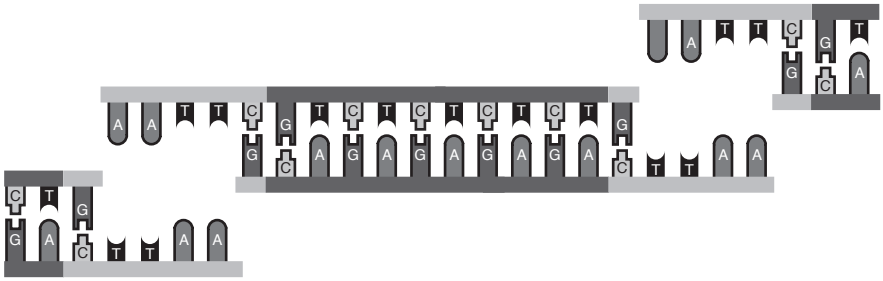
Biotechnology

An Illustrated Guide to select

Genetic Applications

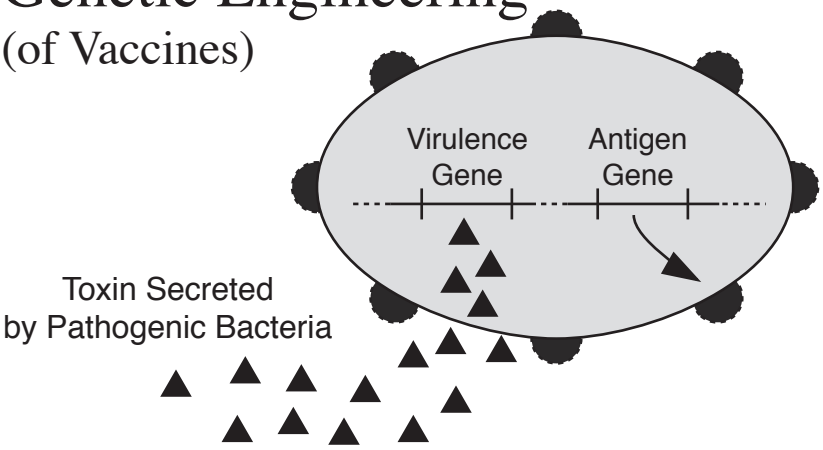
with

Microorganisms



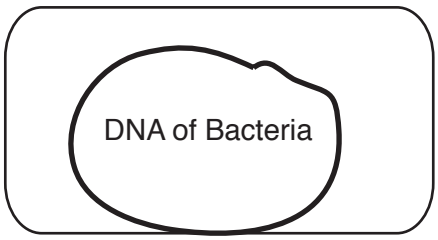
By Noel Ways

Genetic Engineering
(of Vaccines)



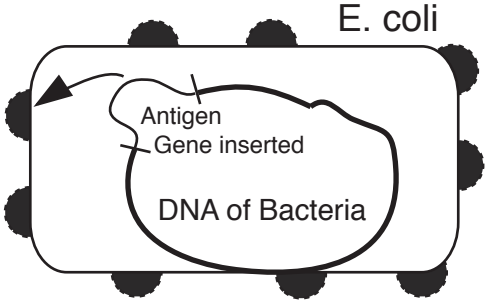
The bacterium at left is both pathogenic and antigenic. It is pathogenic because in produce a toxin that hurts us. It is antigenic because it produces molecules that our immune system recognizes as foreign. Both toxin production as well as antigen production are coded by two different genes

E. coli

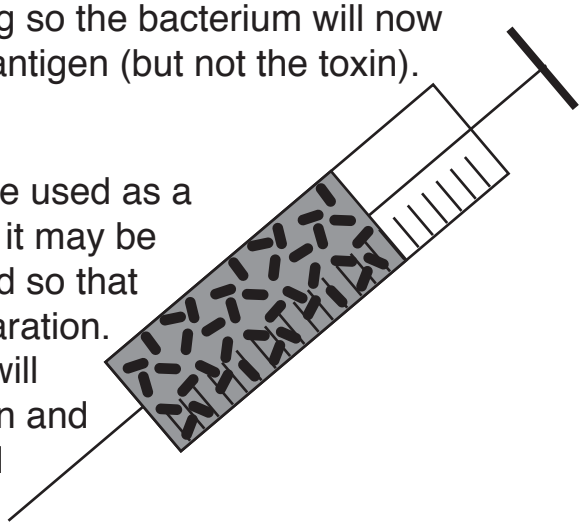


E. coli is a bacterium which normally inhabits our colon (hence the name: coli). As such, it does not harm us, and actually benefits us in several ways.

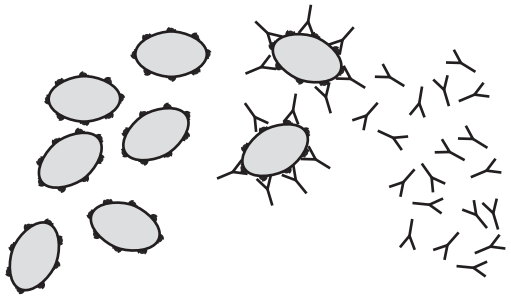
Genetically Engineered E. coli



To produce a genetically engineered vaccine, a genetic engineer might remove the gene that codes for the antigen from the pathogenic bacterium and insert it (and it alone) into E. coli. By doing so the bacterium will now express the antigen (but not the toxin).



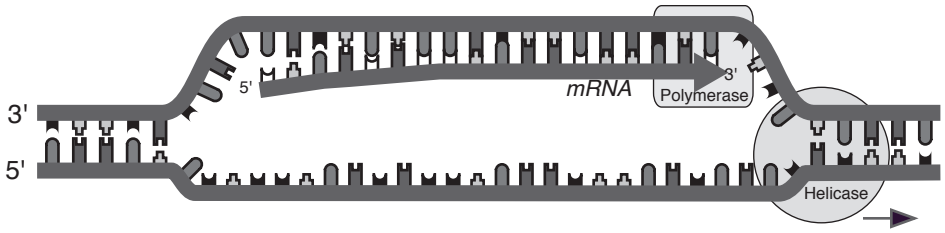
The genetically engineered bacteria can now be used as a vaccine. Since it does not produce any toxins, it may be weakened, but not killed. It may also be refined so that only the antigen is present in the vaccine preparation. Once inside the person, their immune system will mount an immune response against the antigen and destroy the vaccine, but leaving antibodies and memory cells behind incase the perceived threat should return.



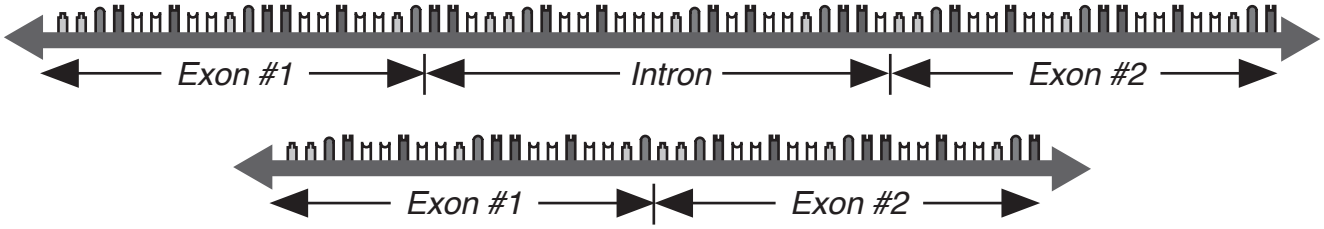
Having been vaccinated, should the original pathogenic bacterium under consideration enter the body, antibodies will bind to it and initiate it's destruction. The person is immune to the disease-causing agent. Furthermore, memory cells may also be activated, which will further boost the bodies own defenses.

Preparation of Human DNA for Insertion into a Plasmid

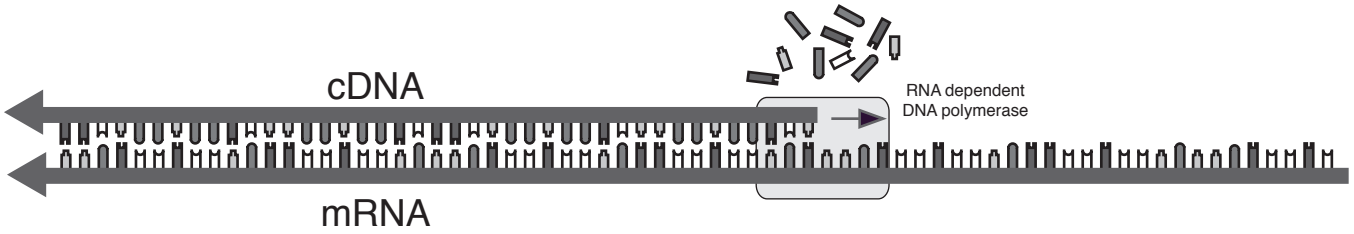
Step #1: Human DNA is transcribed into mRNA within the cell



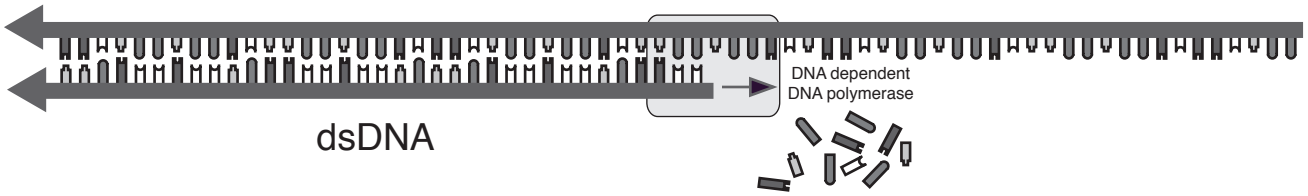
Step #2: Within the nucleus, the introns are removed, and exons united



Step #3: mRNA is harvested. Within a test tube, DNA polymerase (a reverse transcriptase), sufficient quantity of nucleotides, energy, etc are added. The RNA dependent DNA polymerase (again, a reverse transcriptase) now proceeds to make a complimentary strand of DNA from the mRNA.



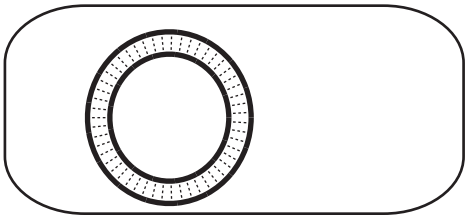
Step #4: Now the ssDNA is isolated and using a different polymerase: i.e., a DNA dependent DNA polymerase, a complementary strand of DNA to the ssDNA is made. The result is dsDNA



Step #5: DNA finished! The new DNA is identical to the DNA that we started with, except that the introns have been removed. Now, restriction enzymes can be added and the desired genes inserted into plasmids, as in the previous example.

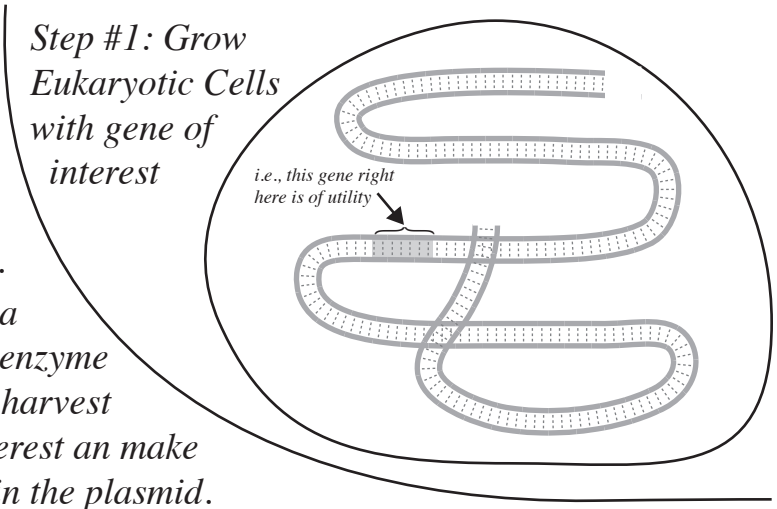
Genetic Engineering Example

Step #1: Grow Bacteria with a Plasmid known to have a sequence specific for a particular and appropriate restriction enzyme.

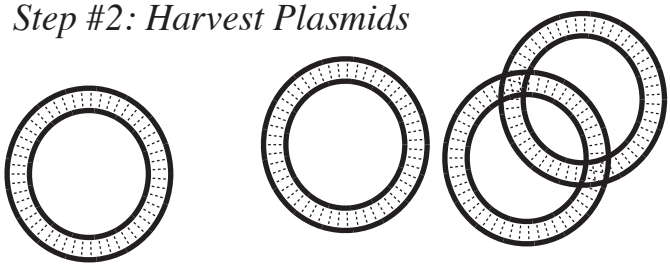


Or choose a restriction enzyme suitable to harvest gene of interest an make one break in the plasmid.

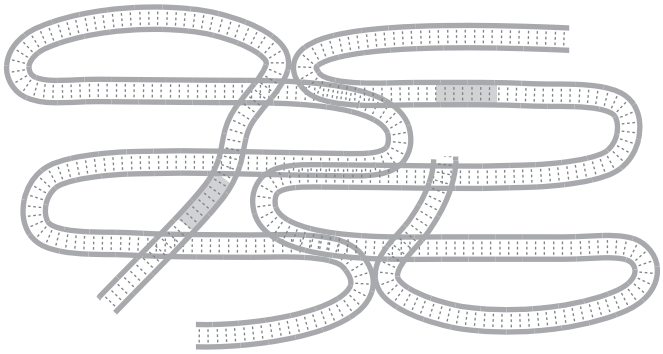
Step #1: Grow Eukaryotic Cells with gene of interest



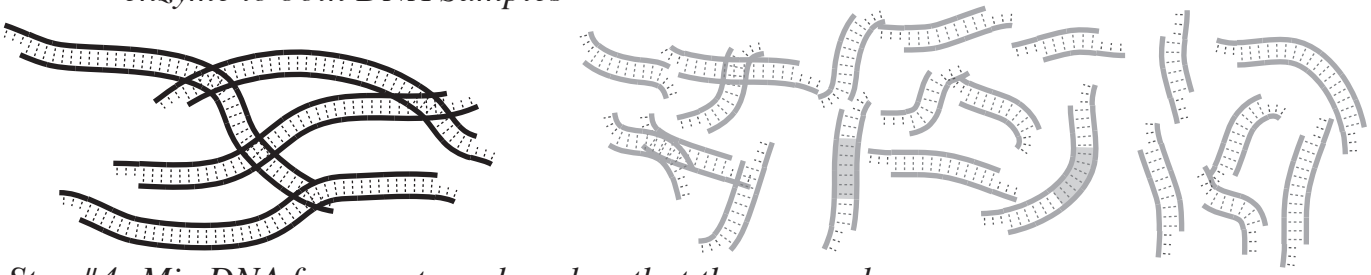
Step #2: Harvest Plasmids



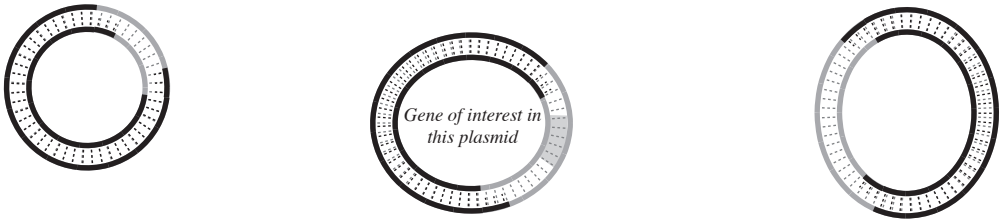
Step #2: Harvest Eukaryotic DNA



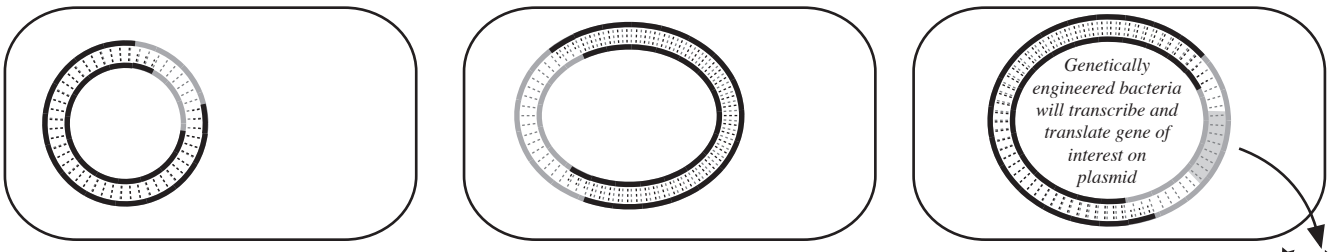
Step #3: Apply the SAME restriction enzyme to both DNA Samples



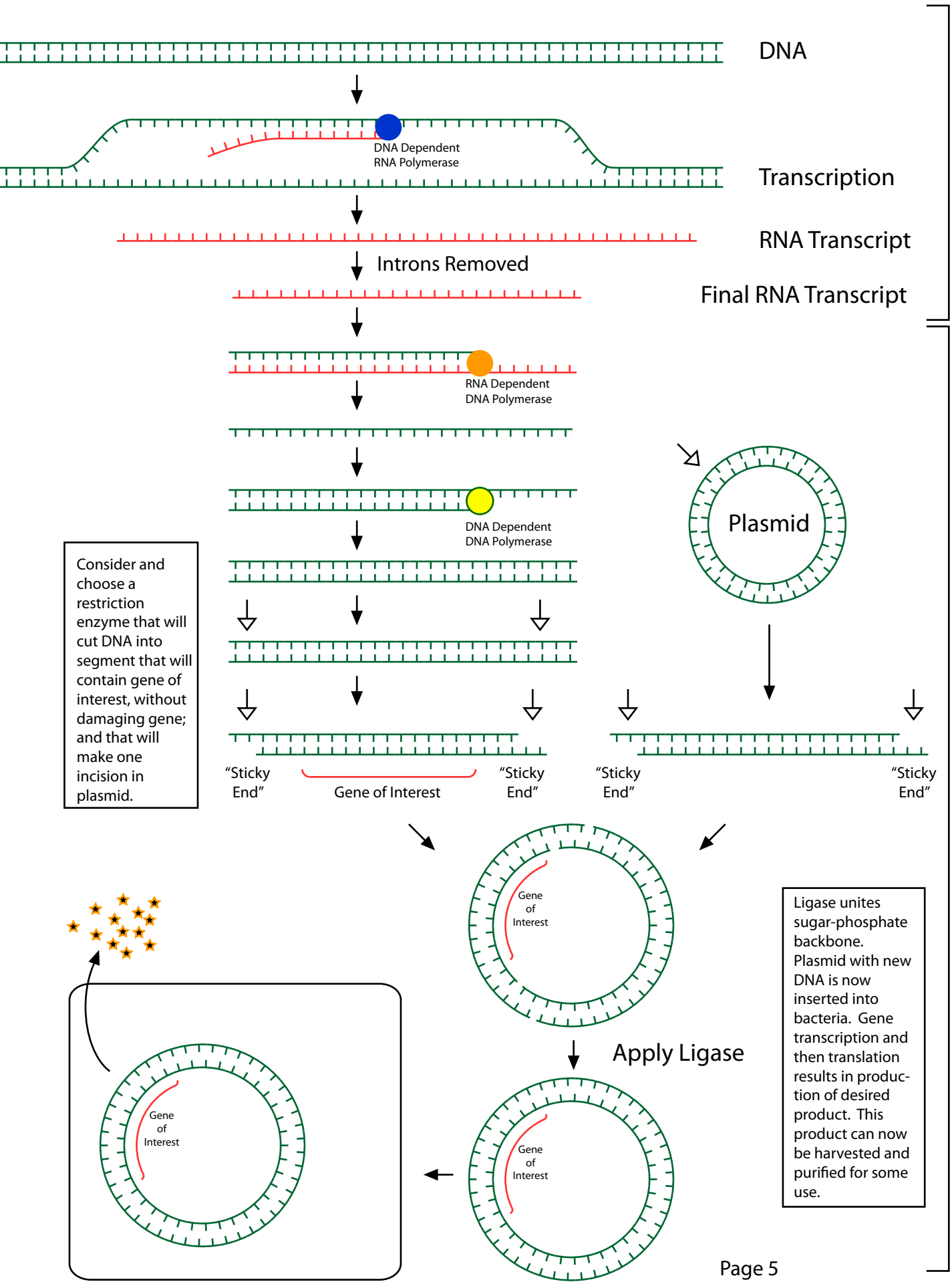
Step #4: Mix DNA fragments and cool so that they anneal. Apply ligase to reform sugar-phosphate "backbone"



Step #5: Insert Genetically modified plasmid into bacteria



Step #5: Identify particular bacteria with gene of interest. When found, grow the bacteria up and harvest the product.


DNA
DNA Dependent
RNA Polymerase
Transcription
RNA Transcript
Introns Removed
Final RNA Transcript
Occurs
In
Nucleus
RNA Dependent
DNA Polymerase
DNA Dependent
DNA Polymerase
Plasmid
Consider and choose a restriction enzyme that will cut DNA into segment that will contain gene of interest, without damaging gene; and that will make one incision in plasmid.
"Sticky End"
Gene of Interest
"Sticky End"
"Sticky End"
"Sticky End"
Occurs
In
Test
Tube
Gene of Interest
Apply Ligase
Gene of Interest
Gene of Interest
Ligase unites sugar-phosphate backbone. Plasmid with new DNA is now inserted into bacteria. Gene transcription and then translation results in production of desired product. This product can now be harvested and purified for some use.