Functional Genomics
Professor S Ganesh
Department of Biological Sciences & Bioengineering
Indian Institute of Technology Kanpur
Lecture No 18
Lab Session: 1

Hello everyone and so far you know in the formal lectures we have been looking at the theoretical aspects as to what really functional genomics mean. So this session is more to introduce how the labs are, so typically what is a lab that you know carry out some of the assays required for the functional genomics. So this is my lab and we have group members close to 12 of them, these include post doctor fellows meaning those who have done Phd, those who have being in Phd and those who are interested in Phd but having some training in the lab.

So what I will show you is this is a typical you know lab set up, so we have equipments and instruments that are required for a genomic applications for characterize the DNA, RNA or protein and to quantify them and do some functional assays. Beyond my lab our department has many other facilities which we routinely use for understanding you know how the genome functions so this session is mainly to give you some idea as to what the lab is like. What are the equipments you have facilities you have and how these are being used right. So as you can see here the lab has several you know wood benches that supports equipment.

So I will go through some of them for example here we have Komal my student is working on aspects that regulate how the translation and of many other messenger are regulate in brain so that is there main project and right now she is looking at some of the transcripts that she has amplified. You can see that this is typically what you call as a gel box where you put a gel and you are able to stain that DNA or RNA and that would give some images to the quality and quantity of the DNA or the RNA what you are looking at. Why not you explain us give us what you are doing?

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Student: So I have send the digestion of this particular construct PC-DNA vector where two restriction enzymes and I have cut it and then loaded it on gel and I am trying to look whether the digestion of this particular vector (())(02:39).

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Professor: So basically this is an instrument which has a UV box which so transilluminator what you call, so when you staining the DNA, RNA with certain stain for example ethylene bromide

are some other such type you know when the DNA or RNA bound die would excite for us in a different way back. So you will be able to see them easily where the DNA and RNA is.

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And what I will show you is some of the images like you can see here, so these are the images which clearly talks about you know the DNA for example we have two bands (())(03:19) size standards with this you are able to you know characterize the DNA but apart from that we have couple of other machines.

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Again I will request Komal to explain this is machine which most of you could have you know studied in the theoretical lessons that we had discussion. This is a PCR machine, a machine that has the polymerase chain reaction but it has some difference but this also can do what is called as real time PCR meaning as quick the products are getting amplified we can in real time the same time you will be able to measure the changes in the you know the amount of DNA that is being amplified right. So this is very sensitive and being used when you are quantifying the difference in the transcript level for example right so that is why it is applicable and then I would ask Komal to explain so this is a reaction going on you can you can Komal you can explain what is the experiment.

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Student: So I will show you with some of my results so this is the this is one of the results of my experiments in which I have I have checked the targets of particular gene tried to look into the transcript level of this targets under particular condition under a in disease model and in while type and I am trying to look at the level of these targets in this disease models and (())(04:44) type.

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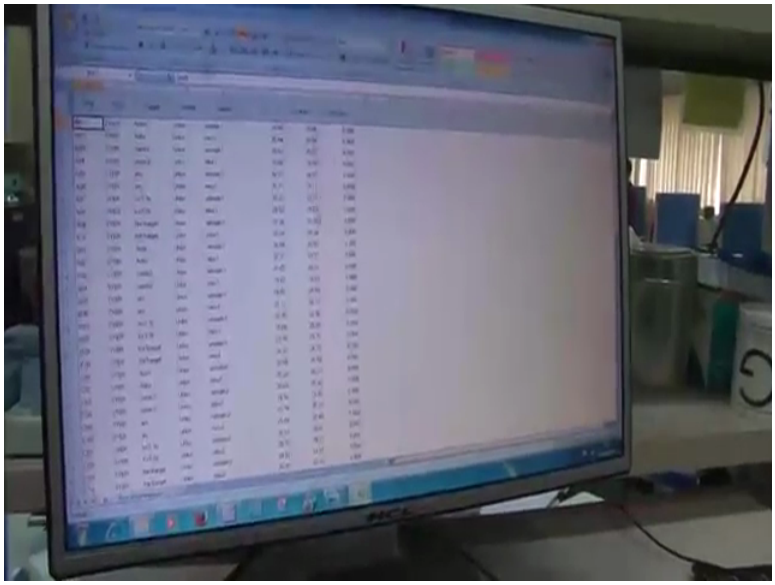

These are the CT values and this shows there is there is no change in level of these targets.

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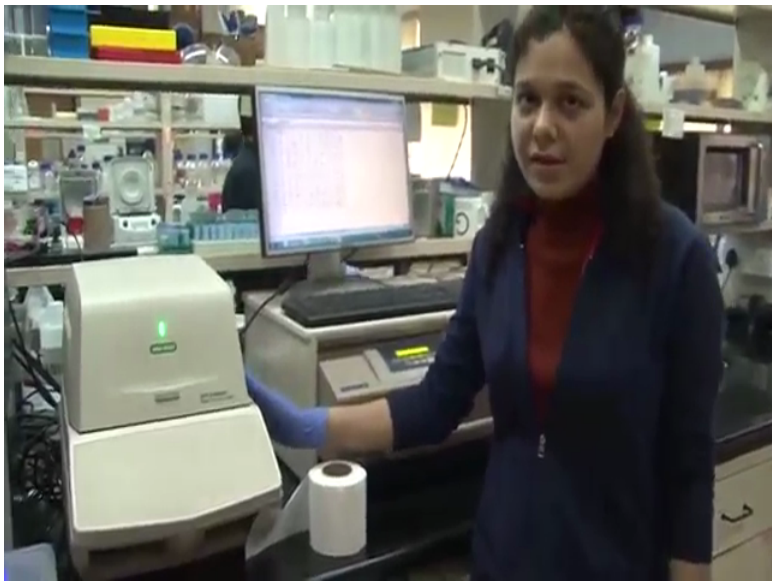

Student: This is how this is how (())(05:05) looks like. We we put the plate here for checking the level of these targets and then after while the reaction is going on while the reaction is going on in real time transcript level of this is required and this is the result that shows that what how the level of my target changes in case of disease and wild type model.

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Professor: Right so that that is one application likewise we have again a high through put machine this machine has you know plate this called as a micro, plate (())(05:46) which is a 96 (())(05:48) so it has 96 for example you can you know have you know test tubes or small tubes

that are 96 we can put it inside and then we can do all sorts of assays for example it can be used for fluorescence, it can read out the fluorescence for example you can grow cells that have a GFP construct under a target so you can see whether the target is amplified or the target is being turned on inside the dilution. So that can be read out or it can have luminescent for example so all you can have even observe it.

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So typically these are the plates that we use, these have we can see that there are 96 wells. So basically you can do reactions in kinetic and you can do you can culture the cells and you can

you know put there for reading so this is how you really put it inside and then this machine would take it and we can set the temperature we can you know enter everything in the computer and it will give you the results.

So you can do observance like spectrophotometer that we have already then you can do a fluorometric reading and and you can do even luminous, these are the three things that you can do there are many such machines some of them we will explain here. So this is typical lab molecular biology lab so we have lab benches and you can see Rohit he is setting up a reaction and we can see that you know you have tubes that are kept over a block.

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This block is at 4 degree so he setting up a reaction for what you call as polymerase chain reaction, so you have all the samples and buffers and then its kept on the ice and that is all you maintain because you do not want to give any thermal shock to the protein when the protein would become ineffective it will expose them to high temperature that is it you know and and if you come here this is Kirti and what she is doing is she is setting up gel.

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So why don't you show this (())(07:55) so this an apparatus wherein you can (())(08:00) for what you call as (())(08:02) so these are very thin gels which you can use to separate for example protein or oligonucleotides or DNA RNA that are very very small in size in bases 100, 50 bases 100 bases and so on so that is what she is setting up this is (())(08:19).

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And these are you know micro centrifuges we can use samples for you know purification of DNA, RNA or for concentration of the protein and we have this.

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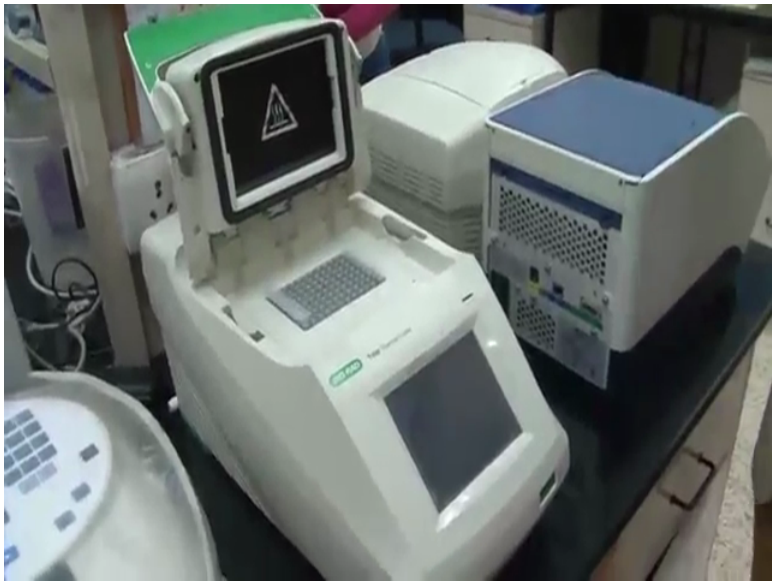

So this again these are regular machines what you call as (())(08:37) chain reaction machines where I will show you one of them. So we can see here again you have a plate in which we can take 96 tubes so therefore we can do reactions of 96 samples. So you can setup the programmes such that you know in a typical in a PCR you have three stages what you call annealing extension and (())(09:00) and you so you can set up as to what should be the temperature at which you expect the primer to anneal extension and (())(09:09) and you give a duration depending on what is the size of the product that you pre amplified. So that is the you know typical lab cutter so we have Varsha an intern we can see she is trying to set up (())(09:29).

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Student: Yeah I have already set the gel right so this is it is already casted now I am going to run my samples I have already completed my RNAi isolation so I am going to check the quality as well as the concentration.

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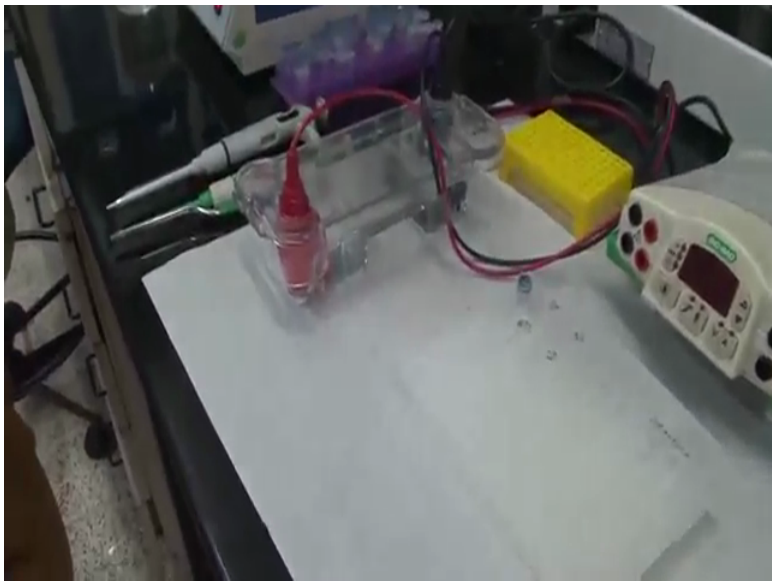

Professor: So this is a typical caste so what you have is a mould in which you know you keep the comb that is where the well kind of structure where you can load the DNA and you melt and leave it there in sort of makes the gel and that can be kept inside this apparatus which is electrophretic chamber so you have a buffer and then keep the gel inside and apply the DNA and then you put them (())(10:09) since the DNA is negatively charged it is a move towards positive side and then you have a gel in between that would you know allow the smaller DNA to migrate faster that is how you you know separate the DNA.

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So you have another (())(10:24) we can we can see this is fluorescence microscope which is normally used to you know look at compartments within the cells to see how and what kind of signals coming, this is Saloni.

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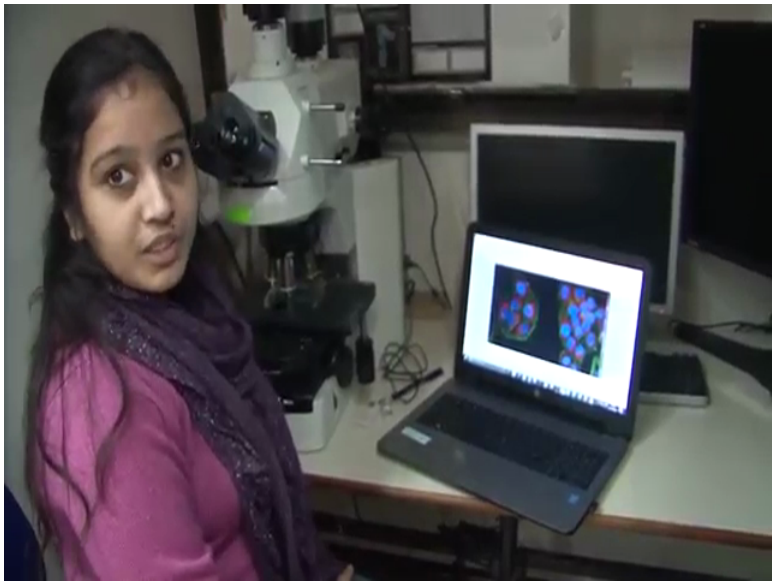

Student: So right now I am imaging for the (())(10:45) I am trying to look for the mitochondria where I am using the (())(10:49) and further loop of this and I have taken the few images here.

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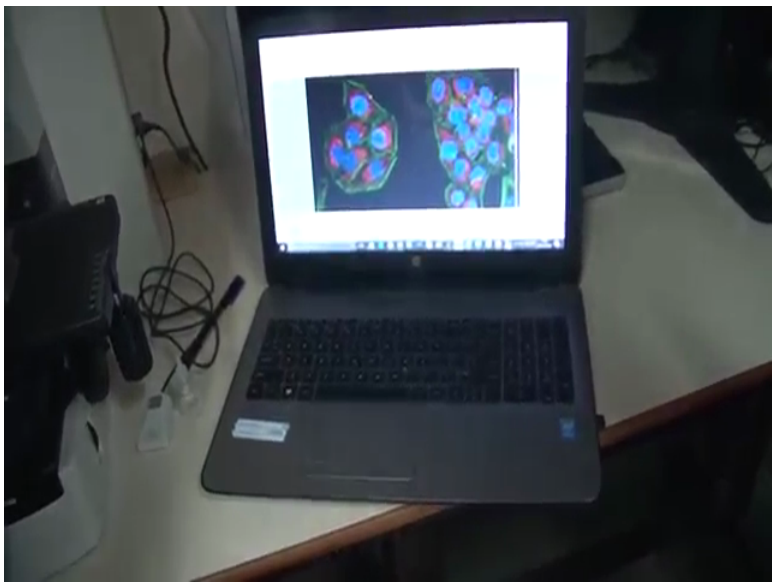

Professor: So you you can see here you know the one that you see in blue colour are the one that are identifying their nucleus of the cell and then you have red colour that is the mitochondria and the green ones are the cellular (())(11:10) compartments. So this is how you look at the cells, we will go and see how the other you know tissues are analysed for example you want to understand function of (())(11:25) you going to understand the functional of any genes so in the reverse genetics if you remember we discuss you delete the gene in the genome and then look at as to what is the function of it in the cell that is what we are looked at, so in the microscope you can look at the cell as to what goes wrong but you can you know use transgenic or knockout technology to generate animal that lack particular energy and try to ask as to what happens as the tissue and organism (())(11:54).

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So here Priyanka is looking at one such aspect that is to look at what goes wrong at the tissue level so this is energy is basically fixed the tissue of from an animal in which a particular gene has been knocked out and she is trying to understand what goes wrong at the tissue level so why do not you explain Priyanka.

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Student: So this is the wax station that we have we for historical analysis we embed the tissue in this tissue embedded as we can see it has. The tissue amyloid is very soft so we need to invade in a system where we can easily process them for cutting out the thin section like we as we know that we need 5 to 10 micro metre of sections for seeing the historical genesis in that.

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So what we do is this is a wax station and in this we embed the tissue in the wax and then this is the cooling plate where we cool the sections. So where you cool the blocks and then after the blocks are cooled enough then this is the cutting section tissue cutting section as you can see we can set the parameters like how much thin sections you want or how much thick sections you want so according to our need. Like right now I need sections that are thin enough that is around 10 micro metres so we cut section of around 10 micro metres and then as we can see here that these are the thin ribbons of tissues in which as you can see this is the peeing tissue of the time processing over here.

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In the block the brain is over here which is embedded in this wax and then this is the 10 micro metre thin section of the tissue which we have to float in warm water which is around 40 to 42 degrees Celsius. So that the tissue is totally spread out and then we just carry it out in the slide, just like this and then we have to dry it overnight at 37 degrees Celsius so that the tissue spreads out more clearly.

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Professor: So this is an explosive facility what we call as culture room so all the mammalian cell, human cell or mouse cells when you do culture for self-biology applications are done here so I

am going to take you through this facility and explain briefly what is there as you can see there is a partition this to mainly to prevent any contamination because such self-culture facilities have the risk of you know attracting microbes and fungus getting contaminated.

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So here is the facility so as you can see here you have a room which is secluded and you have what is called as a bio safety cabinet this is class two like cabinet we are in you know the air that is thrown in t the cabinet is sterilized and then again it gets into and gets filtered so you can see

you have a frame you have all the utensils required for the culture. (())(14:57) you can setting up a culture why do you not explain Bhupander. You can sit and explain.

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Student: Ok as sir told you this is the culture when we culture says yes so right now what I am doing is I have (()) (15:10) that is full of the cells and mutants have been consumed up so right now I am transferring some of the cells to the new cells in which new media has been placed so that they can grow in there and then we can do experiments with these sterilizes, these are immortalised or the cancerous cells they have the property to grow indefinitely unless they run short of the mutants and this is a, so will will show some of this area show this area. So if you you have any (())(15:48) no we not.

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Professor: So these are the what you call as a incubator so basically these are the machines that maintain the temperature 37 degrees not only that it also maintains the carbon-di-oxide ok that is to make the medium PH maintain.

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So I just showing you opening one of that to show you that how incubator looks like and you can see that it has several different types of dishes, you know you have smaller one, bigger one and what you call a t20 flask. Each one has medium and cells are growing various different kinds of cells are grown within that. Use of the cell line is to understand the functions of the genes at the cell level for example gene could be regulating a process the process could be segregation or gene could be regulation a particular process say mitochondria fish meaning mitochondria getting fragmented or fusing is one such event that all trafficking you know there are what we call as endocytosis or exocytosis.

There are vesical that you know fused to new set of function and then to secrete molecules from their well then cell from inside to outside. So these are the things that you can study using the cells and when you want to understand the function of certain genes what you do is you knock down the gene using the RNAi approach we discussed that in the in our theory class. So in that way we are able to knock down certain genes and then look at the functions of you know these genes, so this is the typical cell-culture facility.

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Now we are going to look into how a typical cloning and other such kind of biology is done. So one of the essential component for such work is the freezer, so you can see here this is an equipment that maintains minus 20 inside we can see already these are ice formed so you have for example all the samples that are stored here it would be enzymes, it could be DNA or it could even be you know some of them you know fine chemicals that are required for any of the experiments and we could see here.

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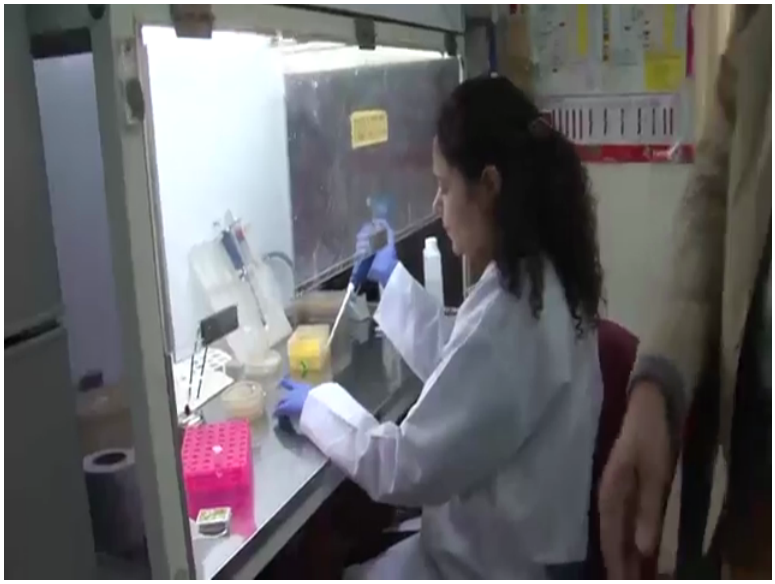

Komal again working using class-1 laminar flow in which the (())(18:06) taken in and you have filter that sterilize the air and throws it out via this this area therefore the chamber within is clean and she is doing an experiment where she is trying to clone it and what you see around are bacterial dishes and Komal why do you not explain.

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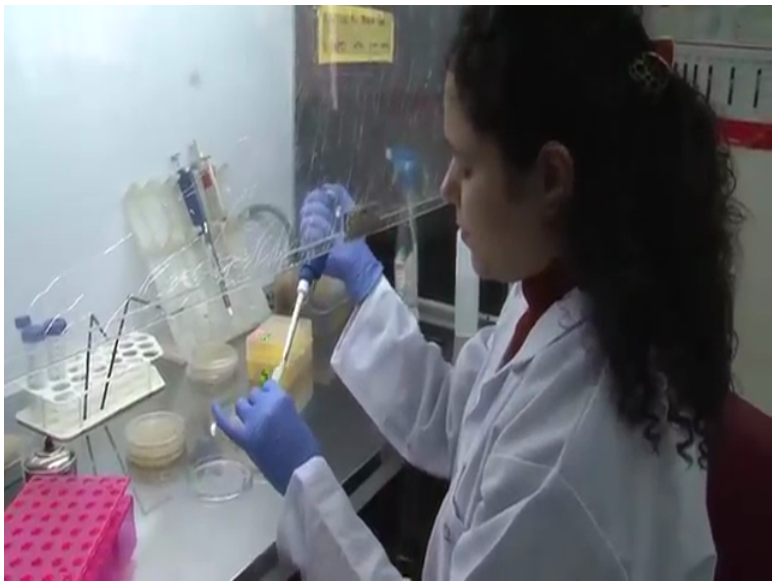

Student: This is ligase makes in which I have cloned in which I have put my vector along with construct and a put ligase enzyme and I am going to plate it on plate which has ampicillin that is (())(18:41) that are specific for my that will specific for my clone.

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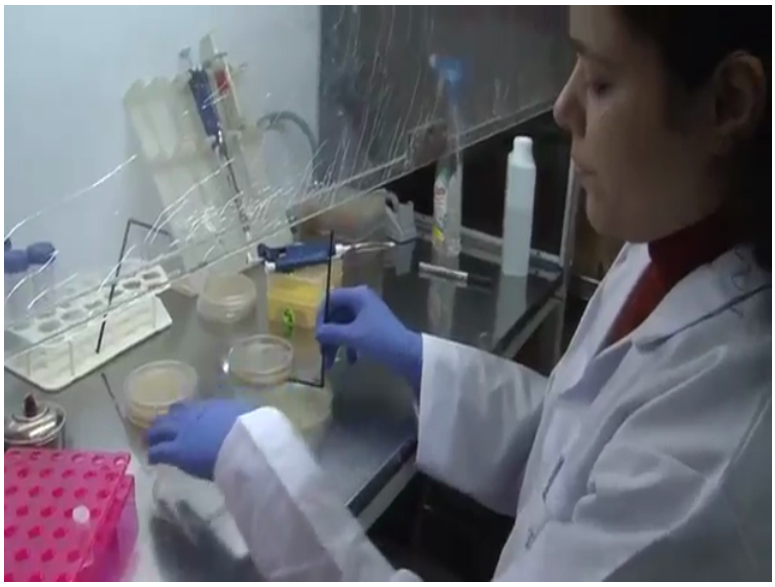

So what I am going to do is to, these are sterilize spatula that will that I use to plate my colonies on this plate. So once the colonies are evenly spread I will just seal the plate and will keep the plate in incubator in 37 degrees Celsius for next 12 hours and look for the colonies that are positive for ampicillin and my clone, so likewise I will create all the colonies I place all the plates likewise and I will put them in incubator.

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Professor: Here is an equipment this is you must have seen in your labs these are what is called as centrifuge but it has more advance features we can you know programmes the machines such that are what speed you want the rotter to you know spin and what should be the speed, how long it should spin and what should be the speed at which it should come to an halt. We can also maintain the temperature of the you know this chamber here it can go up to sub-zero we can maintain here 4 degree, 14 degree for many of the applications. And you can see these are the tubes in which we can you know either you bacterial (())(20:49) or to purify the DNA or RNA and this is a typical equipment that we use for you know purifying most of this biomolecules. .

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So what we are going to show you is a storage device so all the bacterial cell or mammalian cells lines or even you know mouse embryos can be stored for you know several years without losing their viabilities. So this is a possibility and that is a than using a technique called cryopreservation so you mix the cells either E.coli bacterial yeast or mammalian cells with certain medium which is for example (())(21:38) or the anti freezing mediums coming and then you mix them and then store it in liquid nitrogen so Rohit why do you not open it and show.

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We can see its the container which has (())(21:54) we can see that, be careful, so you have boxes inside and then there are tubes that are kept inside and these are you know in freezing condition and normally minus 140, 120 that is the degree Celsius that is where is being maintained and you can revive them any time that you want so when you are not using a particular line it is how it is stored and later you can use it revised down certain protocols again you can culture them and have the cells in running condition. So that is the best way.

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So this is another storage device and just can you just you can see here it shows this champ has not maintained that minus 80 right so the entire chamber inside temperature is minus 80. You can see that so again this is another device for storing you know samples. Anupama why do not you explain.

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Student: Yeah these are the different labs and we have (())(22:58) whichever are required to be store at minus 80 in these boxes. So we can keep them safe for years on this temperature, these are the important parameters samples.

Professor: So it could be enzymes, it could be proteins, it could be again E.coli or cells can be stored in this way.

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So here you have another facility this is also a culture facility right, so you have likewise incubators likewise what you have seen as cell culture facility where you have incubators and then you have a very high and microscope set up. So this you know this set up is such that you can even image cells that are, the cells that are growing.

So basically you have a microscope and you can put a chamber here, this are the chamber in which you can culture the cells and you have all these parameters that you can fill in wherein you maintain the temperature of the you know the chamber, maintain that carbon-dioxide level, maintain oxygen level and then look at the cells when they are when they are growing, so you can look at dynamic changes that are happening within this.

And you can use different fluorescence molecules to tank a various compartments of the cell and then you look at how these compartments you know functions. So to help in setting up the cells for culture and other we have like ways we have bio-safety cabinets and other facilities and we have with us Anshika and Rashmi who are looking at various aspects for example Anshika is looking at some of the noncoding RNAs we discussed about non coding RNAs and some of these noncoding RNAs in the humans is expressed only when the cells are under stress for example the cells are exposed to very high temperature say 42 degrees, 45 degrees.

So it is a stress on the cells and the cell has to sort of mount a response therefore you can survive with that high temperature, she characterised one such RNA which is expressed only when the

cells are under stress that is her work and she would explain some of them here. And then we have Rashmi and she looks at different aspects of this for example she shall respond which is when this cell is exposed to very high temperature. What it does is that it also stops the translation process for a while therefore you do not make abnormal protein so she is looking into that aspects as to how the translation is halted when the cells are under a stress right. So what she both has got looking at is to what are the genes that are involved in this process and therefore we can understand how the process works.

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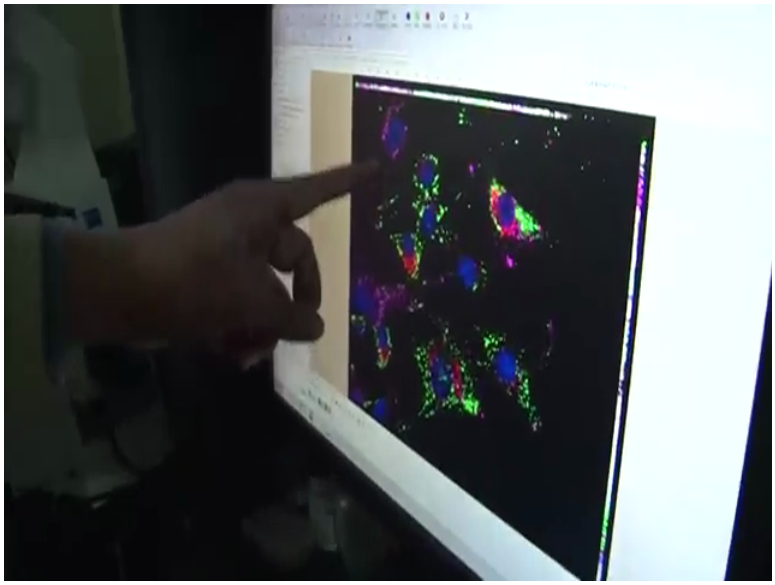

And you can see this image here and we can you have a different colours and these are nothing but you can see that these blue ones are the nucleus and you have the green ones, the green ones are the structures called as stress granules when the cells are under stress. What it does is it puts all the ribosomes which are translating the RNA into a compartment and the halt their translation therefore the cells do not make protein when they are under stress and you know you can explain as to what kind of you know how these equipments can be used to you know understand this functions. We start with...

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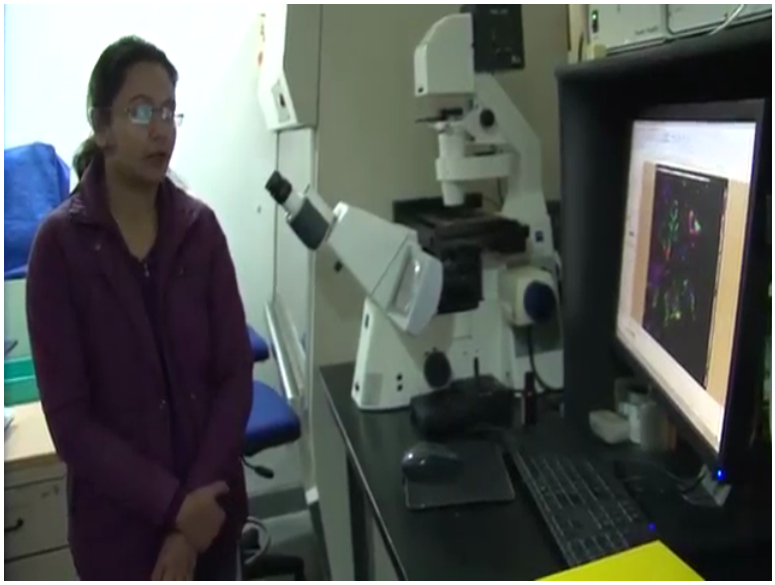

Student: So hello I have been working to study the role of a noncoding RNA in stress response and I have tried to work on the function of this RNA that would function it is playing in the cell and to study that I have been using some techniques in which I can visualize this RNA this noncoding RNA in the cell, one of this that technique is RNA in C2 hybridization. So what we do in that technique is that we use a probe which is a which is which binds complimentary to the RNA in the sense transcript of the RNA present in the cell and hence and that probe is staged to a fluorophore and when it binds to the its complimentary RNA, the and we fluoros the cells with the in a fluorescent microscope then that RNA can be visualized.

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Student: This can be what I just told can be seen in this image this is an RNA in C2 hybridization image in which the blue colour which you see is the dummy and the pink granules that you see are the RNA which are being probed by an antisense oligo against this RNA which is tagged to a, which is tagged to a fluorophore so this is called fluorescence in C2 hybridization and this microscope that you see here it is a, it is a fluorescence microscope that is the basic principle of this microscope and it emits as we begin expose the cells to different wave lengths and according to fluorophore tagged to the proteins or nucleic acids that we want to visualize and the wavelength of light that we emit accordingly. We see coloured signal in the we see a coloured signal, so that is the basic principal of the microscopy of fluorescence microscopy and Rashmi will show tell you more about her work.

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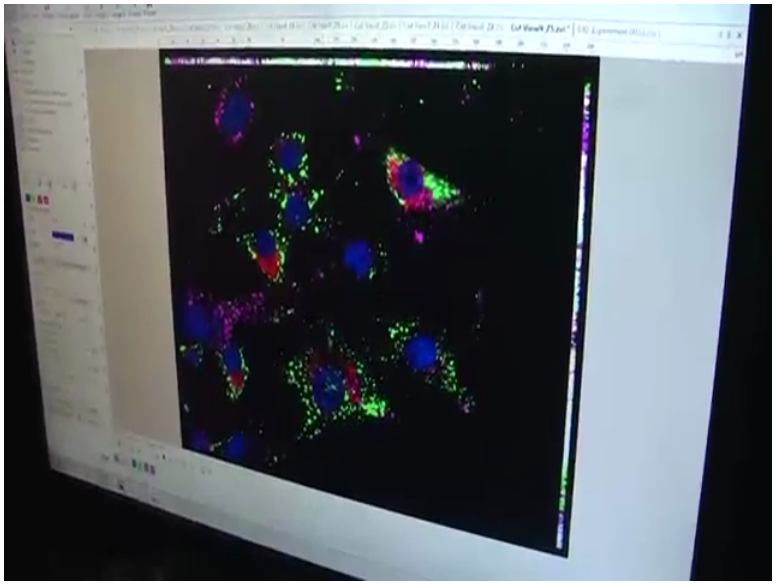

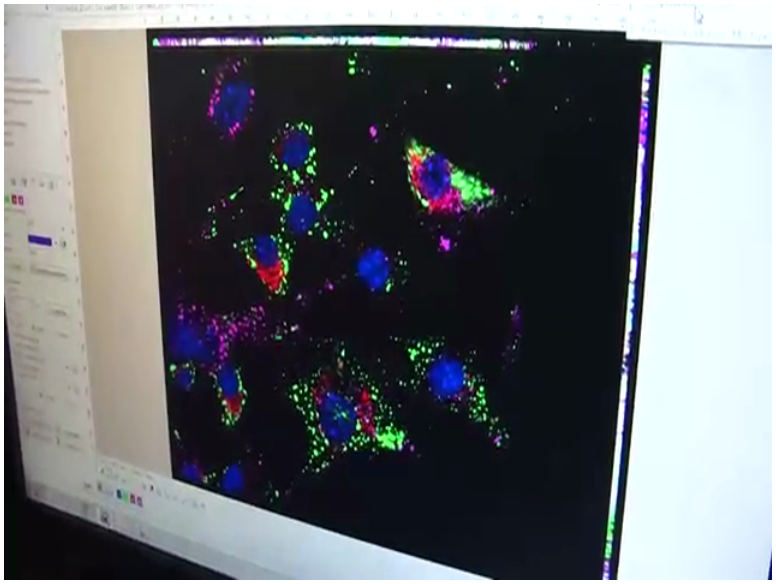

Student: Ok so as already sir told you that cells when exposed to different stresses then they respond to to maintain homeostasis they form stress (())(28:59) by halting translation and that is here this green you can see these are the stress granules, this formed when stress when translation is halted and this normally get whenever this cells are getting recovered they get cleared via autophagy.

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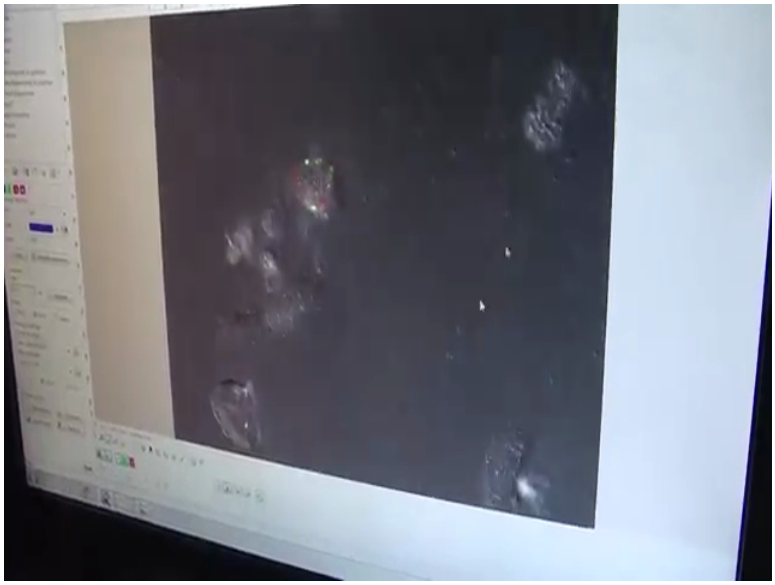

So you can see here I am just looking on this aspect and this pink granules you can see these are the autophagy markers that P62 and apart from this from the microscope we can do live cell imaging as well and here is a that experiment was done and here this it is a DIC image and this green this is over expression constraints GFP tag. This is laforan GFP and this red one it is RFP Ti1 it is another stress granule marker and this cells were exposed to heat shock.

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So for this I have used this live cell imaging whole facility and for this we can use this is a chamber where we keep 35mm glass bottom dish inside this and then we maintain temperature and CO2 via these modules and humidity as well by setting all the parameters and then we can maintain all the parameters and we visualize what happening in the inside this cell.

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Student: Apart from this life cell imaging we have to other microscopes as well. We have one as inverter one and other one is stereo microscope or you can say it is a dissecting microscope so this normally we use to dissect our minute like a brain dissecting brain from the mice embryo and this we use to visualize cells morphology.