RECOMBINANT CLONING

The microbe ***Escherichia coli (E. coli)*** is perhaps the most studied and understood organism on the planet. Most of its genetic and biochemical mechanisms have been determined by scientists, making it easy to manipulate for our benefit. Although this bacterium has received lots of bad press, there is no question that it can be a useful tool in biotechnology. Bacteria store most of their genes (pieces of DNA that code for proteins) in a single molecule of DNA, but they also contain mobile segments of DNA called **plasmids** (see Figure 1). Bacteria often have plasmids present in their cells and use them in case of 'emergencies'; for example, plasmids commonly carry genes for **antibiotic resistance**. These are genes that wouldn't be required under normal growing conditions but are very helpful in the presence of an antibiotic, such as penicillin, ampicillin, erythromycin, etc., which normally will kill the cell.

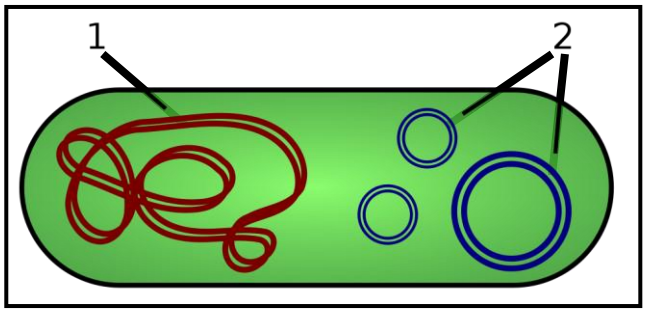

Figure 1: Bacterial DNA and plasmids.
1: Bacterial DNA, **2:** Plasmids
Image source: http://commons.wikimedia.org/wiki/File:Plasmid_(numbers).svg Wikimedia Commons.

These same plasmids (also called **vectors**) are what scientists use to introduce 'genes of interest' into bacteria. A 'gene of interest' is a gene that a scientist may want to study to learn more about the gene's function (i.e., what it does), structure (i.e., what it looks like), or sequence (i.e., the DNA coding).

The process of introducing genes into a vector to form a new DNA molecule which can be replicated in a host cell is called **Recombinant Cloning** (also called **Molecular Cloning)**. Recombinant means that two different strands of DNA that would not normally occur together are combined and cloning means creating multiple copies of genetically identical organisms (**clones**).

There are four major steps in bacterial recombinant cloning. These are:

1. **Ligation Reactions**
2. **Transformation**
3. **Selection** and **propagation**
4. **Isolation**

Let's look at each step in more detail.

1. Ligation Reactions

The first step in this process involves transferring the 'gene of interest' into a plasmid that will be taken up by *E. coli*, but how do scientists get the gene into the plasmid? First, scientists use special proteins called **restriction enzymes** to cut both the gene and the plasmid at specific sites that are complementary to each other. The two loose pieces of DNA find each other with the aid of another enzyme called **DNA ligase** and they are joined (**ligate**) to become a **modified plasmid** (see Figure 2).

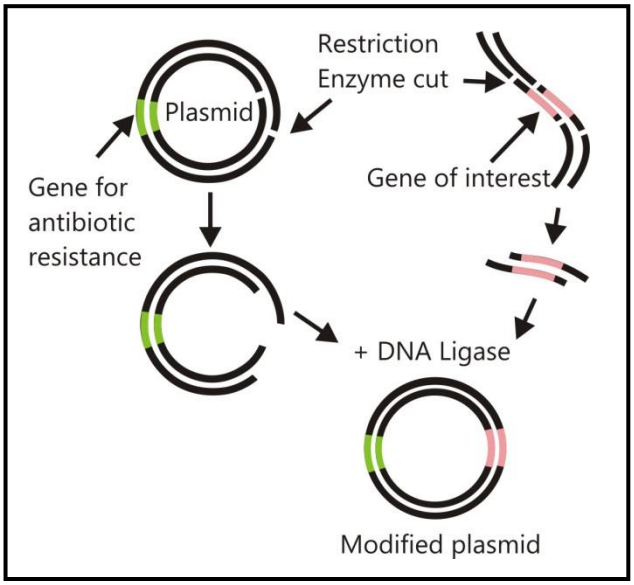

Figure 2: DNA strands are cut and then joined back together with DNA ligase. Image source: Let's Talk Science.

2. Transformation

Now we have a plasmid which contains the 'gene of interest'; however, a plasmid cannot clone a gene on its own – it needs a host system to make copies of the plasmid (and therefore, make copies of the 'gene of interest'). The most efficient host system is bacteria, specifically *E. coli*, because they divide and grow very rapidly. One *E. coli* cell can grow and divide into billions of cells in just 24 hours! If an *E. coli* is carrying a plasmid with the 'gene of interest', every time the bacteria duplicates it will also duplicate the plasmid; therefore, with an overnight growth period there could potentially be billions of copies (clones) of the gene!

So, how can we convince bacteria to become hosts for our plasmids? To begin with, many types of bacteria will naturally take up DNA molecules from their environment, but if we want maximum uptake, we can treat the *E. coli* with chemicals to make it easier for them to take up the plasmid DNA. One way to do this is to grow the *E. coli* with a mixture of the plasmids and salt. The osmotic pressure (i.e., high salt concentration on the outside of the cells, lower salt concentration on the inside of the cells) and the presence of the plasmids will cause the plasmids to enter the *E. coli* cells (see Figure 3).

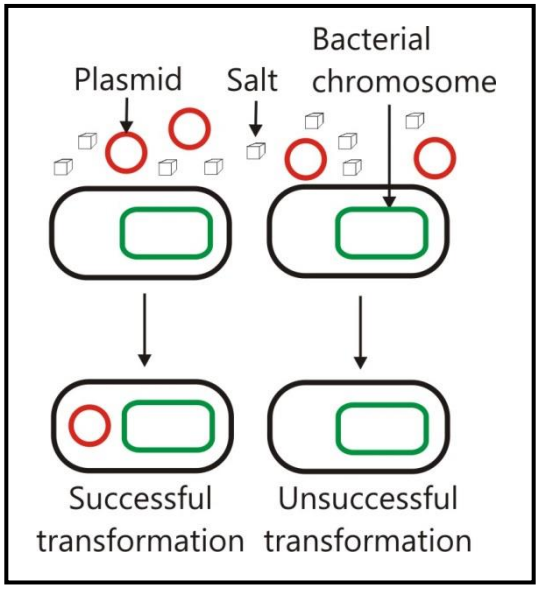

Figure 3: Bacteria are induced into taking up plasmids. Image source: Let's Talk Science.

3. Selection and Propagation

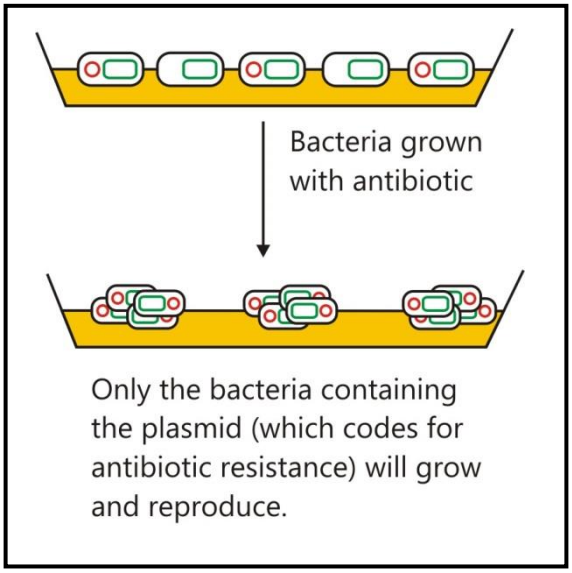

Figure 4: Antibiotic selection of bacteria containing plasmids. Image source: Let's Talk Science.

Unfortunately, as you can see in Figure 3, not all of the cells will pick up the plasmids, so the cells must be screened to determine which cells picked them up and which cells did not. How do we know which bacteria picked up the plasmids and which didn't? The plasmid carries a **selectable marker**, usually a gene that codes for antibiotic resistance (see Figure 4). Since the bacteria are grown in the presence of an antibiotic, bacteria WITH plasmids will be able to live and grow, whereas bacteria WITHOUT plasmids will die (see Figure 4). This is called **antibiotic selection**. Once a **colony** (cluster of bacteria) is shown to have the plasmid (because it was able to withstand the antibiotics), it is isolated and allowed to multiply. This results in many bacteria which contain the 'gene of interest.'

4. Isolation

The final step is to harvest the plasmids (and therefore, the 'gene of interest') from the bacteria (see Figure 5). To do this, the bacteria are **lysed** (the cell membrane is broken open) (A) and the plasmids are separated from the bacterial DNA using an acidic (low pH) solution which is high in salt (because plasmid DNA can withstand these conditions but regular DNA cannot) (B). Finally, the plasmids are separated from all of the other cell parts using **centrifugation** (spinning at high speed) (C).

Recombinant cloning is a highly controlled and effective method of producing such compounds as vitamin C, the enzyme chymosin (rennin) that is used for making cheese and the pigment indigo (indigo gives blue jeans their colour). Today applications of recombinant DNA are found in industry, food production, human and veterinary medicine (see below), agriculture, and bioengineering.

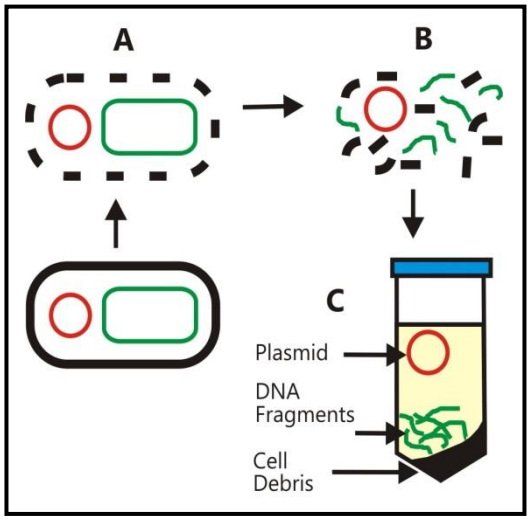

Figure 5: Harvesting of plasmids from bacteria. Image source: Let's Talk Science.

Recombinant DNA in Medicine

Recombinant human insulin…
has almost completely replaced insulin obtained from animal sources (e.g., pigs and cattle) for the treatment of type1 diabetes.

Recombinant human growth hormone (HGH, somatotropin)…
is used for patients whose pituitary glands generate too little HGH to support normal growth and development. Before recombinant HGH was available, HGH for therapeutic use was obtained from the pituitary glands of cadavers (dead people).

Recombinant blood clotting factor VIII…
is used for patients with forms of the bleeding disorder hemophilia, who are unable to produce enough factor VIII to allow normal blood coagulation (clotting).

Recombinant hepatitis B vaccine…
is a recombinant vaccine used in the prevention of the liver infection hepatitis.

Three common tests for diagnosing HIV infection…
all involve the use of recombinant cloning or the products of recombinant cloning.

Thank you to the Let's Talk Science Challenge volunteer writers who provided content in this backgrounder.