Molecular Biology
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Module - 01
Basics of Biological system (Part 1)
Lecture-04 Cellular Fractionation (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 cellular structures of the prokaryotic cell and the eukaryotic cell. And in the previous two, three lectures, we have focused more on to the structure and as well as function of the different parts, what is present in these type of cells. So when we were discussing about the prokaryotic cell, we have discussed about the plasma membrane, we have discussed about the cell membrane, we have discussed about the plasmids and we have also discussed how you can be able to isolate the plasmids and how the plasmids can actually be able to exist in three different forms. And subsequent to that, we have also discussed about the eukaryotic cell.

When we were talking about the eukaryotic cell, we have discussed about the plant cell and as well as the animal cell, we have discussed about the differences between the plant cell and the animal cell. And we have also discussed about the different types of membrane-bound organelles what are present in the eukaryotic cell. So we discuss about the nucleus, we discuss about the mitochondria, chloroplasts, glycosomes, Golgi bodies, endoplasmic reticulums. And at the end, we have also discussed about the plasma membranes.

And while we were discussing about these cell organelles, we were also discussing about their structures, functions, and what is their contribution in regulating the different events within the cell. So in today's lecture, we are going to discuss about how you can be able to study these individual organelles. So although the membrane-bound organelles are not present within the hyperkaryotic system, we can still be able to isolate the different fractions from the prokaryotic cell and we can be able to study those fractions. So let's start today's lecture. So before getting into the detail of these aspects where we are actually going to study how you can be able to fractionate the cells, how you can be able to isolate a particular fraction, let's understand that we have the prokaryotic cell, we have the eukaryotic cell.

And within the eukaryotic cell, you can be have the unicellular eukaryotic cell such as yeast or you can have the multicellular prokaryotic eukaryotic cells such as animal cell, plant cell or the fungi. All these cells are very different from each other. And that's why

the first question comes if you want to isolate a particular fraction of these cells, how you can be able to grow these cells. And if you want to grow these cells, you are actually going to provide them the necessarily nutrition. So let's see what are the different nutrients, what is available for these cells.

So when we talk about the growth medium, so growth medium of a host organism is required to produce the four major basic biomolecules. One is protein, second is carbohydrate, third is lipid and the fourth is the DNA or the RNA. Now when we want to synthesize the proteins in organisms, the proteins are made up of the amino acids and these amino acids are actually going to be made up of the five different types of atoms such as carbon, hydrogen, oxygen, nitrogen and sulfur. In comparison to that, the carbohydrates, carbohydrates are commonly known as sugar. They are made up of the three different types of atoms, carbon, hydrogen and oxygen.

Then lipids, lipids are made up of the fatty acids. So lipids is a derivative of the fatty acids and the fatty acids are made up of the different types of atoms such as carbon, hydrogen, oxygen, phosphorus and sulfur. DNA and RNA mostly are made up of the nucleotides and these nucleotides are made up of the carbon, hydrogen, oxygen, nitrogen and phosphorus. The role of these biomolecules are different. For example, the protein is a building block.

So it actually requires for making the different types of structures. I am sure you might have seen the different types of proteins what are present in the different parts of the prokaryotic as well as the eukaryotic cell. Remember that the different types of receptors what are present onto the plasma membrane or different types of protein complexes are present in the mitochondria for ATP synthesis and the kinds of things. So those are the building blocks. Whereas the carbohydrates and lipids are mostly being used as a source to provide the energy.

Energy in terms of the burning of these molecules so that you can be able to produce the ATP and then this ATP would be you know the going to be the molecule which going to be supplied the or which can actually going to carry the high energy bonds and that actually going to carry the energy from one part to the other part. Lipids are also been mainly been used for the energy. So if suppose our organism want to move from one part of the dish to the another part of the dish or one place to another place, it actually requires a nutrition energy for the locomotion and the energy comes from the mainly from the carbohydrates and the lipids. Similarly we have we require the DNA and RNA. So for DNA, RNA is always been a part of the genomic content of the organisms either it can be DNA or RNA.

So anyway we are going to discuss in detail about the genomes and that time it will be clear in what are the organisms where you are actually going to have the RNA as the organisms. So apart from these three molecules so actually you require a nutrient source which actually can be able to provide you the carbon, hydrogen, oxygen, nitrogen and sulfur and as well as the phosphorus. So if you take a nutrient media or if you require a growth media which can actually be able to supply you the carbon, hydrogen, oxygen, nitrogen and sulfur and if that is actually going to you know so that is good enough for a cell to assimilate the different types of building blocks such as amino acids, sugar, fatty acids or nucleotides and ultimately they are actually be able to generate the biomolecules such as protein, carbohydrates, lipids and DNA and RNA and at the end they are actually going to use these molecules for running their metabolism, running their different types of activities and that's how they are actually going to use this for their growth. Apart from these two these major molecules you also require the minor quantity of the minerals or the vitamins. So considering these you can actually be able to design the different types of growth medium which is going to be used by the host cell or by the prokaryotic or the eukaryotic cell for their growth as well as the deployment.

So these are the different types of atoms what is required to be provided by the growth medium and considering the requirement of these atoms you can actually get these from the different types of biomolecules. So what are the different types of constituents which can be a part of the microbiology medium and mediums. So you can actually have the amino nitrogen which is actually going to be so amino nitrogen is going to be provided by the pectins protein hydrolysates in views and extracts then you require then you can actually have the growth factors. So growth factor can be provided by the blood, serum, e-sect or vitamin or NAD then you require the energy source that can be provided by the sugar, alcohol and carbohydrates and you also require the buffer because maintaining, you know, the pH of the media is also very important for the growth. So that can be done by the phosphate, acetate and citrates.

Then we can also require the mineral salts and metals. So like minerals, metals and cofactors. So that can be provided by these kind of metals. Then you also require the selective agents such as the chemicals or antimicrobials or dye. So that can be more relevant when you want to use these microorganisms for other kinds of molecular biology manipulations.

Then you require the indicator dye. So in some of the media, you also require the indicator dye so that you can be able to see whether there is a change in pH or not. So for that you can use the phenol red or neutral red. And then sometimes you also require the solid media. So solid media you require the agar or gelatin or silica gel.

So considering all these constituents or the source, you can be able to make the different types of microbiology media. So you can have the M9 minimal media, you can have the M63 minimal media, you can have the Lura Bertani and LB media, you can have the LB Linux media, you can have SOB media, YT media, Terephi broth media, Super broth and T pi g. And these are the composition what I have given and these are the bacterial species what you can actually use for cultivation. So you can actually use the M9 media for cultivation of the E.coli and other kind of E.

coli strains. Similarly you can use these kind of media for making the competent cells and all that. So all these terms are probably will be new to you but it will actually go and you will understand these when we are going to discuss about the different types of molecular biology aspects. And for example, this YT media, YT media is required to the production of phage productions and all that. So how the first question comes how you are actually going to prepare a microbiology media. So for preparing a microbiology media, what you require is you require constituents.

So for example, if I take an example, you can actually require the peptone, you can require the E-sexrad, you require the NaCl and so on. So for preparation of microbiology media, you dissolve the component in 1 litre of distilled water and then you cover the top of the flask with a cotton plug and autoclave the solution at 121 degree Celsius for 20 minutes. So that you are going to do in the autoclave. And what are the precautions while you are making the microbiology media. So ratio of media volume and culture flask that is very important for aeration.

So for example, if I am going to make the 250 ml medium, then it has to be prepared in 1 litre flask. So that you can actually have the enough aeration because when you are going to grow the bacteria, bacteria require the oxygen and you cannot open the cotton plugs. So bacteria require a certain amount of oxygen for gaseous exchange so that it can take the oxygen and it can actually be able to release the carbon dioxide and that is actually going to decide what will be the final growth of these particular bacteria. Then you require that the media components should be hygroscopic and while weighing avoid the moisture. So store in a cool and dry place.

So you can understand that if there will be a moisture then the amount of actual powder is going to be lower because you are actually getting the weight of the water as well. So that is actually going to make the media less nutritious for the bacteria. Then while autoclaving, open the autoclave only after it is cold because the water is boiling inside the autoclave and it is actually going to have the vapors. So you have to take those kind of precautions. Then you are actually going to avoid the charring of the media components.

So charring means you are actually going to, you should not burn the media component because if they got burnt, then for example, if the glucose got burnt, right. So glucose is actually going to be get converted into carbon. And this form of carbon is not suitable for any kind of biological system to assimilate, right. So that you cannot use. Then you also require the solid media should be poured in a plate once it is cold because if you pour the media which is very hot, then it is actually going to destroy the other kinds of additives.

For example, if you are adding the antibiotics or if you are adding the selection pressures and all that, then it is actually going to destroy or inactivate them. So the various antibiotic or nutrient supply should be added to the media when the temperature is less than 50 degrees Celsius. So this is all about a critical aspect, how you can be able to prepare the microbiology media. We have prepared a small demo clips to explain you how you can be able to prepare the microbiology media in the lab and how you can be able to do the autoclaving and all that. Hello everyone, in this video we are demonstrating how to prepare bacterial culture prep.

For preparing culture prep, we need three components. One is peptone, yeast extract and sodium chloride. For 100 ml of culture prep, we need 1 gram of peptone, 0.5 grams of yeast extract and 1 gram of sodium chloride. For 100 ml of peptone, we need 1 gram of sodium chloride.

After weighing, we have to clean the spatula and keep it in original position. And during weighing, care should be taken to avoid contact with any of these media components. After weighing the media components, we have to dissolve them in double distilled water. So initially we are dissolving in 80 ml of distilled water. Once the components completely dissolved, we have to make up the volume up to 100 ml.

While it is tilling, we have to prepare cotton plugs for the flask. For preparing cotton plugs, you have to take one thick layer of sheet of cotton. If you have two hands, fold like this. Once the media dissolution is complete, we have to pour into the flask.

We have to pour up to 100 ml. So we use only one third of the place. Remaining space is empty. This is used for aeration purpose and also ensure proper autoclaving. In order to check whether the components are autoclaved or not, the media is autoclaved or not, we use stability indicator.

This is paper based stability indicator. We have to paste on to the flask and we have to autoclave. If the autoclave is properly finished, then we will see the white strips turning into the black one. So this is the indication of the autoclave flask. Now the media

components are completely dissolved.

Now we have to pour into the flask. Once the media preparation is complete, we have to sterilize the media in order to use further applications. This is the typical autoclave where you can see temperature and pressure indicator and these are the pressure knobs and this one is quick pressure release knob. You can use when you are in a hurry, you have to use this one, but I will prefer not to use this one. Let it go on its way.

We have to turn on the autoclave. So you can see here the bulb is glowing. Before keeping the media components to autoclave, make sure that the heater inside the autoclave submerged with water. Now I am going to keep the media components in the basket which we use for the autoclaving. Then keep this one inside the autoclave. While closing the autoclave, make sure that you are closing in opposite direction.

Once the pressure and temperature reaches 121 degree Celsius and 15 kPa pressure, you have to hold on that point for 20 minutes. Then you have to turn off the machine, let it cool down and remove the components. The same procedure you have to while opening, you have to open in opposite direction. To conclude the video demonstration, we have discussed how to prepare bacterial culture media and how to prepare cotton bags and autoclave it. During culture weighing of the media, we have to make sure that the media component should not be exposed to air because those substances absorb the moisture and become liquefied.

So another thing is that for cotton block preparation, we have to take a single layer of cotton, then we have to fold it. After autoclaving, we should not release pressure in a single shot. Let it go and come to normal pressure, then we have to open autoclave. So with this, the video is over and thanks for watching. Now when we talk about the eukaryotic cells, right because you also require the studying the different fractions of the eukaryotic cell, the simplest eukaryotic cell is the yeast.

So you can actually be able to use the some of the yeast media. So you can actually be able to use the CSM media, you can use the YPD broth, you can use the YPGAL, you can use the standard minimal media, and you can use the yeast nitrogen based media and all that. And I have given the composition for one meter, what you can actually be able to use. And this is the application. So for example, the YPGAL media is the standard media for S-survey C for omitting the glucose repurposition and all that.

Similarly, YPD broth media is commonly been used for the yeast media for the maintenance and propagation of pichia pectores and S-survey C, right. So pichia pectores and S-survey C are the most common yeast which are being used in the

laboratories. And method of preparation is almost the same as per the media composition, the consequences are being added in the 900 ml of water and autoclave, then you allow the media to cool down and then you add the 50 ml of filter sterile 40% glucose so that the final concentration will become 2% and adjust the final volume to a 1 liter if necessary. So this is about the yeast media. Now, moving to the next media, which is the mammalian cell culture media, right.

So initially, we discussed about the basic media, right, which is going to be utilized by the unicellular eukaryotic cell. Now you can use the more specialized media which is going to be used for the by the more specialized mammalian cells, we can still, so for the mammalian cell culture media, which is you see this, this is a DMEM media, right. So this is the DMEM media, which is prepared and the composition is that you are going so DMEM constituents are actually very, very high. So you require large amount of amino acids, different types of sugar, different types of other kinds of so it when you are going to buy the DMEM media from the vendor, it is actually going to provide you the powder.

So you require the 13.4 grams of the powder for 1 meter, then you require the sodium bicarbonate which is 3.7 grams per liter. And then you also require the fetal bovine serum which is FBS so 10%, normally you're going to get the FBS of 10, 100%. And then you also require the antibiotics.

So you are actually going to use the 1%. So 100 from the 100x you are going to use a 1x. How you're going to prepare the cell culture media? To explain the method of media preparation, we are taking the example of DMEM media. So add 13.4 grams of dry powder media into the water and mix it to dissolve it completely.

Then you add the 3.7 grams of sodium bicarbonate, mix completely and adjust the pH to 6.9 to 7.1 using the one normal NAH or one normal HCL. Finally, you add the cell culture grade water to the media to bring it to the final volume, sterilize the media using a sterilized filter, merminate filter with a pore size of 0.

22 micron. Then you are actually going to add the supplements such as the antibiotic and serum can be added to the sterilized solution using the aseptic techniques within the biseptic cabinets. This is all about the theoretical explanations. We have also prepared a small demo clips to explain you how you can be able to prepare the media within the laboratory or within the biseptic cabinets. Hello everyone. My name is Bhaumadh Rafi, a research student at IIT, Guwahati in biosciences and bioengineering department.

In this video, we are going to demonstrate how to prepare cell culture media for mammalian cells. For preparing cell culture media, there is a step by step process. First

we need to weigh the components of the media and dissolve it in required amount of water. Then we need to set the pH using ES clip and then we need to filter the media using 0.

22 micron filter to make it aseptic. For further use, we can also adequate the media and store it in 4 degrees. In this video, we will be demonstrating how to prepare mammalian cell culture media. For that purpose, we need aerosol media which is DMEM, double cross modified, eagles media as to the high glucose. We need FBS, fetal bovine serum and we need antibiotic cocktail comprised of streptomycin and anti-cellulone. The vessel media provides inorganic materials and amino acids which are preferred for basic development of cell and the FBS is used for providing both factors to the cell.

We cannot autoclave this media because it might degrade the components of the media. For that purpose, we use 0.22 micron filters. This is a 250 ml bottle top filter.

After we have packed the filter, we have to keep it for autoclaving. We have to keep it for autoclaving. After adding media, we need to stir it on a magnetic stirrer for the components to dissolve completely. We can either use double distilled water or milli-q water but double distilled water is more preferable as it contains more ions than milli-q water. After the media components have dissolved completely, we need to set the pH of the media.

For that purpose, either we can use pH meter or pH strips. In this case, we cannot use pH meter as the bulk of the pH is sensitive to the media components and may get corroded. After the media components have dissolved completely, we will be able to set the pH of the media. We need to adjust the pH of the media. The bright red colour indicates that the pH of the media is in the range of 7.

2 to 7.4. If the colour of the media turns purple, then it indicates that the media is acidic. If the colour of the media turns yellow, then it indicates that the media has become acidic.

Now, we will be checking whether the media falls in the range of 7.2 to 7.4. After the media has been set, we now need to filter the media inside the biosafety cabinet as we have added the constituents in their non-eceptric condition. After the media components have been completely dissolved and the pH has been set, we now need to sterilize the media using membrane filter media. For that purpose, we use class 2 biosafety cabinets which are used for handling mammalian cell cultures. So, this is a typical biosafety cabinet in which we perform the filtration for media.

This is the control panel which is used to operate this machine. This is the on and off

switch. This is the switch for normal light. This is the switch for UV light. Now, we are going to filter the media. For that purpose, we need a suction pump which can be connected to the bottle top filter.

This suction pump is for the purpose of extracting the air from the bottle top filter so that it can be used for the purpose of extracting the air from the bottle top filter. After the media has been filtered, we now need to add a PS and antibody in order to make it 10 percent FPS containing serum. So, we have seen how to prepare cell culture media for mammalian cells. We have seen how to prepare the cell culture media for mammalian cells. So, while you are going to prepare the media, you also have to consider the different types of different types of precautions.

For example, the pH of the media. So, pH of the media should not be between 7 to 7.4. This is the physiological pH. It should not be above to this or it should not be below to this because that is actually going to adversely going to affect the growth and as well as the other kinds of features of the cells. Filtration should be performed at a very low speed so that you should not feel while you are filtering, you should not compromise the pore size of the membrane.

And if you are going to filter at a very high speed, you are actually going to generate the shear stress. And because of that shear stress, you are actually going to increase the pore size. You know that the pore size is pore size of these membranes are 0.22 micron. So if you are actually going to bring very high speed, the pore size may grow up and it becomes like 0.

25 micrometer or it could be even 0.4 micrometer depending on how fast you are growing. So if you become 0.4 micrometer, some of the bacteria which are lower to this value are actually going to enter into your media. Apart from that when you are spinning or when you are filtering at a very high speed, you are also going to take up the air and air is nothing but the which contains the microbiologies. It can contain the pathogenic bacteria.

And you are actually going to add the serum which is heated activated serum. Then you also require the antibiotics and you are actually going to check the contaminations. So when you prepare a media, what you are going to do is you are going to take a small dish and you are actually going to put the 10 ml media and put it into 37 degree Celsius for 2 days. And if that is nothing will grow, right. So then you can observe this plate and if this plate does not show any kind of bacterial growth, then you can imagine that your whatever the media you have prepared is perfect and it can actually be able to good to use for the mammalian cell culture. Apart from that you can also use the insect cell

culture media and this is the recipe what I have provided.

The media preparation method and everything is almost the same as what we have discussed. So this is all about the cell biology and how we can be able to use the different types of techniques for the cell fractionations. So what we have discussed so far, we have discussed about the cell propagations, we have discussed about how you can be able to prepare the different types of media. So we discussed about the microbiology media, we discussed about the yeast media, we discussed about the mammalian cell culture media and we have also discussed about the insect cell media. We have also shown you the couple of demo so that you can be able to prepare these media in your laboratory as well such as the osmotic lysis, thermolysis, sonications, mechanical methods and so on. And once you have broken the cells, you are actually going to get the different types of fractions whether it is a prokaryotic cell or the eukaryotic cell.

You can be able to use differential centrifugation and as well as the density gradient centrifugation to separate the different types of organelles and then you can utilize these fractions for your subsequent studies. For example, you can actually be able to study the mitochondria, you can be able to isolate the nucleus and do the different types of experiments. So with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the biological system. Thank you.