Molecular Biology
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Module - 02
Basics of Biological system (Part 2)
Lecture-08 Cell Cycle and Control (Part 1)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and what we were discussing, we were discussing about the basics of the biological system and in this context so far what we have discussed, we have discussed about the cellular structures. So, initially we discuss about the prokaryotic structures followed by the eukaryotic structures and when we were discussing about the eukaryotic structures, we have also discussed about the different organelles what are present in the eukaryotic cells and how you can be able to separate them with the help of different types of fractionation techniques. We have discussed about the density gradient identification and as well as the differential certifications and in the previous lectures, we have also discussed about the cellular metabolism, so we have discussed about the anabolic reactions and we also discuss about the catabolic reactions. So, within the catabolic reaction, we discuss about the carbohydrate metabolisms and lipid metabolisms whereas within the anabolic reactions, we discuss about the synthesis of the different types of amino acids and so on. So, in today's lecture, what we are going to do is we are going to discuss about the how the cell is utilizing this energy for different types of activities and one of such different activity which is very crucial for the cell is the cell division and the cell cycle.

So, when we talk about the energy, the energy what is present in the biological system is going to be utilized for many types of applications, this energy is going to be utilized for the synthesis of the anabolic reaction and the end product of the anabolic reaction is the synthesis of biosynthesis of product, biological molecules and such as protein and nucleic acid. Most of these things are required for the other kinds of activities. Now, energy is also being utilized for the growth of the organisms. So, when you take a nutrition, you are going to produce the energy and that energy is going to be utilized for anabolic reaction, running the anabolic reactions, so that you can be able to synthesize the different types of biomolecules and that energy is also going to be used for the growth of the organisms.

And when you are doing the biosynthesis of the different types of biological molecules, that also is going to be utilized for the growth of the organisms. Because when you are going to increase the number of cells or when you are actually going to enlarge the growth of the cell, you require the synthesis of the plasma membrane, you are going to

require the synthesis of the nuclear content and then you also require the synthesis of the different types of molecules. So, when we talk about the prokaryotic cell, in the prokaryotic cell, let us take an example of the bacteria for example. So, if you take a bacteria, what happened is that the bacteria is having the chromosome right or the nuclear content right. So, what happened is that when it actually requiring the nutrition from outside, so it is taking the nutrition right, it is taking up the nutrition and then enlarging into the site.

So, it is synthesizing the lipid molecule, it is synthesizing the protein molecules, it is synthesizing the nucleic acids and all that and as a result what is happening is that it is actually going to increase the size right, it is actually going to increase the cell size of the cell and the nuclear DNA also right. When it goes to beyond a certain limit okay, then what happened is that it is actually going to make another copy of the DNA and as a result what will happen is that it is going to have the two copy of the DNA right and once it has the two copy of the DNA, it is actually going to be divided from the center right and that is how it is actually going to have the two different bacteria right and with its own nuclear DNA, its own chromosome right and that is how the bacteria is going to grow from one bacteria to another bacteria and this kind of division is called as binary division right. And binary division could be equal division or it could be unequal division, apart from the binary you can also have other kinds of divisions such as fragmentations and other things of that right but binary division is the most popular division what is happening into the prokaryotic system. Now, here when you are doing the binary division, you are simply cutting the bacteria from the middle and so that both the cells will have the some amount of cytosol and some amount of the nuclear content and ultimately these two are also going to take up the nutrition and then they also will grow right. But since they do not have the organelles, they do not have the membrane bond organelles, it is easy because you do not have to distribute the organelles also.

For example, if it is a eukaryotic cell, you also have to ensure that when you are going to do the cell division or when you are going to divide the cell and produce the two cells, you should have the equal distribution or nearly equal distribution of the mitochondria, you also should have the distribution of the endoplasmic gut equilibrium, you should also have the distribution of the Golgi bodies and so on. So that the both the cells should be sufficient enough or independent in terms of the you know running its metabolisms. And that is very difficult and that requires a precise mechanism so that you can be able to you know do the cell division and that is why the cell division in the eukaryotic cell is well planned and well organized in such a way that you are actually going to have a sequence of events so that you can be able to perform the cell division. And these sequence of events are called cell cycles. So every eukaryotic cell is actually going to go through with these cell cycle stages and then only it actually can be able to you know

increase its number.

So it can actually be able to divide and give you the more numbers. So the cell cycle when you talk about the cell cycle, the cell cycle has the precise different phases, you have the interface, you have the G1, S, G2 and M phases. So the eukaryotic cell undergoes the precise cell cycle and division to produce the two daughter cells. Cell cycle is the series of tightly regulated events leading to the division and duplication. It is a vital process used to single cell called fertilizer egg is developed into the full organisms.

Several division is the crucial event underlining the regeneration and repair in the tissue, liver and heart. In eukaryotic cell, the parent cell is divided simply by the division in the two halves through the process of binary fusion. Whereas in the case of eukaryotic cell, the cell cycle has three important phases, the interface, the mitotic phase and the cytokinesis. So in the interface, you are going to have the synthesis of the genomic content and the tetraplasm. Whereas you can in the mitotic phase, you are going to have the division of DNA into the two halves and the cytokinesis, it is actually going to have the division of the cell.

This means in a cell cycle, you are going to have the interface. So this interface is actually going to prepare the cell for the cell division. And you can also have the S phase where you are actually going to have the synthesis of the DNA and the G1 and G2 phase are actually the and then you also going to have the mitotic phase which is actually going to divide the DNA into the daughter cells and then it also going to followed by the cytokinesis which actually going to divide the cell into the two cells. So let us start discussing about these different phases. So first is the interface.

So interface is preparatory phase required to perform the requisite steps. These are the series of events in the nucleus and as well as in the cytosol of the daughter cell to enable it to enter into the division phase. Division phase, this phase has several phases, there are as follows. So within the interface, you are going to have the G1 phase. So it is also known as the growth phase, it starts from the end of the mitosis and until the beginning of the S phase.

So this is the G1 phase. It starts from the mitotic and it goes up to the end of the beginning of the S phase. During this phase, the cellular proteins, enzymes are synthesized. So during this phase, you are actually going to have the synthesis of the different types of biological machinery, right, you require the DNA polymerase, you require the actin protein, you require the plasma membrane, all those kinds of things are actually going to be synthesized within the G phase. So G1 phase is actually where there

will be a synthesis of cytosol so that it is actually going to help for the cell to enter into the S phase.

Most of these enzymes are required for DNA synthesis in the S phase. Duration of the G1 phase depends on the cell type within the organism. G1 phase is under the control of the p53 gene products, right. So you know the p53 is a transcription factor which actually regulates the cell cycle and the cell division. So the G1 phase is under the control of p53.

And G1 phase, the length of the G1 phase depends on the availability of the different raw material what is required for the synthesis of the enzyme. It also be regulated or it is also being linked to the genetics of that particular cell. So if the cell is very slow growing cell, then the G1 phase probably could be very large because it requires a large quantity of the enzymes and other kind of things. Whereas the fast growing cells, the G1 phase could be short because it has to divide very rapidly. And in that case, the G1 and G2 phase could be very, very small, because it actually is full of nutrition.

So it actually can prepare the G1 and G2 phase very quickly. Then we enter into the S phase. So the S phase is the one cells grow and all factor nucleotides available. It starts the DNA synthesis during the S phase. At the end of this all chromosome present in the nucleus is replicated and the DNA content get doubled.

No change in ploidy. The synthesis of DNA occurs very fast to avoid the exposure of newly synthesized DNA to the mutation. So in the S phase, you are actually going to have the DNA synthesis. DNA synthesis from the pre-existing copy. This means if you have the parent DNA, if you have a parent DNA, that parent DNA will enter and will give you the two DNA strands.

One is parent DNA. One is the original DNA or the parent DNA and the other one is going to be the daughter DNA. Right. And you know that the DNA is, the application is semi-conserved in nature. So the parent DNA is also going to have the new copy and the daughter DNA is also going to be the new copy. This means both of these DNA strands are going to be identical in nature, which means the different DNA, it will be going to have one strand from the parent DNA and the other strand from the newly synthesized DNA.

Whereas in the daughter cell also you are going to have the one strand from the parent DNA and the other strand is going to be from the newly synthesized DNA. And that is how the DNA synthesis is going to occur during the S phase. And at the end of the S phase, the amount of DNA is going to be the 2x. It is not going to be in change the

priority of the cell. It is actually just increasing the DNA content of that particular cell.

So earlier when it was in the GPEN phase, it is actually going to have the X amount of DNA. Whereas in the S phase, at the end of the S phase, it is actually going to have the 2x amount of DNA. Then it enters into the G2 phase. So G2 phase is also the preparatory phase, the growth phase between the DNA synthesis and the mitosis. During this phase, the cells grow and synthesize the protein and cellular machinery required for the mitosis and the cytokinesis.

This means it is actually going to produce large different types of the cyclin proteins and other kinds of protein which is spindle proteins, tubulins and all those kind of things so that they will help into the mitosis and as well as during the cytokinesis. Apart from these two phase, these C phases, we also have a phase which is called as G0 phase. So G0 is actually a non-dividing phase. So it is a non-dividing phase. So after the G1 phase, the quiescent, quiescent means the cell which are non-dividing cells, senescence which means the cell which are very sick or they do not want to divide and non-proliferating multicellular eukaryotic cell enters into the G0 phase because they do not want to divide.

For example, the neural cells, right? And so the cells of the brain, the first cells of the spinal cord, the cells of the neural system, they do not divide because they are terminally being programmed like that. So they will be always remain into the G0 phase and they will never enter into the any of these cell cycle stages. So cells remain in this phase for long period of or the indefinite period as in the case of the neuron cells. It is also common in fully differentiated cells. The fast growing cells never enter into the G0 phase and hence it is not a regular cell cycle phase and the cells are undergoing into the specific conditions they will undergo into the G0 phase.

For example, the RBC, RBC is under the G0 phase because it does not decide, because RBC does not have the nucleus. So it has actually a specific case where a particular type of cell does not have a nucleus and that is why it cannot divide. So it will actually into the G0 phase. Apart from that, the neural cells are also non-dividing cells. So they still have the nucleus, but since they do not get any kind of stimulus from the external so that they can actually be able to decide to divide actually.

So after the G2 phase, you are actually going to have the mitotic phase and then you also going to have the cytokinesis. So mitotic phase, whether it is, so mitotic phase can be divided into two different types of phases. It can be mitosis or the meiosis. So mitosis or the M phase after the G2 phase, the cell enters into the mitosis or the M phase to divide the DNA equally between the two daughter cells. Each mitosis has the four distinct states to precisely divide the DNA content of the cell.

So the purpose of the mitotic phase is that it wants to divide the DNA precisely between the two daughter cells. So it actually can divide in such a way that the both of these cells are actually going to get the equal amount of DNA. So the mitotic phase is also being divided into the four different phases. First phase is called prophase. So during this phase, the nuclear membrane is dissolved and the chromatin condense into the chromosome.

The nucleus nucleolus in the nucleus disappear. In the beginning, each cell has two centromere, each cell has one centromere, which replicates along with the DNA to give rise a pair of centrosome to coordinate downstream events. Each centrosome has microtubule to form the spindle and assist in the distribution of the nuclear content during the mitosis. Centrioles are considered to organize a microtubule assembly, but they are not essential. So this is what you are going to see here is the different types of phases within the during the mitosis.

So you what you see here is the mitosis. This is the prophase, then metaphase, then anaphase and telophase. During the telophase, both the chromosomes, both the DNA strands are going to be separated and that is how it is, then you are going to have the cytokinesis. So then it is actually going to divide and get you. And what you see here is the, you know, the different types of cells. And this is a typical pattern what you are going to see because some of the cells are under the M phase, some cells are under the prophase, metaphase, anaphase and all that.

So this anyway, we are going to discuss in detail when we are going to talk about the experimental top setup. So after the prophase, you are going to have the metaphase. So in the metaphase, so once you have, you know, loosened nucleus, so during the prophase, what you have done is your first thing what you have done is you have removed the nuclear membrane, so that the DNA what is been synthesized and the two copies of the DNA what is present inside the nuclear is now free for distribution, right. And then you are actually going to, you know, pick up the DNA, right. So you're going to divide the chromosome into the two parts.

So in this phase, the two centromere start pulling the chromosome using the attached centromere towards the end of the cell, which means what happened is that you are actually first going to dissolve the nuclear membrane, so that the magnetic content what is present inside this is actually going to be freely be accessible by the cellular machinery. So what happened, this is what you're going to happen during the prophase. In the beta phase, what will happen is that the chromosome is actually going to be attached and it starts pulling the chromosomes, right. So you have two copies of the

chromosome of the same chromosome and it is going to be start pulling on to the end of the cell, right.

So imagine that you have this as a cell, right. So one end will enter into this side, the other DNA will enter into this side. And that will happen with not with the one chromosome, but it will happen with all the chromosomes, right, all the chromosomes will be going to fold into the two poles of the cell. So as a result, what will happen is that the chromosomes are aligned along the metaphase plate or the equatorial plane. Since the pulling power of both the centrosome is almost equal, it eventually arranged the chromosome on the metaphase plate. So this is what's going to happen, it is actually going to arrange all the things on to the metaphase plate.

The alignment of the chromosome along with the metaphase plate is crucial event to decide the entry of cell into the another phase which is called as the anaphase. The signal required for this control is created by the mitotic spindle checkpoints and all these checkpoints are being controlled by the cycle independent cell cycle proteins. Then we have the anaphase. So the protein attached to each chromatids are cleaved and the sister chromatids are separated as the daughter chromosome. So chromosomes linked on the metaphase plates are pulled by the microtubule and move towards their respective centrosome.

Although the exact mechanism of generating the force required for the centrosome movement is unknown, but it is suggested that the rapid assembly and breakdown of microtubule may provide the force for this movement. At the end of this says that chromosomes are being prepared for the distribution between the two different types of cells. And then we have the telophase. So in the telophase, in this phase, the daughter chromosomes moved and attached to the opposite end of the cell, right.

So this is what this is the telophase. The nuclear membrane forms around each set of the separated chromosome daughter chromosomes and the nucleothorolus reappears. In this event, the several processes during the prophase are reversed to give the two daughter nuclei. So this is what going to happen. After this, you are going to have the cytokinesis.

Cytokinesis means the division of the cellular content. At the end of the telophase, the mitosis is over, but the cell division requires the distribution of the cellular content equally between the daughter cell. In the animal cell, a cleavage photo is formed between the along the metaphase plate and divide an individual nuclei as the separate cell. During this process, it is ensured that besides nuclei, all other cellular organelles should be distributed equally between the daughter cell. Whereas in the plant cell, the

cell plate is formed and divide the cellular content between the daughter cells. So cytokinesis is a very, very important step or very, very important events which actually going to divide the cellular content, such as mitochondria, endoplasmic reticulum, Golgi bodies, centrosomes, paraxesomes and all those kinds of things.

So that both of the components are actually going to be equally competent or equally independent so that they can be able to run their own metabolisms. Now, so these are the four different stages what are present into the cell cycle. You have the we started with the G1, then the cell are actually going to prepare for the cell division, cell is going to be prepared for the DNA synthesis during the G1 phase. So there will be a synthesis of DNA polymerase, nucleotides and all that kind of. Once that is ready, the cell will enter into the S phase and within the S phase, it is actually going to do the replication.

And once the cell has replication, it is actually going to receive the X amount of DNA from the G1 phase. And that is actually going to be get converted into 2X amount of DNA during the S phase. That 2X will enter and go to the G2 phase and then it will enter into the mitosis. So during the G2 phase, it is actually going to synthesize the machinery what is required for performing the M phase or mitosis. And in the mitosis, then it is actually going to divide the nucleus and it was actually going to distribute between the mother cell and as well as the daughter cell.

It means the amount the DNA content is again going to be 1X at the end of the mitosis. And then there will be a cytokinesis so that it is actually going to produce the daughter cell and it is also going to have the mother cell which will enter into the cell cycle. So if I want to study this particular type of phenomena during the using the some of the analytical tools, what we require is we require a machinery so that it can be able to differentiate the cellular content. So you know that from this to this, the cell will actually grow.

So it actually going to change the size also. And it is also going to change the DNA content. So we require a machinery so which can actually be able to monitor the size and it also can monitor the DNA content of the cell. And that is how you can be able to, you know, identify the different types of cells what are present in the G1 phase, S phase, G2 phase and M phase. And within the M phase also you can actually be able to decide which cells are under the prophase, metaphase, anaphase or telophase. So one of the classical technique what you can actually be able to do for studying the size and the DNA content is called as the flow cytometry.

And the flow cytometry is a very, very robust tool to study the different types of cellular properties. So before getting into the detail of how you can be able to study the cell cycle

using the flow cytometry and how you can be what are the different types of protocol what you have to follow, I would like to show you some of the basics of the flow cytometry. So flow cytometry is a very robust tool which actually studies the different types of activities. So it actually can measure the density, size, it can also be able to tell you the sector what are present in the cell surface and it also can be able to, you know, differentiate the cell based on the metabolic reaction. How it actually happens? It happens because it has a cell analyzer and cell analyzer is actually streamlining all the cells into a small chamber and once the cell will exit out of this chamber, it is actually going to be illuminated by a laser.

So once it is going to be illuminated by the laser based on the size or based on the all these properties like the cell surface receptors or the metabolic reaction, it is actually going to give to the signal to the different types of detectors. So it can actually give you the size to the detector for measuring the size, it can actually give the signal to the detector for measuring the density or it actually can give the signal to the different types of fluorescence signal. And accordingly, you can be able to know which cell has the B cell receptors or which cell has a T cell receptor and so on. And based on these kind of informations, you can actually ask the machine and it can actually be able to collect these cells which are would be having the desirable features. So to do this job, we have a very detailed instrumentation part where you are actually going to have the different types of components and the cells are actually going to flow into a chamber or into a flow and once they reaches into the center and it is actually going to be illuminated by a laser.

And once it is going to illuminate by the laser, the cell is actually going to reflect the signal and it is actually going to be captured by the different types of the photomultipliers and these photomultipliers can give you the signal for the different types of properties. So it can be give you the information about the size, density and all other kinds of things. So it actually has the 3 main components, you can have the flow system which is called fluidics, you can all have the optical system and you can also have the electronic system which means whatever the signal you are going to get, it is actually going to process the signal and it will give you the readable signal. So as far as the flow system is concerned, it is going to have the flow cell and that flow cell is going to have the central core which is sample is going to injected and then also going to have the outer sheet membrane and it is going to have that and because of the hydrodynamic focusing, it is actually going to focus the cell in such a way that they will be actually going to travel in a single stream and that is how the single cell is actually going to be illuminated by the lasers present at the end of the tube. As far as the optical system is concerned, the light source used in the flow cytometer could be either the laser beam or it can also have the arc beam.

You can also have the organ lasers which are actually going to give you the 488 nanometers wavelength. Although in modern flow cytometers can actually be able to have the different types of lasers, can have the 488 lasers, can have 280 lasers and all that. So this is actually making the things more robust so that you can be able to collect more information about the cell. Then we also have the different types of detectors. So you can have the detectors for the forward scattering, you can also have the detectors for the cell size, reflective index and so on.

Then you also have the detectors for the side scatters and then you also have the intensity and whatever the intensity you are going to get, this is going to be the signal for the cytosolic content of the structure. And then you also have the electronic system. So electronic setup is going to be convert the information or the photons to the photoelectrons and these measure the amplitude area and the width of the photoelectron pulse and it amplifies the pulse either linearly or the logarithmally and then it supplies the amplified pulse. Then we also have the different types of plots what you are going to show into the flow cytometry. You can have the single color histogram, you can have the two color dot plot, you can have two color contour plot or you can also have the density plot.

And in the data acquisition when you are actually going to prepare a sample for the flow cytometry, what you are going to do is you are first going to do a you know trial run or test run actually right. And using the test run you can be able to set up the voltage, you can be able to set up the gain in such a way that it should actually you know your untreated sample should be in the center of the graph okay. And so that you can be able to monitor the moment in the left or the right directions right. Apart from that you can also do the gating right and gating is actually nothing but the selection of a subset okay.

Selection of a subset. So selection of only a certain population of cell for analysis on a plot right and it allows the ability to look at the parameters specific to only to the subset which means suppose I am doing the you know the whole blood right, I am doing a whole blood analysis right and I have collected this as like FL1 versus or suppose site scatter versus FSC right. So I am going to get this right and I am not interested in all these, I am only interested in macrophages. So if I go with this particular type of gate, it is only going to give you the information about the macrophages or lymphocytes or lymphocytes. So all these different types of cell type can be collected and then you only get the information about that. Although you are doing the complete proved sample, you are doing the blood analysis, but it only going to give you the information about the macrophage or the lymphocytes.

And you can actually have the different types of gates, you can have the rectangular

gate, you can have elliptical gate, you can have polygonals, quadrants, histogram and so on. So to explain you these things in more in detail with a you know with an instrument, we have prepared a small demo clip where the students are actually going to explain you all these steps in more in detail. Hello everyone, in this video we will be discussing about flow cytometry, its equipment and the software related to it. Flow cytometry is a basic technique in which the chemical or physical characteristics of a cell or a population of cells are determined by the instrument.

In this process, the cells are suspended in a fluid, mostly saline, 0.9% saline and is passed through a beam of light and then the physical and chemical properties are recorded. Coming to the equipment, this is a standard flow cytometer equipment manufactured by BD Biosensors, BD Fax Caliber. So basically there are 3 parts in this machine, the fluidic chamber, the sample induction port and the fluidic panel. In the fluidic drawer, there are basically 2 tanks.

This tank is used is known as the sheet fluid tank and this is a wastage reservoir. In this one, we have to pour 0.9% saline which is used as the sheet fluid and this is which passes to the sample and then on to the detector. Coming to the sample induction port, in sample induction port only certain types of tubes are known, are used which are known as the Fax tubes. In this sample injection port, we have to change the sample like this. Coming to the fluidic panel, as you can see there are 6 buttons, the low, medium, high, run, standby and prime.

We have to always remember that whenever we are changing the sample, the machine should always be on standby and when we are acquiring the data, it should be on run. The low, medium and high buttons represent the speed or the speed with which the machine sucks the sample. For low, the machine sucks the sample at 12 microliters per minute, for medium at around 35 microliters per minute and for high around 60 microliters per minute. This prime button is used when for example, if the sample injection port is stuck with air bubbles or is stuck with like samples like which are having doublets or debris. So, in order to analyze the data of the Fax, we use the software known as the CellQuest Pro.

We can find its icon in the toolbar as shown in the window. After we open the CellQuest Pro software, the first thing we need to do is acquire and then connect it to cytometer. After connecting it to cytometer, two windows pops up, the acquisition control and the browser and title. In the browser and title, we can save the data as well as the data file. This acquisition control is used to acquire the data and set the setup button. When the setup button is on low, we can try and error the data in this main panel and when we remove the setup and acquire the data, only then the data will be saved.

One more thing we have to remember is after connecting to the cytometer, we should make sure that in the instrument, the tank is pressurized and it should not be depressurized. So, when the tank is pressurized, only then the sheet fluid will flow through the instrument. Then coming to the things required for acquiring the data, we need a parameter description, we need counters, we need detector and amps, we need status. So, coming to the detector and amps, as we can see that there are multiple detectors like FSC, SSC, FL1, FL2, FL3 and FL4.

The FSC and SSC are related to the forward scattering and the side scattering. The forward scattering tells us about the cell size, like how large the cell is and the side scattering tells us the complexity or the granularity of the cell. The FL1 channel is for the green fluorescence, FL2 is for red fluorescence, FL3 is for red or orange and FL4 is for red fluorescence. As we can see in the voltage, we can see that there are 5 voltage gates like E00, E01, E02, E03 and E-1. For cancer cells, we mostly use E-1 because the cell size is large and as the cell size decreases, the E01, E02 increases. For example, the E01, E02, E03, these are used for smaller cells such as RBCs or microbes or macrophages.

We can control the voltage using this tool bar. I will show you how to do that when we acquire the data. In the status panel, we can see that the status is showing standby, which means that we are not yet acquiring the data and the system is on standby. In the counters, we can see that the total events per second is 0 and the events per second is 0 because when we start, it will change. Coming to the plots, the most basic plot in FACTS is the dot plot.

No data is valid without the dot plot. As soon as we plot the analysis dot plot, the inspector dot plot window pops up. In this, we have to click at the corner of the analysis dot plot and then in the plot type, we have to do acquisition and analysis. And then we can change the X parameter or the Y parameter using one of these options. For this demo, we are just showing how to acquire the data using a cancer cell line.

Now, I will show how to acquire the data. Before acquiring the data, we should make sure that how many number of events we want to record before stopping. The default is set to 10,000. So we will reduce it to 5,000 just in case just to see the data how it is going and then click okay. And after that, on the fluidics panel, we have to press run in order for the machine to set the sample from the FACTS tube.

As soon as we press the run button, we should press acquire and then only we can see the data. As you can see that, we can see that there are multiple cells being shown here

in the near the 00 and they are showing away from the access as well. So we can change the direction of the flow using this according to our requirement in order to set the population. So let's say that I want to stop the data after 5,000 events. So before that, we need to save the data. So let's say that we are going to save the data in this file and then the data file can be written as, then click okay.

And if we remove from the setup, then we can actually save the data. As we can see here, the total events are being recorded like till now 500, 600 events are recorded and the events per second are 75. As we have seen on the fluidics panel that there are three buttons, the low, medium and high. This actually decide how many events are being recorded per second. For example, if we press the low, then the events per second will be recorded low because we are actually taking low amount of sample from the fax tube.

And if you press high, the number will increase. It also depends on the concentration of the sample. So mostly if the concentration is high, we should opt the low button in the fluidics panel so that the complete number of events will, so that the instrument can record complete number of events in a right manner. Now as we can see that as we have sent the total events to 5,000, after 5,000, the data looks like this. So the machine stops recording the data after 5,000 events because we have set to that. So after that, if you want, like this one, let's say that if this was the untreated and we have acquired the data of the untreated at a particular voltage and amp gain in order to acquire the data of the treated samples, we should not change these parameters or else we will not know what is actually difference between the untreated and the treated samples.

So after this, let's say that we have completed the experiment. So after this, we need to watch the system so that the next person or when we use the next time, it will be easy for us to operate. So for that, we have to clean the system with 2% sodium hypochlorite solution and we have to remove from the setup so that it does not save the data. Now I have changed the sample from the sample to the 2% sodium hypochlorite. As we can see that some of the cells will still be there in the sample induction port and that needs to be cleared.

So for that purpose, we are washing the system with 2% sodium hypochlorite at high pressure. We have to keep washing the system till the events per second remain 14 or 15 for a longer time. So while handling the FACTS instrument, there are some precautions to be taken. For example, when we change the sample in the sample injection port, we should always make sure that the system is on the standby mode and also when we are done analyzing the data, the system should always be in the standby mode and after using the instrument, we should always depressurize the sheet fluid tank because if it is not depressurized, it may actually harm the system. Now we are going to see how to

process the raw data which we have just acquired in the FCS Express Data software.

So far we have seen how to acquire the data in the FACTS equipment. After we acquire the data in the FACTS equipment, we have to process the data in the software known as the FCS Express 5 flow software. So to begin with, after we open the software, we open the new layout. After we open the new layout, a window appears in which variable options are there such as home, insert, gating, batch, format, text, data, multi-cycle view. So in the home tab, we can see that we can take a new page according to our needs like blank title according to our requirements and also we can check the layout. We can also take the layout which is best suitable for our plots and we can also change the size of the plot with respect to our requirement and if you want to change the orientation, we can use portrait or landscape.

And also in the insert, we can use whichever data, whichever plot which we want to use, for example histogram or multi-cycle DNA or proliferation or dot, density, color, contour, scatter, etc. So the most basic plot is the dot plot which is essential to every facts data. So after that, we can also do gating. So gating is a mechanism in which we can segregate two or more different populations from a given population of cells.

For example, if you want to segregate the debris from the singlet and the doublet cells, we can use gating. So gating can be of different types like ellipse, rectangle, polygon free form. And also we can do the quadrant analysis. For example, in acridine orange, propidium iodide or annexin 5 propidium iodide staining for the live and dead cells, we can use quadrant analysis in which the first quadrant shows the live cells and so on. In the batch, in the format, we can select which format which we want to use.

Anyhow, all the options will be enabled once we load the data. So let's load the data and see how it looks. So after we input the file into the software, a window appears in which we can use different types of plots such as dot, density, color, contour, etc. So the dot, the density, color dot, contour, these all and the scatter plot, these all are the same plots but in a different way of representation. So let's see how does that look. So for example, I have taken an untreated sample obtained on a FL2A channel.

So FL2A channel is responsible for red fluorescence. So we can see that this is the multi-cycle DNA plot and this is the histogram plot as we have taken it on the FL2 channel. So we will switch to FL2A and this is the scatter plot. This is the density, the contour plot. This is a color plot and this is a density plot and the final one is the color plot.

So the first one is the FSC versus SSC. This is a dot plot. This is a basic dot plot which

represents how the data looks. For example, as the population of the cells goes away from the 00 mark, we can say that the granularity as well as the size of the cells increase. So if you want to exclude that from the data, we can exclude that using gating option. Also as we can see that the second plot which is the density plot, it is almost as same as the dot plot but with respect to the density. For example, the blue color in the middle represents a denser population and as it goes outward, the population of the cells decrease.

So the same dot plot can be represented in a contour plot as shown in the third plot and the fourth plot is the histogram in which we can see the difference, the variable intensities for the different cell cycle phases and the fifth plot is the DNA cell cycle plots in which we can see different phases of cell cycle like G1 as G2. The scatter plot is just another representation of the dot plot in which the events are just highlighted in a respectable way. And also the last plot is a color plot. Actually in a color plot, we can see two different populations assigned with a different color but because we have used only one dye, so only one color is visible. So in this way, we can check the different plots and also if you want to see the statistics and also the details of the plot, we just have to click right and then press the format option and we can compensate the data, we can cut, copy, paste.

And in the format option, we can actually do a variable number of things like changing the border color, overlaying the data, the dot options, the size, the background, the axis. We can do a whole lot of options which will be helpful for us in order to present the data in a more appropriate manner. So this is all with respect to the FCS-CFI Express software.

Hopefully, this was helpful for everyone. So with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to study or we are going to discuss some more aspects related to the biological system. Thank you.