# Biotechnology and Gene Manipulation

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# Introduction

- Biotechnology involves the exploitation, genetic manipulation and alterations of micro-organisms or biological systems to make commercial valuable products and that also involves fermentation and various upstream and downstream processes.

# Introduction

- Microorganisms produce an amazing array of valuable products such as macromolecules (e.g. proteins, nucleic acids, carbohydrate polymers, even cells) or smaller molecules and are usually divided into metabolites that are essential for vegetative growth (primary metabolites) and those which give advantages over adverse environment (secondary metabolites).
- They usually produce these compounds in small amounts that are needed for their own benefit.

# In General, Biotechnology Techniques

*Gene Manipulation*

- Identify a gene from *another species* which controls
- A trait of interest
- Or modify an existing gene (create a new allele)

# In General, Biotechnology Techniques

*Gene Introduction*

- Introduces that gene into an organism
- Technique called *transformation*
- Forms *transgenic organisms*

# Recombinant DNA

The manipulation and combination of DNA from two sources.

- Bacterial DNA +  human gene for insulin
- Plant DNA + bacterial DNA - *Agrobacterium tumefaciens*
- Mouse DNA + human DNA = transgenic

# Recombination

- Insert a foreign gene into a host.

  Plasmid ( for example, exogenous DNA) into the bacterial cell – transformation or transfection-organism referred to as transgenic or recombinant

- Goal – To produce many copies ( clones) of a particular gene
- Reporter gene – tags gene of interest – to identify the presence of a gene.

# Genetic Manipulation for Biotechnology

- Molecular genetics can be used to manipulate genes in order to alter the expression and production of microbial products, including the expression of novel recombinant proteins.
- The compounds that are isolated from plants or animals can be synthesized by genetic manipulation of different micro-organisms to enhance the production and by environmental and other manipulations, even up to 1000-fold for small metabolites can be increased.

# Genetic Manipulation for Biotechnology

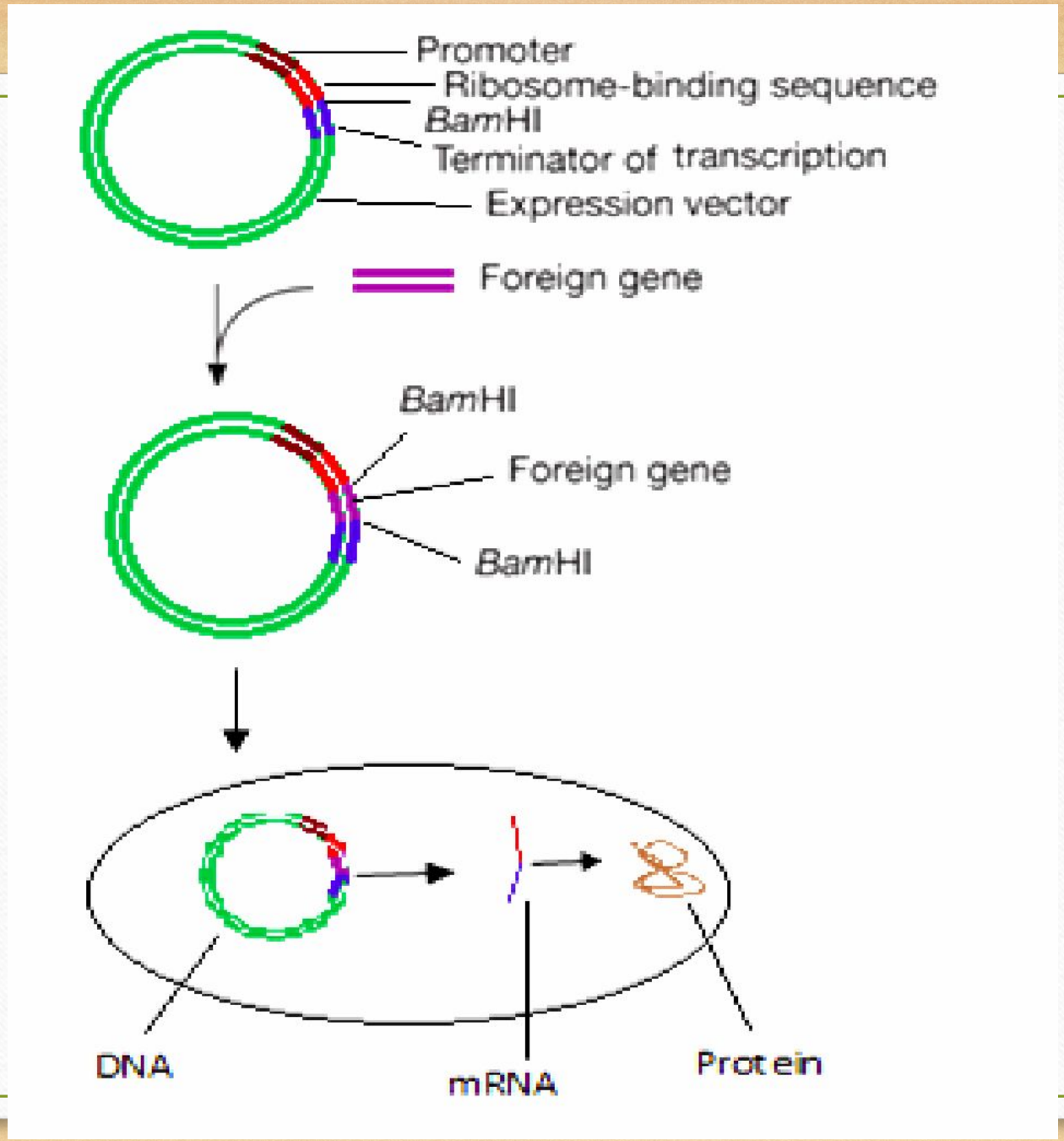
- The advent of recombinant DNA technology (also referred to as gene cloning or *in vitro genetic manipulation)* has dramatically broadened the spectrum of microbial genetic manipulations.
- With the advancement of recombinant DNA technology, many novel host systems have been **explored** to produce commercially important products like therapeutic proteins, antibiotics, small molecules etc.

# Genetic Manipulation for Biotechnology

- The basis of this technology is the use of restriction endonucleases, polymerases and DNA ligases as a means to specifically cut and paste fragments of DNA.
- Similarly, foreign DNA fragments can be introduced into a vector molecule (a plasmid or a bacteriophage), which enables the DNA to replicate after introduction into a bacterial cell.
- The ability to modify and clone genes accelerated the rate of discovery and the development in biotech industries.

# The basic steps in DNA cloning involves

- A fragment of DNA is inserted into a carrier DNA molecule, called a vector, to produce a recombinant DNA.
- The recombinant DNA is then introduced into a host cell, where it can multiply and produce numerous copies of itself within the host.
- The most commonly used host is the bacteria, although other hosts can also be used to propagate the recombinant DNA.




**Expression of a foreign protein in a microbe**

# Potential applications of genetic manipulation

- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IX for hemophilia B
- Human growth hormone (GH)
- Erythropoietin (EPO) for treating anemia
- Interferons
- Interleukins

# Vectors

- Plasmids
- Viruses
- Particles ( DNA coated bullets)
- Exogenous DNA

# Characteristics of a Vector

- Can replicate independently in the host cell – contains an Ori
- Has restriction sites in the vector- Polylinker cloning region
- Has a reporter gene that will announce its presence in the host cell.
- Is a small size in comparison to the host chromosome for ease of isolation

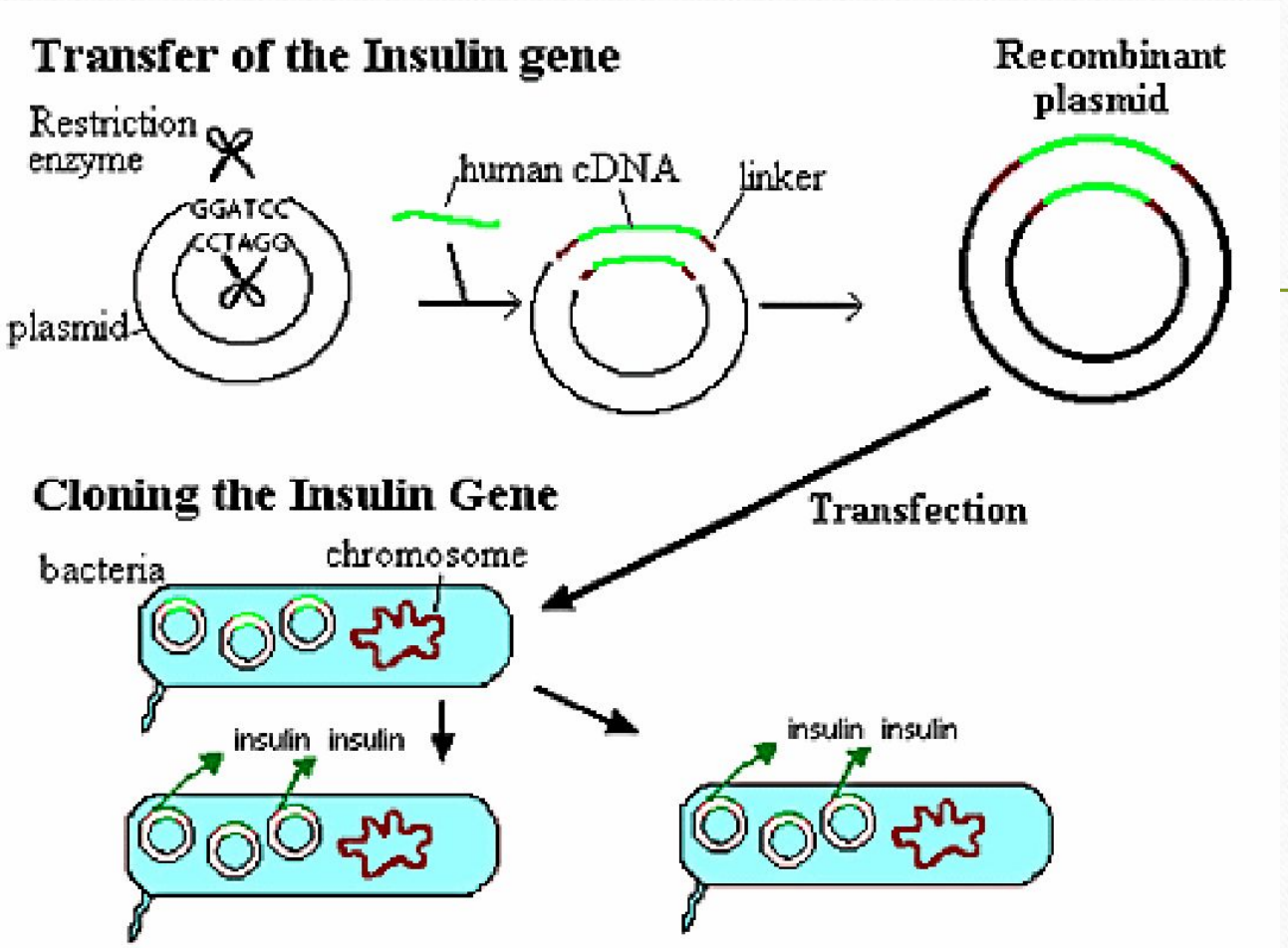
# Restriction Enzymes

- Cut Plasmid with restriction enzyme
- Cut gene of interest with restriction enzyme
- Splice together gene of interest

# Production of recombinant enzymes:

1. Enzymes of industrial importance: Amylases, Proteases, Chymosin, Catalases, Isomerases recombinant Lipases.
2. Enzymes used for analytical purposes, such as glucose oxidase (GOs), alcohol dehydrogenase (ADH), hexokinase, cholesterol oxidases, horseradish peroxidase (HRP), alkaline phosphatase etc.
3. Enzymes of medicinal importance: Trypsin, Asparaginase, Proteases, Lipases etc.

# Production of Insulin




Transfer and cloning of the Insulin gene

# Production of Monoclonal Antibodies