**Biotechnology and Genetic engineering**

Dr. Abeer Fauzi Murad Lecture(5)

**Overview: DNA cloning**

**Introduction**

When you hear the word “cloning,” you may think of the cloning of whole organisms, such as Dolly the sheep. However, all it means to **clone** something is to make a genetically exact copy of it. In a molecular biology lab, what’s most often cloned is a gene or other small piece of DNA.

If your friend the molecular biologist say that her “cloning” isn’t working, she's almost certainly talking about copying bits of DNA, not making the next Dolly!

**Overview of DNA cloning**

**DNA cloning** is the process of making multiple, identical copies of a particular piece of DNA. In a typical DNA cloning procedure, the gene or other DNA fragment of interest (perhaps a gene for a medically important human protein) is first inserted into a circular piece of DNA called a **plasmid**. The insertion is done using enzymes that “cut and paste” DNA, and it produces a molecule of **recombinant DNA**, or DNA assembled out of fragments from multiple sources.

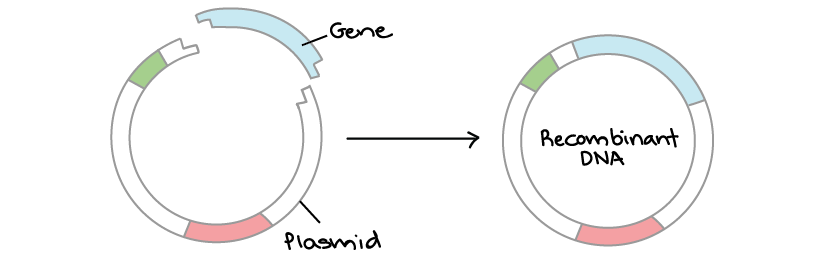


Diagram showing the construction of a recombinant DNA molecule. A circular piece of plasmid DNA has overhangs on its ends that match those of a gene fragment. The plasmid and gene fragment are joined together to produce a gene-containing plasmid. This gene-containing plasmid is an example of recombinant DNA, or a DNA molecule assembled from DNA from multiple sources.

Next, the recombinant plasmid is introduced into bacteria. Bacteria carrying the plasmid are selected and grown up. As they reproduce, they replicate the plasmid and pass it on to their offspring, making copies of the DNA it contains.

What is the point of making many copies of a DNA sequence in a plasmid? In some cases, we need lots of DNA copies to conduct experiments or build new plasmids. In other cases, the piece of DNA encodes a useful protein, and the bacteria are used as “factories” to make the protein. For instance, the human insulin gene is expressed in *E. coli* bacteria to make insulin used by diabetics.

As you may know if you have friends or family members who are diabetic, the hormone **insulin** plays an important role in human health. In a healthy person’s body, insulin is produced in the pancreas. It travels through the bloodstream and binds to cells of the body, allowing them to take up the sugar glucose.

In a person with type I diabetes, the cells of the pancreas that produce insulin are damaged or destroyed. People with type I diabetes must regularly inject themselves with insulin from another source to prevent dangerously high levels of blood sugar (and to allow sugar to enter the cells of the body as fuel).

(For many years, all of the insulin used by type I diabetics came from the pancreases of pigs and cows that had been slaughtered for meat. The insulin molecule is similar between humans, pigs, and cows, so pig or cow insulin can substitute for human insulin in the bodies of type I diabetics.

Starting in the 1980s, however, scientists developed new techniques to make human insulin using *Escherichia coli* (*E. coli*) bacteria. Basically, they turned the bacteria into tiny insulin-producing factories. This **recombinant** insulin, or insulin made by combining DNA from multiple sources (humans and bacteria), is now the primary treatment used by type I diabetics.

**Steps of DNA cloning**

DNA cloning is used for many purposes. As an example, let's see how DNA cloning can be used to synthesize a protein (such as human insulin) in bacteria. The basic steps are:

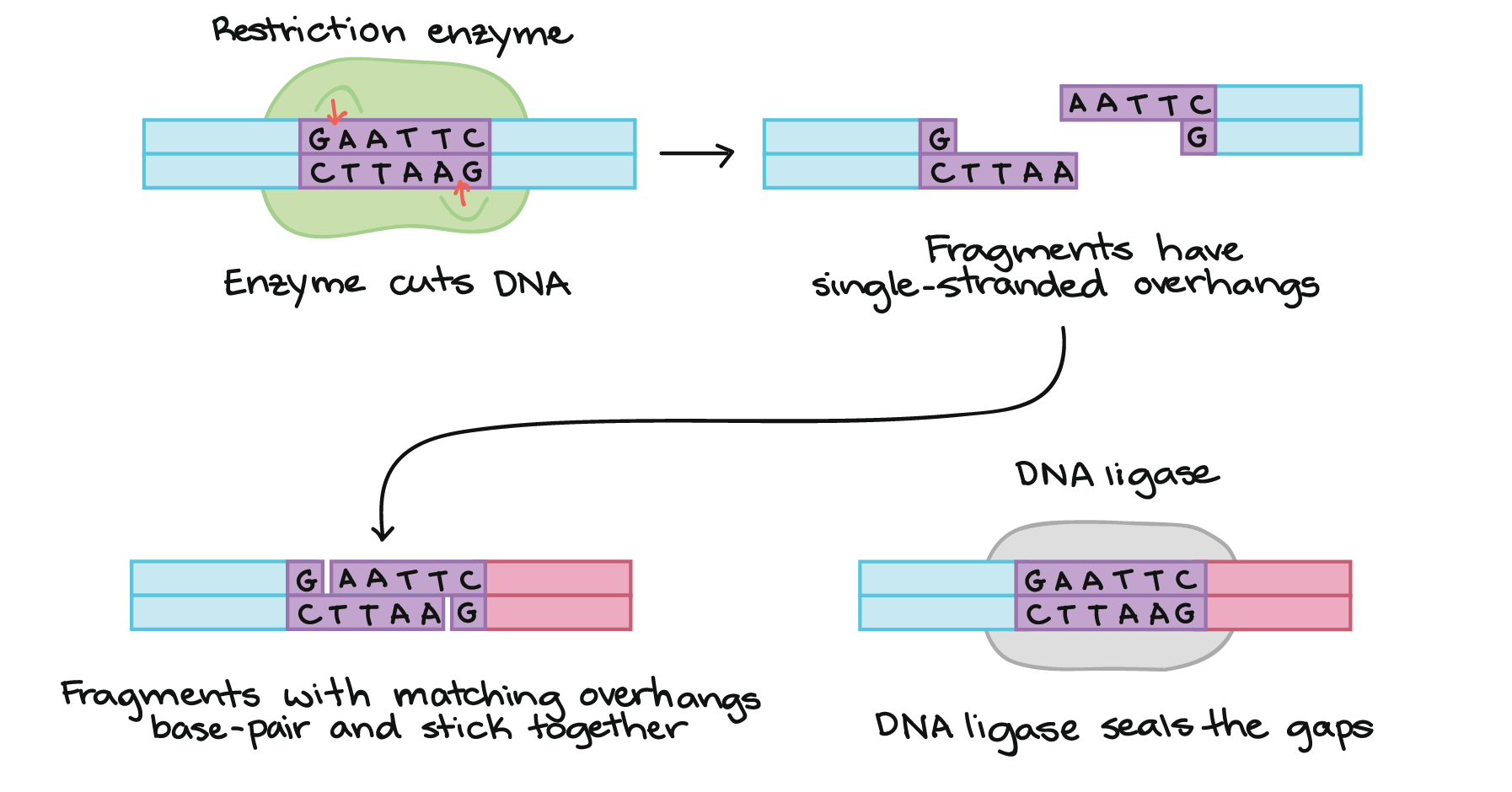
1. Cut open the plasmid and "paste" in the gene. This process relies on restriction enzymes (which cut DNA) and DNA ligase (which joins DNA).
2. Transform the plasmid into bacteria. Use antibiotic selection to identify the bacteria that took up the plasmid.
3. Grow up lots of plasmid-carrying bacteria and use them as "factories" to make the protein. Harvest the protein from the bacteria and purify it.

Let's take a closer look at each step.

**1. Cutting and pasting DNA**

How can pieces of DNA from different sources be joined together? A common method uses two types of enzymes: [restriction enzymes and DNA ligase](https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/restriction-enzymes-dna-ligase).

A **restriction enzyme** is a DNA-cutting enzyme that recognizes a specific target sequence and cuts DNA into two pieces at or near that site. Many restriction enzymes produce cut ends with short, single-stranded overhangs. If two molecules have matching overhangs, they can base-pair and stick together. However, they won't combine to form an unbroken DNA molecule until they are joined by **DNA ligase**, which seals gaps in the DNA backbone.



Our goal in cloning is to insert a target gene (e.g., for human insulin) into a plasmid. Using a carefully chosen restriction enzyme, we digest:

* The plasmid, which has a single cut site
* The target gene fragment, which has a cut site near each end

Then, we combine the fragments with DNA ligase, which links them to make a recombinant plasmid containing the gene.

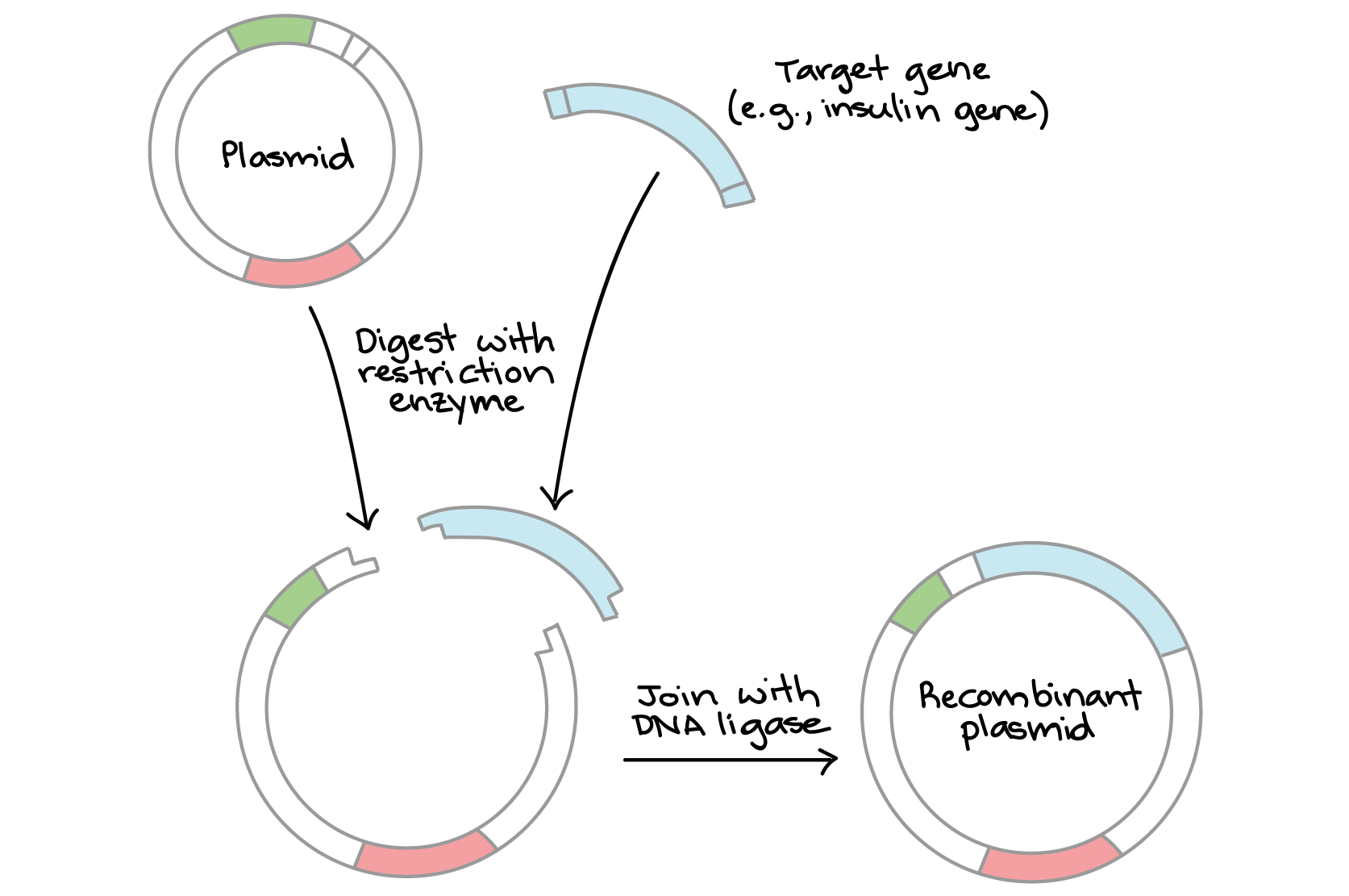


Diagram depicting restriction digestion and ligation in a simplified schematic.

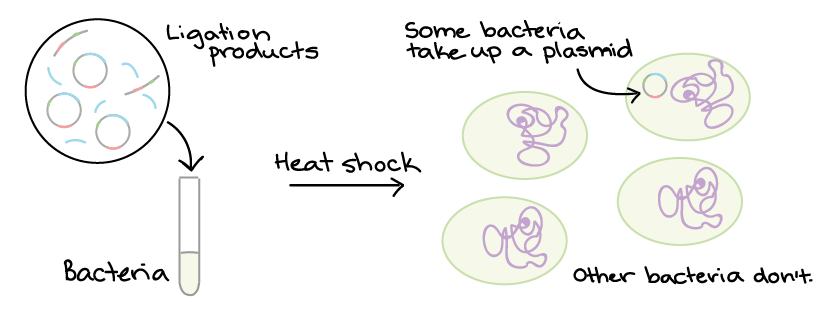
We start with a circular bacterial plasmid and a target gene. On the two ends of the target gene are restriction sites, or DNA sequences recognized by a particular restriction enzyme. In the plasmid, there is also a restriction site recognized by that same enzyme, right after a promoter that will drive expression in bacteria.

Both the plasmid and the target gene are (separately) digested with the restriction enzyme. The fragments are purified and combined. They have matching "sticky ends," or single-stranded DNA overhangs, so they can stick together.

The enzyme DNA ligase joins the fragments with matching ends together to form a single, unbroken molecule of DNA. This produces a recombinant plasmid that contains the target gene.

**2. Bacterial transformation and selection**

Plasmids and other DNA can be introduced into bacteria, such as the harmless *E. coli* used in labs, in a process called **transformation**. During [transformation](https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection), specially prepared bacterial cells are given a shock (such as high temperature) that encourages them to take up foreign DNA.



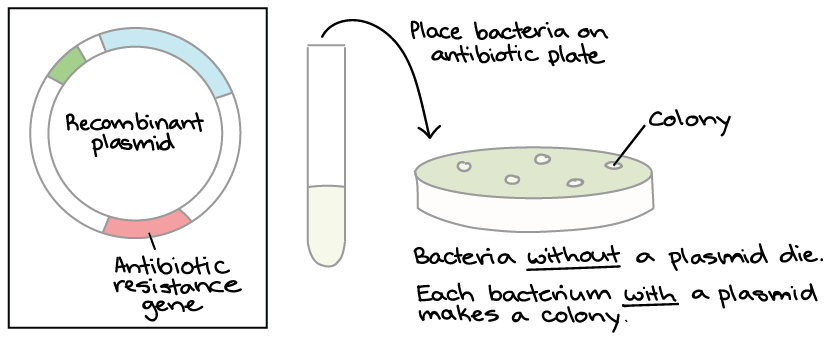
The DNA produced by ligation (which may be a mix of desired plasmids, side-product plasmids, and linear DNA pieces) is added to bacteria. The bacteria are given a heat shock, which makes them more apt to take up DNA by transformation. However, only a tiny minority of the bacteria will successfully take up a plasmid.

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[[Why does a heat shock make bacteria take up DNA?]](javascript:void(0))

Great question! Oddly enough, no one seems to be totally sure of the answer, although heat-shock transformation is a very common technique in molecular biology. In general, it's thought that the heat shock changes the fluidity of the membrane and/or causes pores (holes) to form, making it easier for the DNA to get across and enter the cell^1​1​​start superscript, 1, end superscript

A plasmid typically contains an **antibiotic resistance gene**, which allows bacteria to survive in the presence of a specific antibiotic. Thus, bacteria that took up the plasmid can be [selected](https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection) on nutrient plates containing the antibiotic. Bacteria without a plasmid will die, while bacteria carrying a plasmid can live and reproduce. Each surviving bacterium will give rise to a small, dot-like group, or **colony**, of identical bacteria that all carry the same plasmid.



Left panel: Diagram of plasmid, showing that it contains an antibiotic resistance gene.

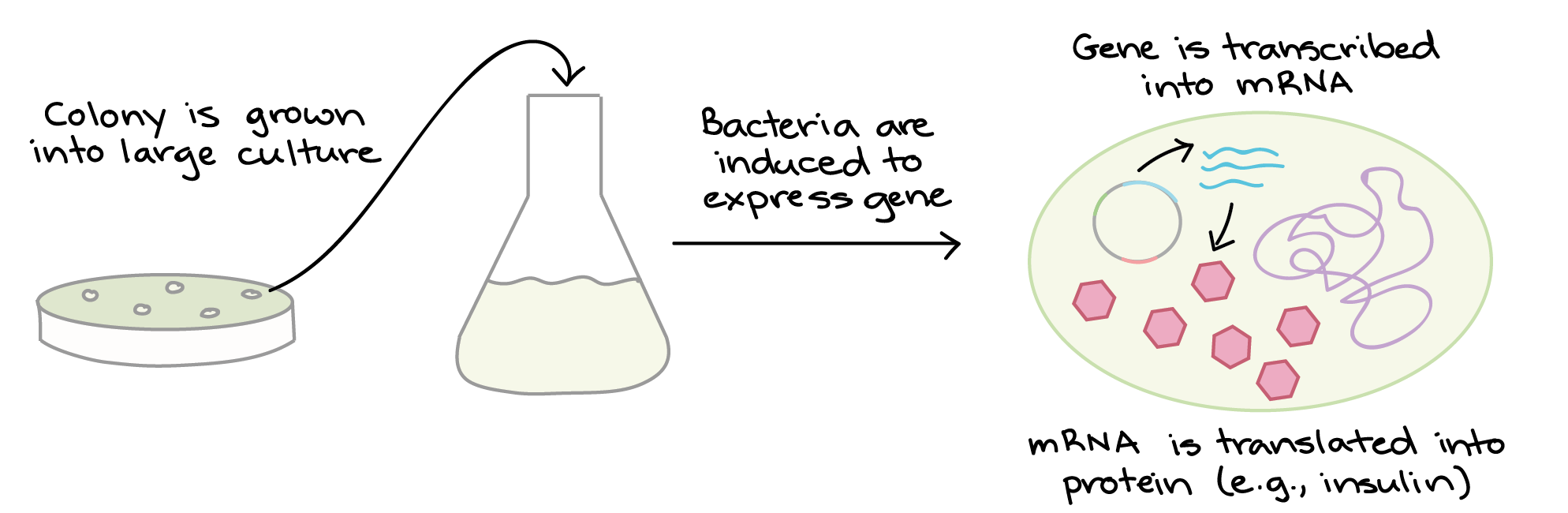
Right panel: all the bacteria from the transformation are placed on an antibiotic plate. Bacteria without a plasmid will die due to the antibiotic. Each bacterium with a plasmid makes a colony, or a group of clonal bacteria that all contain the same plasmid. A typical colony looks like a small, whitish dot the size of a pinhead.

Not all colonies will necessarily contain the right plasmid. That’s because, during a ligation, DNA fragments don’t always get “pasted” in exactly the way we intend. Instead, we must collect DNA from several colonies and see whether each one contain the right plasmid. Methods like [restriction enzyme digestion](https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/restriction-enzymes-dna-ligase) and [PCR](https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr) are commonly used to check the plasmids.

**3. Protein production**

Once we have found a bacterial colony with the right plasmid, we can grow a large culture of plasmid-bearing bacteria. Then, we give the bacteria a chemical signal that instructs them to make the target protein.

The bacteria serve as miniature “factories," churning out large amounts of protein. For instance, if our plasmid contained the human insulin gene, the bacteria would start transcribing the gene and translating the mRNA to produce many molecules of human insulin protein.



A selected colony is grown up in a large culture (e.g., a 1-liter flask). The bacteria in the large culture are induced to express the gene contained in the plasmid, causing the gene to be transcribed into mRNA, and the mRNA to be translated into protein. The protein encoded by the gene accumulates inside of the bacteria.

Once the protein has been produced, the bacterial cells can be split open to release it. There are many other proteins and macromolecules floating around in bacteria besides the target protein (e.g., insulin). Because of this, the target protein must be **purified**, or separated from the other contents of the cells by biochemical techniques. The purified protein can be used for experiments or, in the case of insulin, administered to patients.

**Uses of DNA cloning**

DNA molecules built through cloning techniques are used for many purposes in molecular biology. A short list of examples includes:

* **Biopharmaceuticals.** DNA cloning can be used to make human proteins with biomedical applications, such as the insulin mentioned above. Other examples of recombinant proteins include human growth hormone, which is given to patients who are unable to synthesize the hormone, and tissue plasminogen activator (tPA), which is used to treat strokes and prevent blood clots. Recombinant proteins like these are often made in bacteria.
* **Gene therapy.** In some genetic disorders, patients lack the functional form of a particular gene. Gene therapy attempts to provide a normal copy of the gene to the cells of a patient’s body. For example, DNA cloning was used to build plasmids containing a normal version of the gene that's nonfunctional in cystic fibrosis. When the plasmids were delivered to the lungs of cystic fibrosis patients, lung function deteriorated less quickly^2​2​​start superscript, 2, end superscript.
* **Gene analysis.** In basic research labs, biologists often use DNA cloning to build artificial, recombinant versions of genes that help them understand how normal genes in an organism function.

[another](javascript:void(0)) example

For instance, researchers studying [neurons](https://www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/v/anatomy-of-a-neuron) in fruit flies might use DNA cloning to assemble a **reporter construct** for a neural gene. In this construct, the regulatory region (promoter) of the gene might be pasted in front of a gene encoding a fluorescent protein. When transferred into a fly, the "reporter gene" would be expressed in the same neurons as the neural gene itself, causing those neurons to glow (fluoresce) under UV light.

These are just a few examples of how DNA cloning is used in biology today. DNA cloning is a very common technique that is used in a huge variety of molecular biology applications.