BIOTECHNOLOGY

- **Any technological application that makes use of Biological systems, living organisms or derivatives thereof to make or modify products or processes for specific use (UN convention on biological diversity)**

RECOMBINANT DNA TECNOLOGY

- **Recombinant DNA is DNA that has been created artificially.**

- **DNA from two or more sources is incorporated into a single recombinant molecule to achieve certain desired goals.**

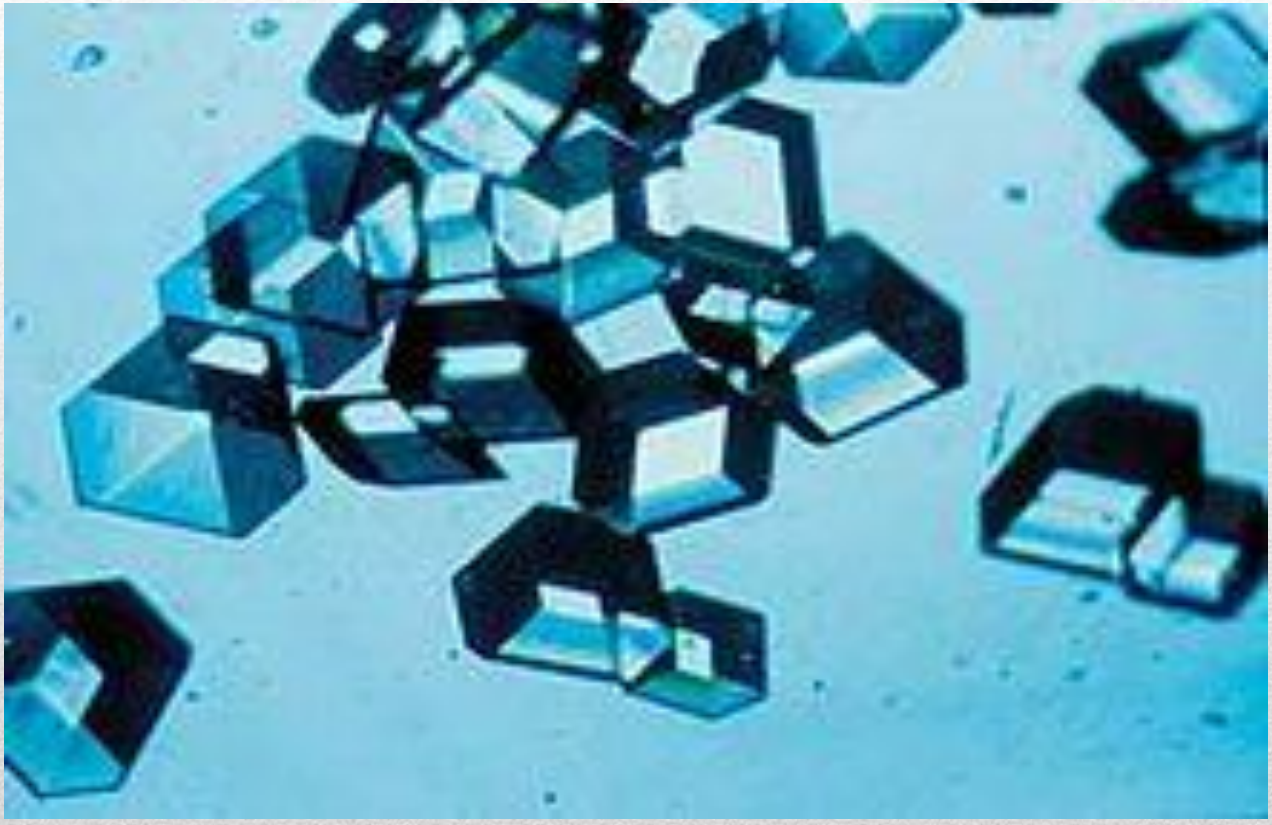
HOSTS USED IN rDNA TECHNOLOGY

s/n	Host
1	*Escherichia coli*
2	*Bacillus subtilis*
3	*Streptomyces*
4	*Saccharomyces cerevisiae*
5	*Pichia pastoris*
6	*Aspergillus*
7	*Animal cells (CHO, SP20/NSO)*
8	*Insect cells: Baculovirus system*
10	*Transgenic Animals*
10	*Transgenic Plants*

Hosts commonly used in recombinant DNA technology

HORMONES

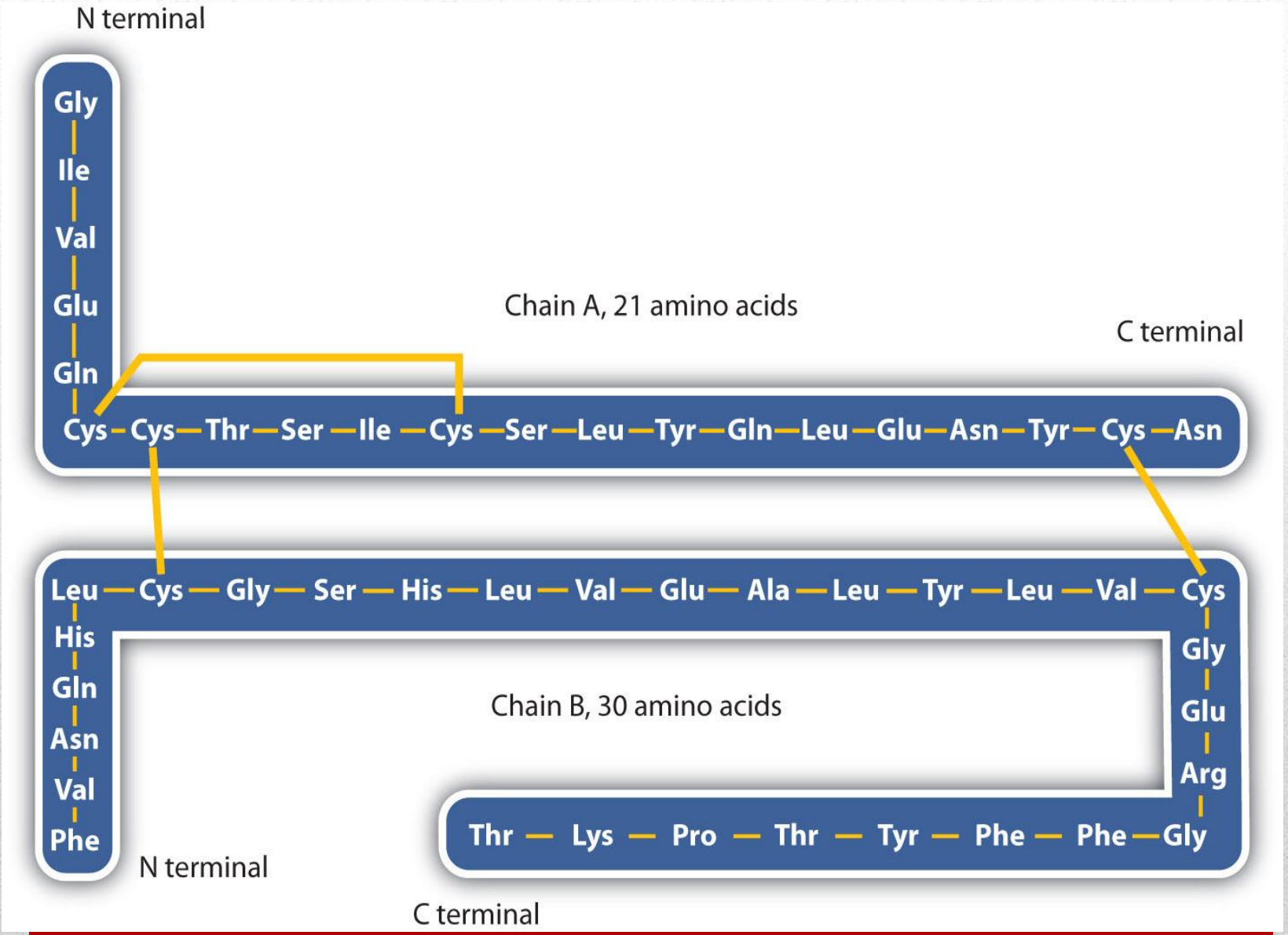
- **Hormones are a class of signaling molecules**
- **produced by glands in multicellular organisms**
- **transported by the circulatory system to target distant organs to regulate physiology and behavior.**



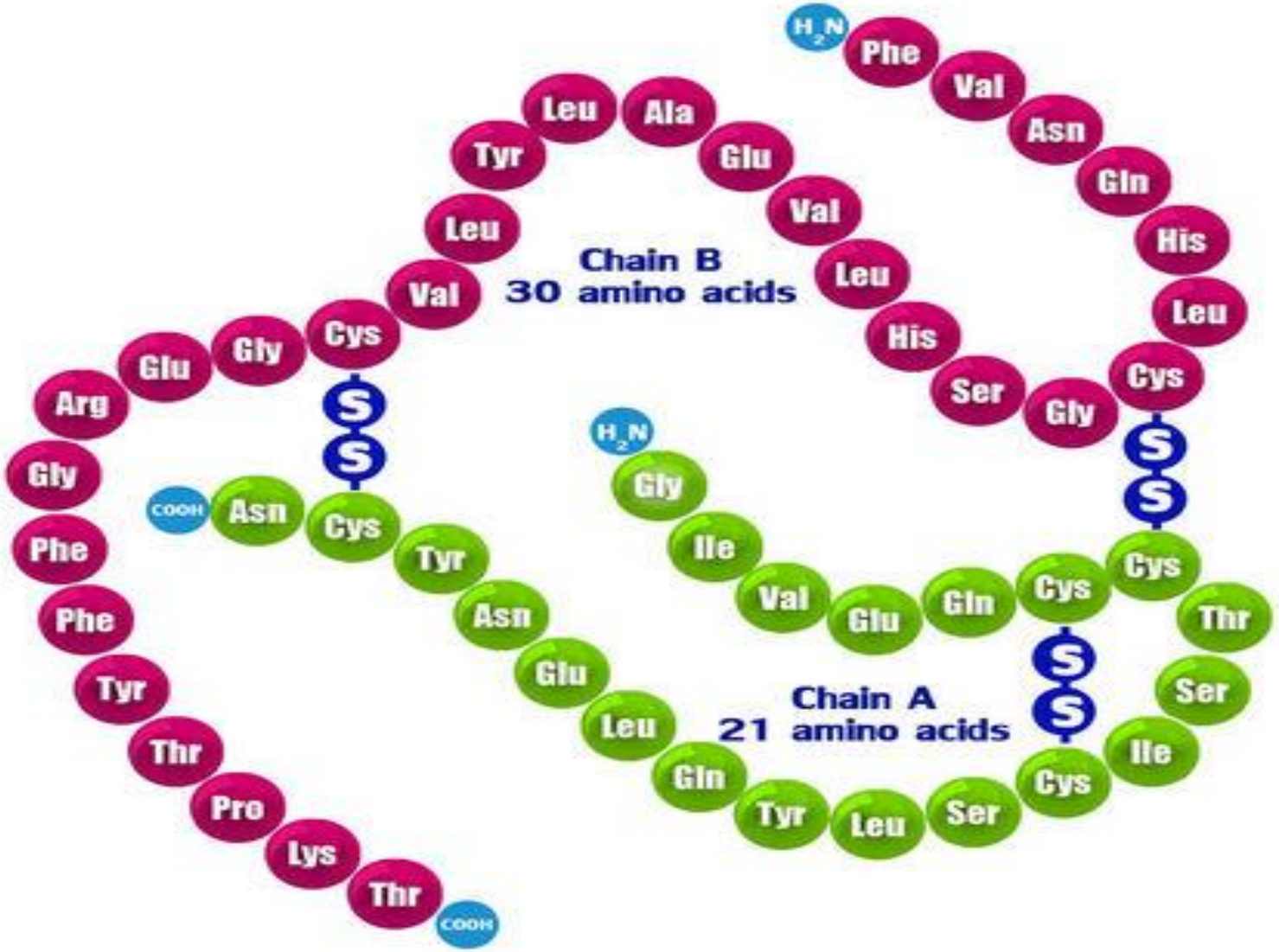
Insulin crystals

INSULIN

- **Insulin is a hormone produced by ß cells of the islets of langerhan in the pancrease.**
- **It is a small protein consisting of**
- **an alpha chain of 21 amino acids linked by two disulfide (S—S) bridges to a**
- **beta chain of 30 amino acids.**


N terminal
Gly
Ile
Val
Glu
Gln
Chain A, 21 amino acids
C terminal
Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys
His
Gln
Asn
Val
Phe
Chain B, 30 amino acids
Gly
Glu
Arg
Thr Lys Pro Thr Tyr Phe Phe Gly
N terminal
C terminal

Human Insulin



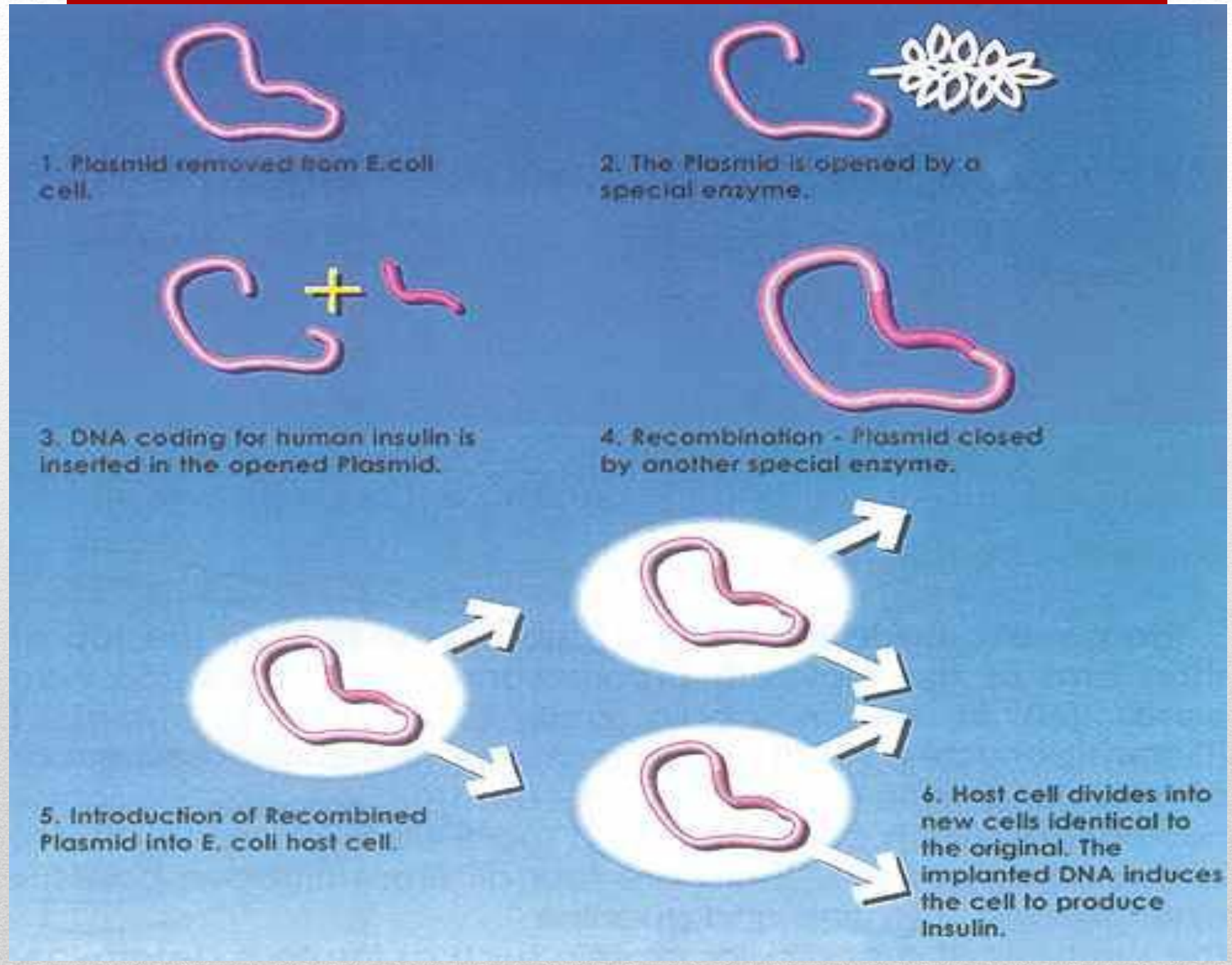
MAKING INSULIN FROM rDNA TECHNOLOGY

METHOD 1

Insulin chains are grown separately.

Manufacturers require two mini genes;

- **Genes for the A chain &**
- **Genes for the B chain**
- **Since the exact DNA sequence of each chain is known, they synthesize each mini-gene's DNA in an amino acid sequencing machine.**


1. Plasmid removed from E.coli cell.
2. The Plasmid is opened by a special enzyme.
3. DNA coding for human insulin is inserted in the opened Plasmid.
4. Recombination - Plasmid closed by another special enzyme.
5. Introduction of Recombined Plasmid into E. coli host cell.
6. Host cell divides into new cells identical to the original. The implanted DNA induces the cell to produce insulin.

- **These two DNA molecules are then inserted into plasmids**

- **Manufacturers first insert the plasmids into a non-harmful type of the bacterium *E. coli* next to the lacZ gene. LacZ encodes for b-galactosidase.**

- **The recombinant, newly formed, plasmids are mixed up with the bacterial cells. Plasmids enter the bacteria in a process called transformation. Manufacturers can add to the cells DNA ligase, an enzyme that acts like glue to help the plasmid stick to the bacterium's DNA.**

- **The bacteria synthesizing the insulin then undergo a fermentation process at optimal temperatures in large tanks in manufacturing plants.**

- **The millions of bacteria replicate roughly every 20 minutes through cell mitosis, and each expresses the insulin gene.**

- **After multiplying, the cells are taken out of the tanks and broken open to extract the DNA.**

- **One common way this is done is by first adding a mixture of lysozome that digest the outer layer of the cell wall,**

- **then adding a detergent mixture that separates the fatty cell wall membrane.**

- **The bacterium's DNA is then treated with cyanogen bromide, a reagent that splits protein chains at the methionine residues. This separates the insulin chains from the rest of the DNA.**

- **The two chains are then mixed together and joined by disulfide bonds through the reduction-reoxidation reaction. An oxidizing agent is added. The batch is then placed in a centrifuge to separate cell components by size and density.**

- **The DNA mixture is then purified so that only the insulin chains remain.**

- **Manufacturers can purify the mixture through several chromatography, or separation techniques.**

- **Procedures used include an ion-exchange column, reverse-phase high performance liquid chromatography, and a gel filtration chromatography column.**
- **Manufacturers can test insulin batches to ensure none of the bacteria's E. coli proteins are mixed in with the insulin.**
- **They use a marker protein that lets them detect *E. coli* DNA.**
- **They can then determine that the purification process removes the E. coli bacteria.**

ANALOG INSULIN

- an analog, a chemical substance that mimics another substance well enough that it fools the cell.

- Analog insulin clumps less and disperses more readily into the blood, allowing the insulin to start working in the body minutes after an injection.

- **Humulin** (an analog insulin) does not have strong bonds like other insulin and thus, is absorbed quickly.

- Another insulin analog, called **Glargine**, changes the chemical structure of the protein
- to make it have a relatively constant release over 24 hours with no pronounced peaks.


NDC 0088-2220-33
Lantus®
insulin glargine
(rDNA origin)
injection
100 units/mL
(U-100)
DO NOT MIX WITH
OTHER INSULINS
USE ONLY IF SOLUTION
NDC 0088-2220-33
Lantus®
insulin glargine
100 units/mL (U-100)
FOR SUBCUTANEOUS
DO NOT MIX WITH

- **Recombinant human growth hormone is generally produced by inserting the human growth hormone gene into plasmids of *E.Coli* bacteria.**

- **Recombinant bacterial cells are cultured and human growth hormones produced by these bacteria are extracted from the extracellular media.**

- **Human growth hormones are also produced and extracted using animal cell culture and Chinese Hamster Ovary (CHO) Cells**

- **They are nowadays used in treating patients with renal carcinoma and also to treat children who are suffering from human growth deficiency**

LIMITATIONS

- During the production of the human growth hormone in the body it is attached with signal peptide with 26 amino acids.
- This signal peptide is cleaved in the body before releasing the human growth hormone to do their biological function.
- *E.Coli* bacteria does not have the mechanism to cleave signal peptide chain.

New Approach:

- To resolve the interruption of signal peptides in the production of human growth hormone, the base sequence coding for signal peptides of around 26 amino acids along with neighbouring 24 amino acids is cut in the coding cDNA using restriction enzyme EcoR1.

- The 24 amino acid sequence of human growth hormone is Deleted cDNA is freshly prepared and ligated to the remaining human growth hormone cDNA.

- **This coding cDNA is then integrated into the plasmid, then inserted into E.Coli bacterial cell**

- **These recombinant bacterial cells are cultured, and then human growth hormones are extracted from the media.**


NDC 44087-0006-7
Serostim® 6 mg
[somatropin (rDNA origin) for injection]
6 mg (approximately 18 IU)
For subcutaneous injection

Other application of recombinant DNA technology is in the production of Tissue Plasminogen Activator and Folicle stimulating hormone (FSH).