Transformation $CaCl_2$

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 second part in the bacterial transformation lab where we are going to be looking at the $CaCl_2$ specifically. There are a lot of really awesome online tutorials for this as well. In fact (2'00") the DNA Learning Center at Cold Spring Harbor has an

excellent site. If you want to check the notes and bibliography for this URL, I will give it to you right now. However it might not pass over well if you cannot write down all the characters. It is dnalc.org. When you go to the dnalc.org, in the upper right corner of the home page, there is a little area called “resources”. Press on that link. After that you have pressed on that link, you will get to what is called the “Biological Animation (2’30”) Library”. This Biological Animation Library has about fifteen activities under it and two of them are bacterial transformation. Go ahead and check those both out because they are both very informative. They can give you some visual cues to the auditory stuff that I am going to be talking about now. Also remember to take a look at the notes for this particular lab as there are a few quick visual elements to the lab to help you understand what is going on.

The idea of this lab is to explain what we mean by competent bacteria and how we create these competent bacteria. “Competent” (3’00”) simply means capable or able and what we are doing is that we are making the bacteria more capable or able to take in DNA and therefore be transformed. This normally occurs in the bacterial world but not very often. So you need large amounts of bacteria in contact with each other for large amounts of time. Incidentally, this is exactly how bacteria acquire antibiotic resistance in hospitals because that is where you find large amounts of bacteria in contact with each other for large amounts of time. Eventually, they are going to “figure it out” (3’30”) and take a piece of plasmid that gives them antibiotic resistance. In fact this process of transformation is exactly how antibiotic resistance has been created in a number of common bacteria and there is visual evidence of evolution in action. Unfortunately it is

evolution that has not done us any good as far as humans are concerned but it has made the bacteria's life easier enabling them to live without being threatened by antibiotics.

With that said then, let's go ahead and look how $CaCl_2$ helps to encourage (4'00") bacterial transformation. There is actually about four or five major ways it does this. First, $CaCl_2$ is a divalent cation (just a fancy way of saying that when it is in solution, the calcium has a +2 charge). That +2 charge can actually attract and hold two phospholipids and so by doing so; it takes those individual phospholipids and puts them into groups of two. This makes them twice as big. If you can imagine people in an elevator and if you have skinny people, and heavier people (4'30"), well, the skinny people would move by each other a little easier. The heavier people would have a little bit of a harder time moving by one another. This is exactly how it works with the phospholipids. If you have two of them linked together, they don't move as easily as they did when they were by themselves. So this makes the membrane slightly rigid. We also make the membrane more rigid by icing the sample down.

So the second major point is that the $CaCl_2$ is always kept very, very cold. By keeping the sample very very cold, we further decrease the membrane's (5'00") fluidity. We also have a situation where the $CaCl_2$ is hypertonic. To put this another way, there is more salt in the solution surrounding the bacteria than there is in the bacteria itself. As a result, water leaves the bacteria. So this third major point is that the bacteria are in a hypertonic solution. As a result of loosing water, they are able to crenulate or shrink down. They sort of pucker-in looking a lot sort of like a raisin if you will, (5'30") after this treatment.

Also the fourth way in which the $CaCl_2$ helps is that the calcium itself will interact with the plasmidal DNA. The plasmid DNA or the small little circular pieces of DNA that you are trying to insert is round (sort of like a hula hoop or ring) and is held open as a ring because there are negative charges all the way around the ring. Remember that negative charges repel one another so the ring is going to stay open because they are not likely to collapse down because they all have the same (6'00") charge and hence are repelling one another. When you add $CaCl_2$ to the mix, the calcium ions get in there and help everything to collapse down. As a result, the plasmid becomes much smaller. When this plasmid becomes smaller, we find a situation where the plasmid can enter more easily.

Again, just a recap, $CaCl_2$ in a nice treatment has four major factors: It has a divalent cation, helps to grab two phospholipids and slows down the membrane motion, (6'30") has a cold solution that further helps to slow the membrane movement. It is also helping the DNA plasmid itself to shrink down, and it is hypertonic to the media causing the bacteria to crenulate or pucker as it looses some of the water that it has in there.

For the next step then, it is a heat shock , and this sounds like it is exactly what it is. It is a shock of heat. A very quick raise in 5 degree Celsius for bacteria is almost life threatening. While to you and I, it may feel like the difference between bath water (7'00") and warm bath water, to bacteria, it is the difference between life and death. That small change in temperatures causes the bacteria to swell very rapidly. As it swells (remember that is has a brittle membrane), it is very likely to crack or to have small fissures open on the surface. This usually occurs on a part of the bacteria known as the adhesion zone. That is not important but I just wanted you to know specifically where

this is happening. What happens is that we can see small openings. Now imagine the bacteria will swell up. As it is starting to swell up, these little cracks (7'30") occur and it is going to pull in water very quickly through those cracks. As it does so, it is very likely to pull in a plasmid. As a result of this heat shock, it has to swell up, crack, suck in a plasmid, and then stop itself from rupturing completely, and reseal. Now if that sounds crazy, you are probably right. It is pretty crazy. A lot of bacteria do not survive. Huge amounts of them burst. In fact, half a million to 5 million burst. When you are working with 10 million or 15 million bacteria, you can afford (8'00") to lose a few. In fact, you will discover in your bacteria counts later that you may have started off with a loop full of bacteria which easily could be a million or plus and you will only end up with a hundred or so that survived this process. So while the heat shock is a brutal process, it nonetheless helps to get the bacteria to take in the plasmid. This process along with $CaCl_2$ treatment was one of the first ways in which we worked out the processes of transformation.

Additional technologies exist; however they usually require (8'30") expensive equipment or more elaborate reagents and are not necessarily more effectives unless there is some specific need for them. Remember that this is the 2nd part in the bacterial transformation lab. Between the first one which focuses on techniques and this one which focuses on theory,you should be well prepared to answer any question that your professor might throw at you.

This concludes our podcast episode for the day. If you would like to get more podcasts, they can be attained at (9'00") www.canyons.edu/host/biotechoutreach/

If you would like specific information on a range 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 (9'30") kathy.flynn@canyonsedu

Information on our engineering program can be reached via David Martinez, engineering department chair, 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 (10'00")

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……..