## Prokaryote reproduction and biotechnology

How prokaryotes reproduce by binary fission. Use of E. coli bacteria in molecular biology.

## Key points:

Prokaryotes (bacteria and archaea) reproduce asexually through binary fission. Most prokaryotes reproduce rapidly.

Due to their fast growth and simple genetics, E. coli bacteria are widely used in molecular biology. In the laboratory, a gene can be transferred into E. coli bacteria on a small, circular DNA molecule called a plasmid. The plasmid is taken up by the bacteria in a process called transformation. The transformed E. coli bacteria can be used to make many copies of the plasmid. In some cases, they will also express the gene on the plasmid and make protein.

Let's say you have one bacterium. How can you get more identical bacteria? How quickly can you get them? And, most importantly, why on Earth would you want a whole bunch of identical bacteria?

Let's fast-forward to that last question: some bacteria, most notably Escherichia coli (E.coli), are widely used in molecular biology labs. There, they serve as little "factories" that churn out many copies of a desired DNA molecule, or many molecules of a needed protein (such as the insulin used by diabetics to regulate their blood sugar). The more bacteria, the more of the DNA or protein product that can be made. Two features that make E. coli very useful in the lab
are its rapid reproduction and its generation of clones, or genetically identical bacteria. Let's take a quick look at how E. coli and other prokaryotes reproduce. Then, we'll examine their applications in biotechnology.

## How do prokaryotes reproduce?

Prokaryotes reproduce through a cell division process called binary fission. Like mitosis in eukaryotes, this process involves copying the chromosome and separating one cell into two.

Binary fission is an asexual form of reproduction, meaning that it does not involve production of eggs and sperm or mixing of genetic material from two individuals. Except in the case of rare mutations, or changes in DNA sequence, binary fission produces daughter cells that are genetically identical to the mother cell.

## Prokaryotes reproduce fast!

Prokaryotes in general reproduce much faster than multicellular eukaryotes. This can be measured in terms of generation time, or the length of time from the birth of one generation to the birth of the next. For humans, a typical generation time might be in the neighbourhood of 20 years. For a typical bacterium, that might be closer to 20 minutes! As a matter of fact, the E. coli bacteria that live inside your gut, and that are widely used in laboratory research, can produce a new generation every 17 minutes or so .

Not all bacteria are quite this quick, and some pathogenic ones, such as Mycobacterium tuberculosis, have a generation time over 12 hours. Still, prokaryotes in general are fast multipliers, which means their populations can grow very rapidly-in a
natural environment, or, in some cases, in a test tube in the lab.

## Bacteria in molecular biology

Bacteria that reproduce quickly and are easy to grow in the lab make good model organisms for many scientific studies. E. coli, for instance, is one of the most widely used organisms in biological research. Although you may have heard of E. coli as a food contaminant, harmless strains of E. coli are used in biology labs worldwide. In fact, many basic biological processes, like the mechanism of DNA replication, were first discovered in E. coli.

## E. coli as DNA and protein factories

Today, E. coli are sometimes used as tiny "factories" to synthesize DNA or proteins. Researchers can insert a gene of interest into E. coli cells through a process called transformation (uptake of DNA from the environment), which is described further in the article on prokaryote genetic variation. In such experiments, the gene of interest is typically borne on a piece of circular DNA called a plasmid, which can be copied by the bacterium and passed on to its offspring.


Once they contain the plasmid with the gene of interest, the E. coli cells will replicate it and pass it along each time they divide, making many copies of the plasmid DNA. If the plasmid contains the right control sequences, the E. coli can also be instructed to transcribe and translate the gene of interest, producing protein. For example, most of the insulin used by diabetics is produced in E. coli cells using this strategy.

## Steps of transformation

In a typical transformation experiment, the target gene (blue DNA above) is first inserted into a plasmid. In addition to the target gene, the plasmid also contains a gene that provides resistance to a particular antibiotic (red DNA above). If the goal is to use the bacteria to synthesize protein from the gene, the plasmid will also contain a promoter, or control sequence, that allows the target gene to be expressed in bacteria (green DNA above).

When copies of the plasmid are mixed with E. coli cells and the cells are heat-shocked (exposed briefly too high temperature), a small fraction of them will take up the plasmid. All of the E. coli are then spread on a nutrient plate containing the antibiotic. The purpose of the antibiotic is to only let bacteria with the plasmid survive and grow.

E. coli lacking the plasmid will be killed by the antibiotic. E. coli that contain the plasmid, however, can survive and reproduce (thanks to the antibiotic resistance gene in the plasmid). Each resistant cell will form a colony of genetically identical bacteria, which appears on the agar plate as small dot. An antibiotic resistant colony can be analysed (checked by other methods to confirm it contains the correct plasmid), then grown up to make a large culture of identical, plasmid-bearing bacteria.

What use is a large culture of plasmid-bearing bacteria? Sometimes, researchers need many copies of the plasmid DNA for use in another experiment, and they can extract this DNA from the culture.
Alternatively, if the plasmid contains the right promoter, the bacteria can be induced (instructed) to express the gene and synthesize protein. This technique is used to produce some medically important proteins, such as insulin and human growth hormone.

