

Bacterial Transformation

Why do scientists transform bacteria?

To get a gene they are interested in into an inexpensive organism that can then make many copies of it. The genes often code for proteins they are interested in studying.

What does "transformation" mean?

Transformation is when the scientist changes the genetic components of another organism by putting additional genetic information into it.

What are some main points to the procedure?

1. Be sterile so you don't grow up stuff that should not be grown.
2. If something should be on ice, put it on ice. Bacteria should be on ice while sitting in CaCl_2 .
3. The heat shock is supposed to literally shock the bacteria. During this time, little holes open in the bacterial membrane that let the plasmids enter the bacteria. 42°C for 50 sec.
4. Write on the EDGES of the petri plate- not through the middle of it.
5. Put the bacteria on ice IMMEDIATELY after the heat shock so the holes can close up and the bacteria can recover.
6. If you can give them 10 minutes with LB to recover before plating, that is good. If not, it is not the end of the world, you'll probably just have fewer colonies when you check your results. If you have 10 mins for recovery, please use all 10 mins.
7. Only put about 100 μL of transformed bacteria on each plate. Putting more than 100 μL can lead to satellite colonies.

What is a plasmid?

A small circular piece of double stranded DNA.

How do plasmids exist?

Some exist naturally. Bacteria routinely share their plasmids with other bacteria, thus antibiotic resistance is able to spread. There are genes for antibiotic resistance, most often found on plasmids. As more and more antibiotics are used, the bacteria that don't have the plasmids die making more space for the bacteria that do have the plasmids. Thus by using antibiotics when we're not really sick (or when we have a viral infection) we are encouraging bacteria that have antibiotic resistant plasmids to proliferate.

What do scientists do with plasmids?

Many years ago, like 30 years, scientists noticed bacterial behavior (phenotype) changed based on whether or not a small piece of circular DNA was a part of it or not. In time, scientists learned how to manipulate these plasmids (small circular pieces of DNA that started out as naturally occurring), to clone a gene of choice. Scientists learned about restriction enzymes- enzymes that occur naturally in bacteria as a defense mechanism. Bacteria produce enzymes to cut up DNA of invasive organisms so that their cell or growing space will not be overtaken. Scientists studied these restriction enzymes (RE) and before PCR, RE were all the rage. Now that we can afford to do PCR, RE are old school. Been there, done that...

If you want to get your gene of choice in a plasmid, you may still need a restriction enzyme to open up a space in the multiple cloning site (MCS), but we do not use RE for diagnostic or identity purposes anymore.

What is up with our lab?

We are using a plasmid that was altered in two significant ways

1. It has a gene in it that codes for a green fluorescent protein
2. It has a special promotor that works in the presence of arabinose

In addition, it is like any other lab-based plasmid- it has antibiotic resistance.

We are going to go through a series of steps to get the plasmid in the bacteria. We are going to plate the bacteria on 4 different plates. Each plate has a purpose. On some plates something is supposed to grow, on some it is not. You are going to have to think about how the plasmid works, what is in each plate, and determine what your results mean. THINK THINK THINK

Make a data table like this one in your lab notebook and fill it out BEFORE doing the transformation. What do you anticipate your results will mean?

Fill this out as part of your pre-lab

type of plate	What it means if bacterial colonies grow on the plate	What does it mean if the colonies glow under uv light?	What it means if bacterial colonies do not grow on the plate	What do you expect the actual result will be?
LB plate only + bacteria that do not have the plasmid				
LB plate with ampicillin (an antibiotic) + bacteria that do not have the plasmid				
LB plate with ampicillin + bacteria that do have the plasmid				
LB plate with ampicillin and arabinose + bacteria that do have the plasmid				

Write your answers in your lab notebook.

LB is the name of the nutrient broth. It is bacteria food. (And fungus food which is why you need to keep your work space sterile.)

After the transformation, when we have results, you will want to make a data table to record your results.

Some words or notation that may help you:

- Lawn- when the entire plate is covered with bacteria. It looks like a swarm of bacteria are growing there.
- TMTC- too many to count. Only use this if it is true. 25 colonies are not too many to count.
- Counting colonies- if you have lots of them, divide the plate in 4 regions. Count the colonies in one region and then multiply the number by 4.
- Satellite colonies- tiny colonies that surround your transformed colonies. They will not glow green when the transformed colonies can.