thing that we do is will set it up.

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Look into the microscope and will aim a micro injector onto the embryo, so this gives us manoeuvres ability top and bottom left and right and with each press there is a pressure going into the pneumatic pump which forces DNA into the tissue. We have a die as you would have seen earlier there it is a blue die in which we use so that we can see if the DNA is getting into the embryo properly or not and then when it is finally done, we have two electrodes here and we direct the DNA towards the positive end because the DNA is negatively charged and from the other end we press it just at the bottom of the yolk.

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So we place one electrode at the bottom of the egg and the other one would be on top and then we have another switch here which we operate with our light which would have this electroporated unit which is...

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Now set at 10 volts on as you can see it's, the on time is 50 micro seconds and the off time is 9115 microseconds. So it's the pulse rate of the voltage pulse that we have given (())(21:29) 5 cycles that is 5 seconds. So with one press with the second count so its one full pulse of 5 second which goes in and we use it for putting the DNA into the cell. Once it gets inside the cell we put it back up and we keep it for incubation. Now obviously it takes around 8 hours for the genes say we use a fluorescent gene, the GFP or (())(22:06) so it takes around 8-10 hours for it to be shown so what I can do is I can show you some images which finally will find out the limbs. So your

electroporating in day 2 I will just show a limb which is having a green fluorescent protein so let the entire rim completely green.

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So as you can see we have put in green fluorescent protein into developing as you as it seen earlier its only a streak at that point of time but if you let it stay on for a longer time like this is an day 8 embryo. So this was a limb in which I have incorporated the archive GFP it's a viral rector with green fluorescent protein along with it. So because it's a viral vector and it's a virus essentially. So it spreads as long the limb and now the entire limb which I had electroporated this fluorescent green label as you can see this is the green as you can see and this one is other one which does not have any such green fluorescent protein so instead of fluorescent protein I have used here I can use different genes to study different aspects of bone development and there is several other like I have used different genes who have different kind of phenotype and study how they are and the similar thing is you brain development.

Student 4: Hi Nilesh.

Student 10: Hello.

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Student 4: So with respect to fish genomics as a best model because it's how do how do you maintain this fish model and how do you adding the genome of this.

Student 10: For (())(23:43) and pressure (())(23:47).

Student 4: That can be used in this.

Student 10: It can be used as (())(23:51) is optimised for system the killing matters then there are (())(24:01). A while ago we we have (())(24:08)

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Student 4: And why this one is specifically red. Student 4: Like the natural colour of the fish.

Student 10: No that is not natural colour this is alpha acting (())(24:20)

Student 4: Ok oh this is an RFN model so red colour and protein, and this one.

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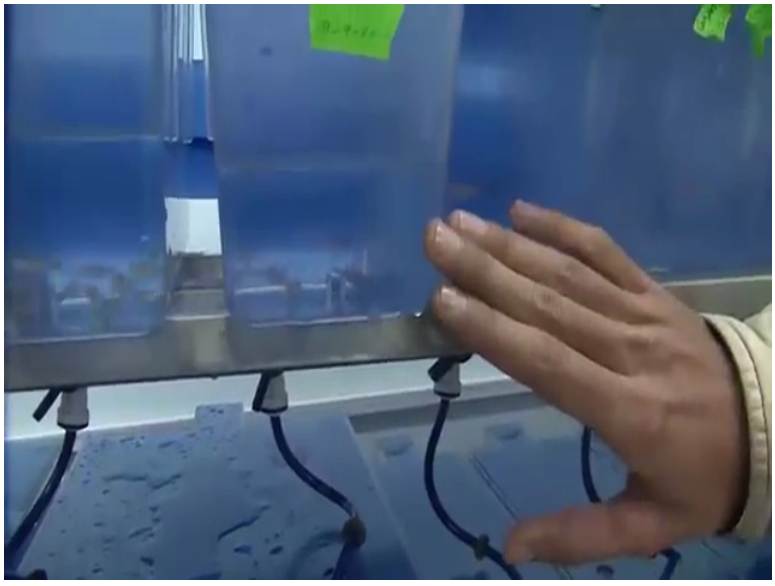

Student 10: This is a CASPer model basically the skin is transparent in this model so all the internal organs are visible you know (())(24:41). In a live organism you can see.

Student 4: Can you show one fish some that list transparency of the body that means.

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Student 10: Sure, so this individual is a female (())(25:24) one white portion towards the case of the ends. The front portion which is a bit yellowish that contents liver and nearby organs pancreas, you see red like a red line going from front to the back that red line is the (())(25:26) fish kidney.

Professor: So hope you enjoyed the lab trip, so the idea was to give you then am over view as to what are the facilities people use for genomic applications and with this session concluding the turn over course called Functional Genomics, I hope you have enjoyed the course and if you have any queries or doubts you are most welcome to write to us. We will try to address back to your loss would be you know taking up one last hang out session so we will will be to interact more.