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 lectures on transformation. What we are going to focus on the first part is on the basics of the lab and how you accomplish it. The second session will talk about the process of (2'00") transformation using calcium chloride ($CaCl_2$). We will talk more about the theory of how $CaCl_2$ works.

Well, the first thing that you want to do is to become familiar with some of the terms used in bacterial transformation. For instance, a "transformed cell" was a term used by Griffith and Havory to describe a cell that had acquired new characteristics. They had presumed that this was DNA because they had gone through a series of experiments to ensure that the transforming agent had to be DNA. So they eliminated proteins, sacharrides, sugars, (2'30") even RNA in some of the experiments. They were pretty sure that DNA was the transforming agent and as a result, they gave strong evidence to the fact that DNA is the molecule of inheritance.

Also, you have to learn about terms such as plasmids, vector, and competent cells. These are terms used to describe small a piece of circular DNA that is known as a plasmid. Vector is simply a way of getting something into a cell. So the vector could be a plasmid. We could put for instance a specific piece (3'00") of DNA into that vector and get it into the cell. So for instance, plasmid might have an insulin gene on it and inserting it as a vector for the insulin gene so we could get that insulin gene into bacteria and have them produce human insulin. We have other ideas to cover such as competent cells. These are cells that are more capable of taking in the plasmid. In our $CaCl_2$ treatment discussion we will give thorough consideration on how we get that done.

What we are going to work on here is to become familiar with (3'30") the basics of the technique and what we are going to be doing. Essentially, this is a very detailed lab, and the fact that it covers sterile techniques and working with small quantities of DNA, you want to make sure that you read the steps of the lab completely before you start the lab. The most important aspect of sterile technique is that it is an aseptic technique, which means that there is no contamination, but in a nutshell, it means

informed technique. Most common mistakes are made when, the students do not know what they are doing, (4'00") and they start doing something "half way" and discover that they have screwed it up. So basically read the entire protocol before you start the lab. Have all of the reagents, everything; in front of you. Make sure that you know what each piece is and what each piece does before you open anything. Make sure that you have your Petri dishes, your microfuge tubes, your inoculating loops to collect your bacteria. Have everything you need in front of you and you know what is going on. Get everything set up directly in front of you and have everything to the left of you (if you work from left (4'30") to right). If you feel more comfortable working from right to left, then put everything on the right of you. Whatever side that you prefer, that is going to be the sterile side. As you use the different items, you are going to then put it over to the right which means that they have been dosed with bacteria or contaminated. By working left to right, you are going to help to minimize the contamination that might occur if per say you were to just sort of put things randomly around the lab bench. Also you wan to consider a few other things.

Work very quickly. The less time, the less time (5'00") for contamination. That is simple math. If it takes you 20 minutes to do something, you have got 20 minutes for possible contamination. If it takes you 5 minutes, you have got 5 minutes for possible contamination. So work very quickly. Also do not leave any container or any microfuge tube, or any Petri dish open any longer than you absolutely need to. When you are working with a bacteria (this is *E. coli*), you want to remember that this is microbiology so a little bit goes a long way. You need to use just a single colony of bacteria form the stock petri dish. "Single colony". I want to repeat that. More colonies will not help your

results. In fact, having more colonies will make your results worse because it will give you more strains of (5'30") bacteria. By working with a single colony, we know that we have a single strain and the results are more likely to be interpretable in the long run. Also as I said before, make sure that you work quickly and minimize the numbers of steps. Watch where the tips of the different pieces of equipment are going. It is very easy to (6'00") casually, let say a tip, touches the ground or touches the edge of something or maybe you leave a microfuge tube left open longer than you should have. During down time, when you have something on ice or when you are putting something into the water bath, make sure that you think about what are the other steps that need to be done.

That said, when you do finally come to the point of the heat shock, the timing here is extremely important. So pay close attention to the protocol and follow the timing exactly. If it says 45 seconds, it means 45 seconds (6'30"), not 47, not 43. When you get all of the transforming done and you have all of the control samples done, you are going to be plating all the bacteria out. Again, speed is of the essence here. You want to put the bacteria in there. Use the spreader to spread the bacteria around. Make sure that you let them sit so that there is a chance for the agar to absorb the extra liquid media. Then you are going to take (all the plates should have been labeled by now) and store all the plate upside down (7'00") and bundle them into a group of four using a piece of masking tape to hold them together. As I said before, this technique is ideal for this bacterial transformation and is not just a lab activity itself but also refines techniques in sterile technique. Again, read all the protocol before you start the individual lab exercise. Keep everything on the left hand side as it is clean. As you work toward the right hand side, it

becomes dirty or contaminated. If anything becomes contaminated or you are concerned that the tip might have touched something or (7'30") a sample might have been open for too long, you can always ask the Professor for a little more sample. Remember that a little goes a long way. Microbiology means small amounts. Do not use more than is needed. These quantities and times are very vigorously controlled. What might seem like fairly small amounts of time or small amounts of reagent to you; from a bacteria scale are enormous and the numbers have been very closely worked out. So again, stay very close to the protocol when address the issue of time. Also there would be opportunities (8'00") in the lab for you to read ahead and keep on top of it, especially when you are icing the specimen. When the specimen is on ice, it can usually stay on ice for any amount of time. However when it is in heat shock, you want to make sure that you pay very close attention to exactly how long that heat shock is done.

Now once you have got all of your bacteria plated out, you should have four plates. You will have a Luria broth with plasmid, a Luria broth without plasmid, a Luria broth ampicilin with plasmid, and a Luria broth ampicilin without plasmid. One of these will contain the transformants. (8'30") The other three are controls. You will then be able to find out from your lab instructor what the roles of each one of these controls are. The reasons for all these controls are simple. You come in tomorrow morning and there are no bacteria growing anywhere, you are really out of luck if you did not make controls. With these controls, you are able to say: "Well, maybe it was the heat shock. Maybe the ampicilin was bad." Each one of the plates gives you a piece of information so that when you go back and try to figure out what you need to do, you can have less work to do (9'00") because you did the appropriate controls. Now remember that this is the first part

of the lab section on transformation talking about the process. In the second one, we are going to be looking specifically at the $CaCl_2$ treatment and how that works.

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 (9'30") 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 (10'00"), 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 (10'30") seriously, we need science students, seriously……