Probing the Cell

Transformation Lab Hints

These podcast episodes were made available by a generous grant from the State Chancellor's office under the SB-70 Quick Start Grant program. In a nutshell, this grant is going to allow us to bring science to students who are interested in doing science, specifically in the area of biotechnology. What you may not be aware of is that the United States is seriously hurting in science. We don't rank within the top 20 nations. California is 49th out of 50 states. 13% of students (00'30") entering 4 years schools graduate with a degree and less than 2% do a science degree on top of that. Simply put, a little bit of science can go a long way! Use this Biology 107 course as a gatekeeper course. It will introduce you to a variety of applications. Make sure that you focus on the material. These lab exercises are pertinent and relevant, and will help you to multitask and really work effectively in a real job setting. They can also help you prepare for quizzes and they have significant (1'00") lecture overlap. So make sure that you spend some time reviewing these podcasts. Texts for the podcasts are available on the biotechnology outreach site @ www.canyons.edu/host/biotechoutreach/

Remember that 107 (1'30") is a significant course. Try to make your mark in this course. If you do well in science, you will continue

to do well in other academic courses and ultimately get a career and options that are at your choosing. **Remember that science is good for you!**

This is the first in a two part series of lab in the lab entitled "Probing the Cell". The first one is actually entitled "Probing the Cell" and the second part of the lab which is on a second podcast (2'00") is called "Cell Membranes and Osmosis". Please make sure that you refer to both podcasts to get a clear picture of what is required in this lab. This aspect of the cell involves the fact that some molecular biology is relatively abstract. You rarely get to observe things directly. That is to say that we do not get to see the organelles themselves without the help of microscopes or we cannot often tell what an organelle is doing without the assistance of let say a chemical test or some other sort of sensing instrument. (2'30") This is unlike other branches of science when you can often observe the animal in the field or perhaps make direct measurement of some larger organism. This abstraction requires that we use a relatively broad range of tools in order to look at cellular processes. Although there is quite a range of them, a few of them dominate.

First off, we use microscopy which we will be using in this lab. We also use chemical analysis which allows us (3'00") to test for the presence or the absence of certain chemicals. We further can use

what is called the cell model which is something we will be looking at in the diffusion osmosis lab which is on the other podcast. Lastly, we can look at centrifugation. The idea here is that we can use a spinning sample to separate substances by exploiting centrifugal force in the fact that different things settle at different rates. For the purpose of this lab titled “Probing the Cell”, we will be using microscopy (3’30”) in combination with chemical analysis and centrifugation to isolate and characterized a handful of organelles.

The first thing you want to do is to take a few minutes to refresh your memory on some of the nature of cellular organelles and some of the big differences between plants and animals, particularly the fact that plant cells have a cell wall, central vacuole, and chloroplast.

In addition to this, we are going to be looking at a pea cell. What we are going to be doing is that we are going to break open a pea cell, isolating, and investigating the different organelles inside. (4’00”) Although there are a range of organelles inside a pea, five of them are commonly seen in an exercise like this. The reason that they are commonly seen is because they are large enough, abundant enough, or can be visualized using technology readily available in the lab. We can sometimes see a thin transparent nucleus. We can often see the cell wall which appears as a regular hexagon almost honey comb like appearance underneath the scope. (4’30”) We can also see

plastids. Plastids are specialized organelles that are common in seeds, which contain large amounts of starch. We further can see mitochondria and chloroplasts which can also be tested for using simple chemical processes or identified under the microscope.

What we will be doing then is that we are basically going to spin the samples after breaking open the cells. Upon spinning them, you will get two fractions. Fraction will either be the pellet which is the fraction that is settled to the bottom sometimes called the sediment and (5'00") the other portion called the supernatant. Upon each fraction (the pellet and the supernatant), we will do microscopic analysis, chemical tests, and in some cases we will combine the two. We will do a chemical test, then examine under the microscope to see what it is we have found. We will do this twice. We will spin the sample once and then we will spin the sample again at a higher RPM in order to see what other organelles we get out of the sample. Let me give you a couple (5'30") of hints on the theory of centrifugation then I will give you a couple of pieces of information on how to work the lab quickly.

Basically, centrifugation involves spinning samples (the sample will appear in the sediment or pellets according to a number of factors). First off is the revolutions per minute (RPM). The faster the sample is spinning, the more likely a particle will make it to the

bottom. Also the time. The longer the sample is spinning, the more likely a particle will make it to the bottom. Lastly, there are factors such as particles size and density (6'00") which all have affects. Obviously, larger particles will make it down quicker than smaller particles. Denser particles will make it down faster than less dense particles. There are a variety of other derivative techniques. In fact, centrifugation is so sophisticated that it can be used to separate DNA strands from one another and even separating viral capsids (very, very small particles). That said then when you are working with the centrifuge; make sure that you always balance the centrifuge. It is very very important. (6'30") When you balance the centrifuge, if you put a sample on one side of the centrifuge, you put another sample on the other side that has equal weight. It is not enough just to put another vial on the other side. The other vial has to contain fluid which approximately equals the weight of the other. Remember that these centrifugation forces are extraordinary powerful and if the centrifuge is not balanced, the device can break sending shards of metal and other debris flying everywhere. As I said, it is a very dangerous device.

Basically (7'00"), what you are going to be doing then is that you are going to be examining the pea extract, or plastids, cell wall, chloroplasts, nucleus, and mitochondria looking at both the

supernatant and pellets from a variety of different spins. Make sure that you label everything clearly. Here are a couple of hints to make sure that everything moves smoothly. As I said, make sure that all samples are labeled. When you pour off the supernatant, do so steadily and slowly. Try not to disturb the pellet (7'30") below. When you need to get to the pellet, use a pipette; simply do a scrapping of the pallet and put a little on the slide or whatever is it that you need to use to further examine the sample. Remember that this is micros copy. So when you are looking at centrifugation samples or at the crude extract, you need very little, (a very small smear is all that is necessary). Also when you are looking for the samples under the microscope with respect to their iodine reactions, (8'00") (remember that iodine will react with the starch and it should give you a positive for the presence of plastid) these iodine particles only appear blue or darker if you have got the microscope light field right. So when you are working with the microscope and you see a lot of brown spheres, make sure that you have adjusted the light because chances are that those spheres are plastids and should stained a blue or even sometimes a darker color (black almost) if they are properly illuminated from below.

They are couple of other (8'00") quick hints regarding time management. Make sure that you delegate. If someone is doing the

microscopy work or setting up the chemical analysis, the other person could be getting the centrifuges set up. Make sure that you do not leave your centrifuges unattended. Also, clean as you go. This is a very messy lab. The sucrose solution that you use for buffering as well as a varitety of the other reagents makes for a very messy lab. So do not let any of the extra samples sit around (9'00") for too long because they become very sticky and hard to deal with. Then you will also notice than in your lab manual on page 7 is an excellent flow chart to help you understand the key steps, what is it that you used to look for what samples? Now again, remember that using microscopy, sample analysis, centrifugation to separate and identify the different substances. You will also be using two types of chemical analysis. As you will see, you want to know where the samples were? (9'30") Were they in the sediment, in the supernatant? Where they in the first spin, in the second spin, in the crude extract? So on and so forth. Once you have got page #7 panels completed, you should be able to figure out what are the relative sizes of the organelles. Now how do you think you are going to do that? Well, simply put, samples that appeared early on in sediment, chances are that they are larger. If they did not appear, chances are that they are smaller.

Remember that this is the first of a two part series of lab and if you have a few minutes, make sure that you go over to cell membrane

osmosis podcast so that you can get a complete picture of what that lab is all about.

This concludes our podcast episode for the day. If you would like to get more podcasts, they can be attained at www.canyons.edu/host/biotechoutreach/

If you would like specific information on a range (10'30") of programs in technical science, College of the Canyons leads the area in technical science training. If you want information on chemistry, you can contact Kathy Flynn, chemistry department chair, at (661) 362-3998 or reach her at kathy.flynn@canyons.edu

Information on our engineering program can be reached via David Martinez, engineering department chair (11'00"), at (661) 362-3007. His email is david.martinez@canyons.edu

Lastly, you can reach Jim Wolf, biology program director, at (661) 362-3092 and Jim's email is jim.wolf@canyons.edu

Remember to continue pursuing your career in biotechnology and to apply all of the things that you have learned because seriously, we need science students, seriously……