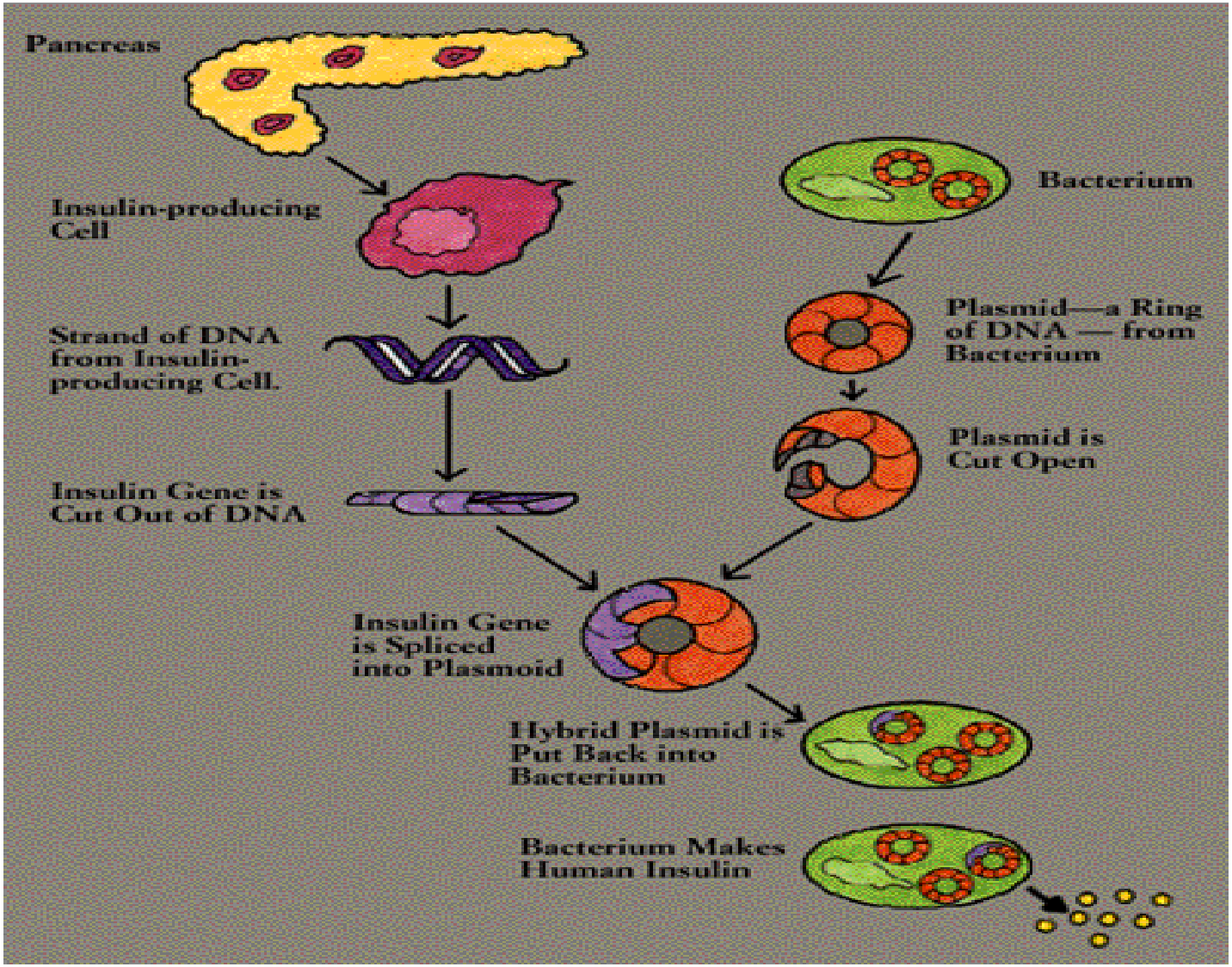
FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Production of recombinant Insulin

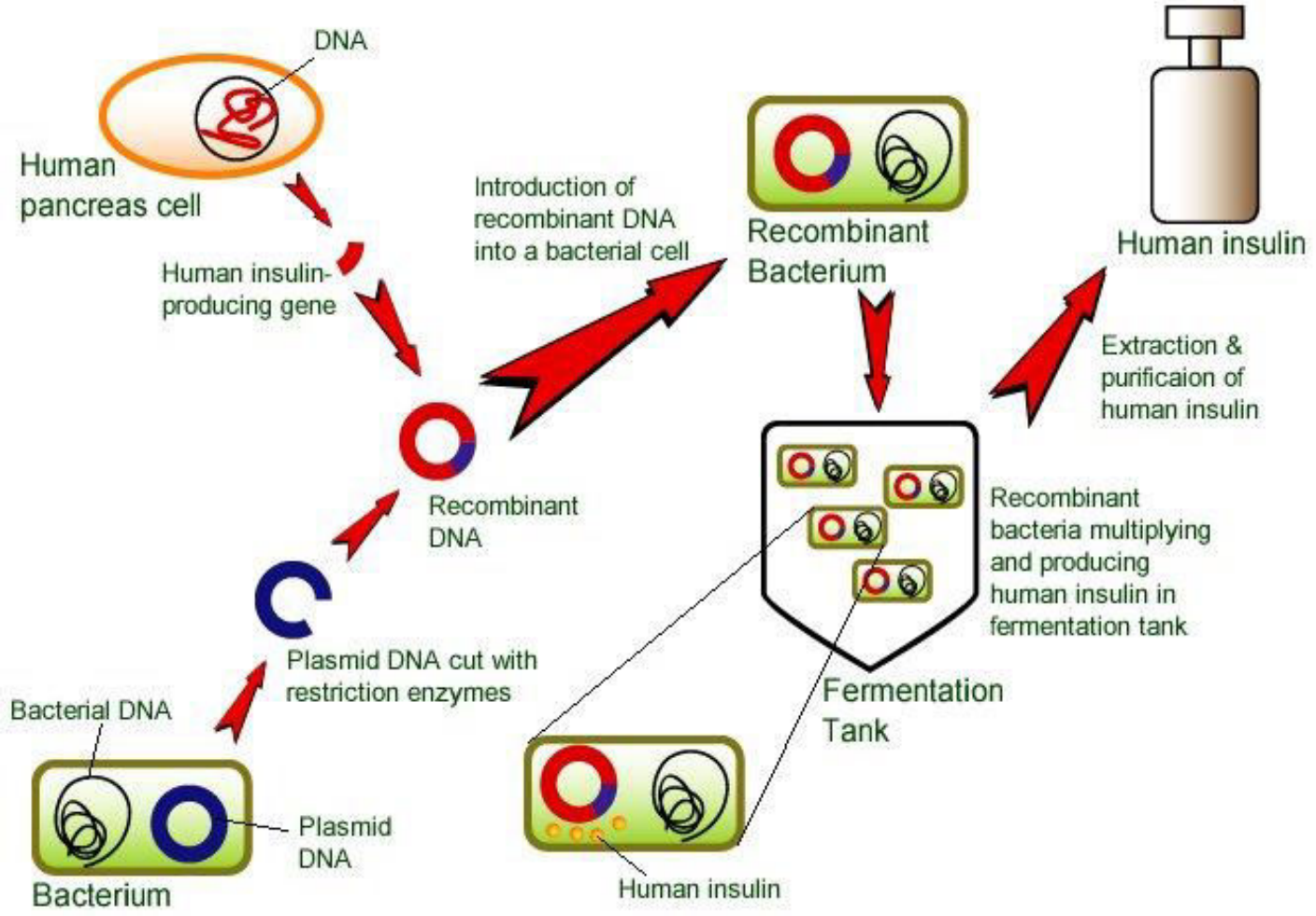
Insulin is a hormone central regulating carbohydrate and fat metabolism in the body.

Insulin is secreted by the Islets of Langerhans of pancreas which catabolizes glucose in blood.

Insulin causes liver cells, muscle cells and fat tissue to take up glucose from the blood and store it as glycogen in the liver and muscle.


Pancreas
Insulin-producing Cell
Strand of DNA from Insulin-producing Cell.
Insulin Gene is Cut Out of DNA
Bacterium
Plasmid—a Ring of DNA — from Bacterium
Plasmid is Cut Open
Insulin Gene is Spliced into Plasmoid
Hybrid Plasmid is Put Back into Bacterium
Bacterium Makes Human Insulin

Human Insulin Production



Structure

Insulin consists of two polypeptide chains, Chain A (21 amino acid long) and B (30 amino acid long). Its precursor is proinsulin which also contains two polypeptide chains, A and B, and is connected with a third peptide chain –C (35 amino acid long).

Production of Insulin

⦿In the Islets of Langerhans, insulin accumulates in secretary vesicles as a single polypeptide chain called proinsulin.

⦿Before secretion into the bloodstream the third C chain of the proinsulin molecule is excised, leaving the A and B chains joined by disulphide bridges as the active insulin.

⦿*E. coli* is not capable of removing the C chain.

⦿There are several strategies for producing insulin from bacteria, but the most successful is to synthesize the A and B separately and then join them together.

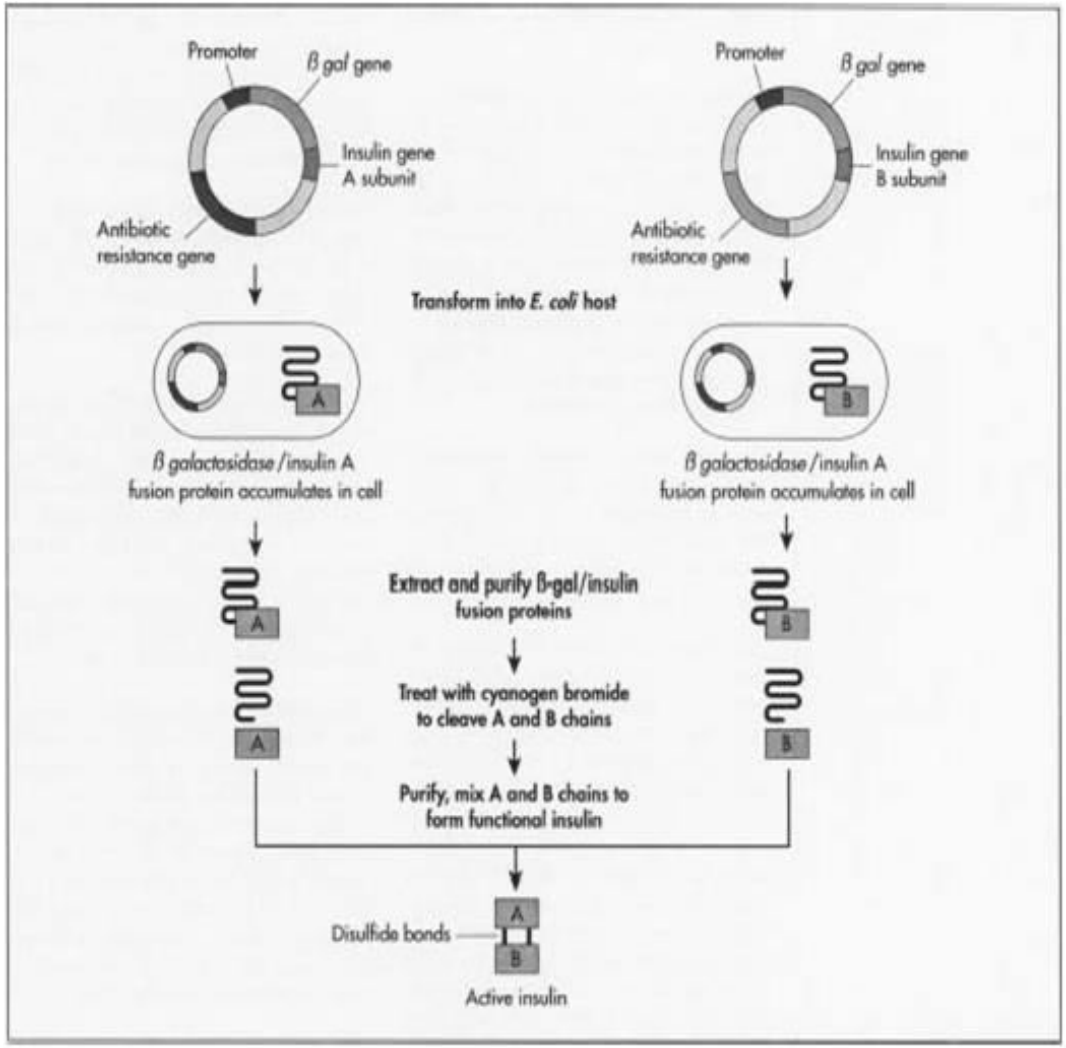
Production of Recombinant Insulin

The gene sequence of determining the A chain has been fused to the ß-galactosidase gene (lac Z) of *E.coli*. The whole lac-Z-A chain fusion is cloned into pBR322. Bacteria with this plasmid synthesize ß-galactosidase with the insulin A chain.

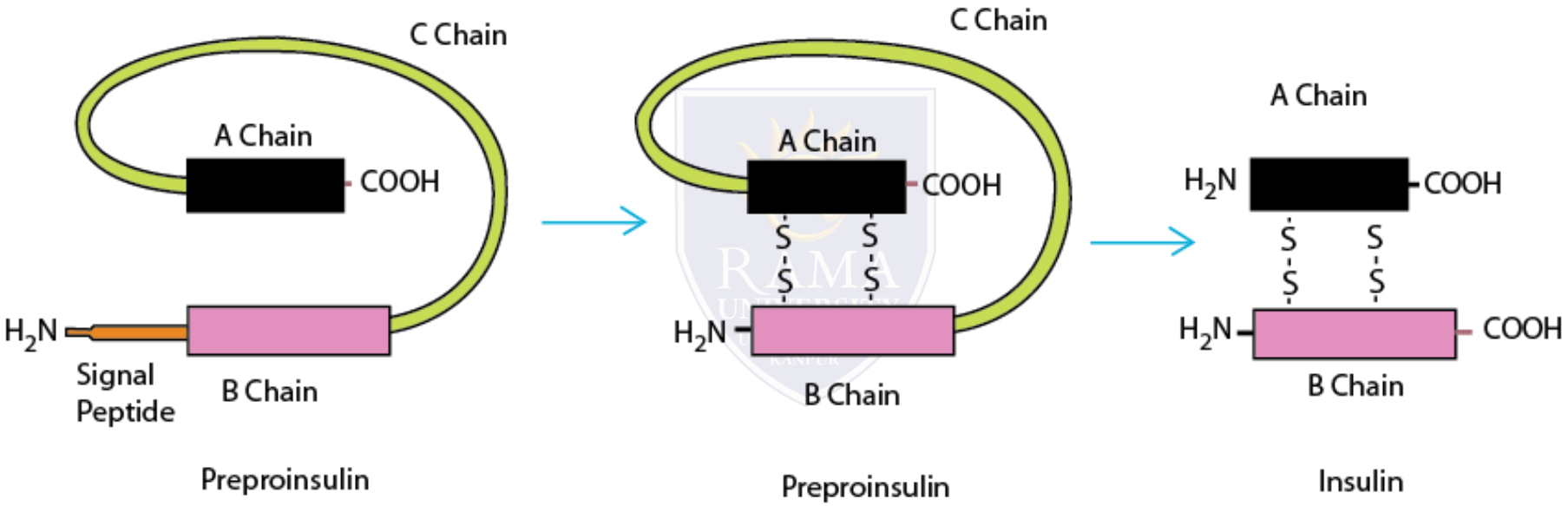
The B chain is produced in an identical manner.

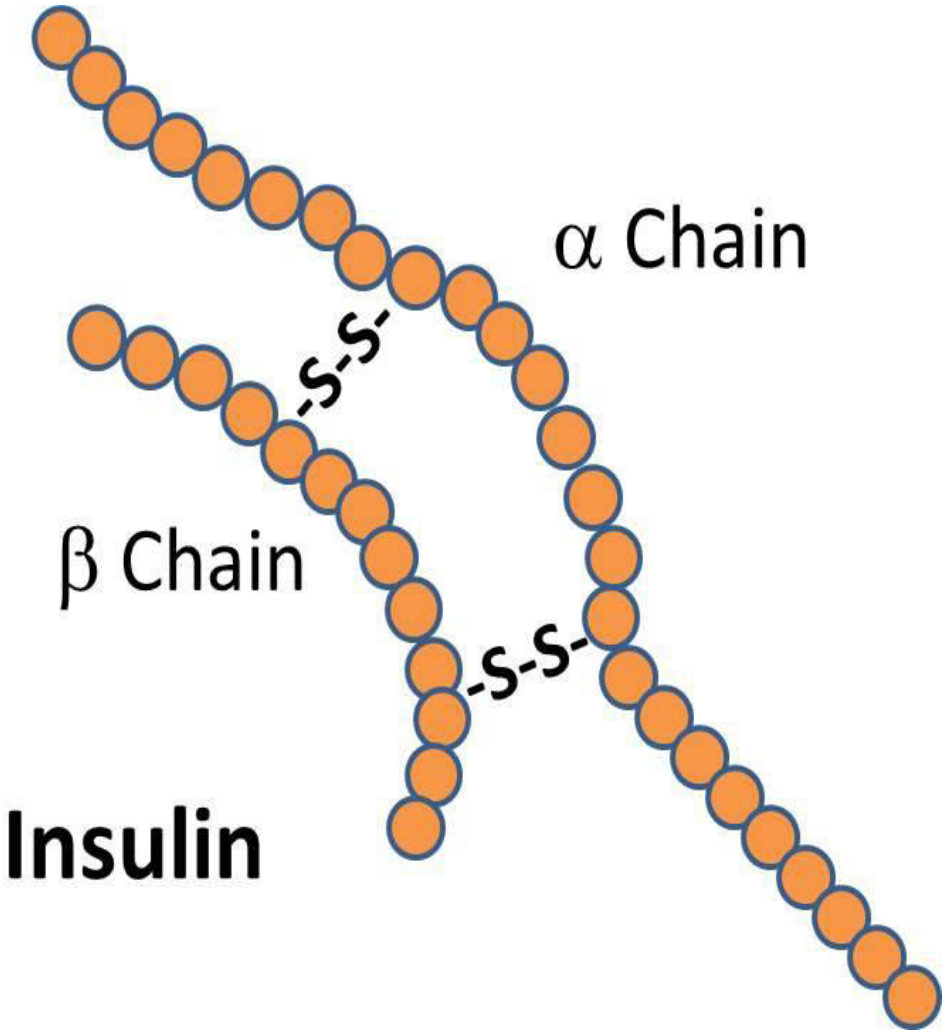
After purification of the two chains they are mixed , oxidized and then reduced which allows the disulphide bridges to form and active insulin to be produced.

Production of Recombinant Insulin

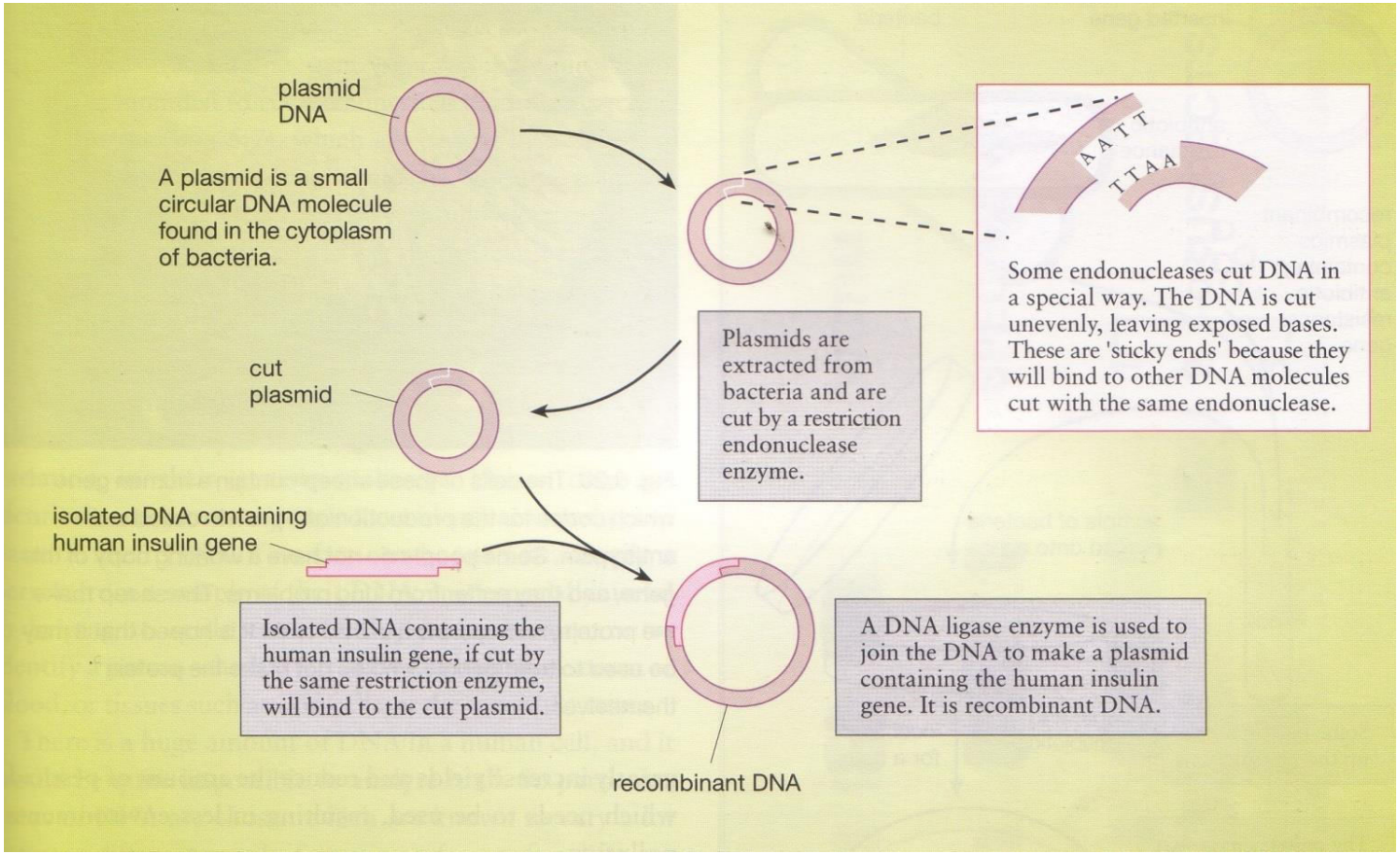


Production of Recombinant Insulin




α Chain
-S-S-
β Chain
-S-S-
Insulin

Inserting the vector into the required organism (*E. coli).*



Inserting the vector into the required organism (*E. coli).*

The **recombinant plasmid** is inserted into the bacteria by the process of transformation.

The recombinant bacteria are sorted by growing them in the presence of an **antibiotic**. The bacteria which survive are the ones which have taken up the plasmid.

They are said to be **transformed**.

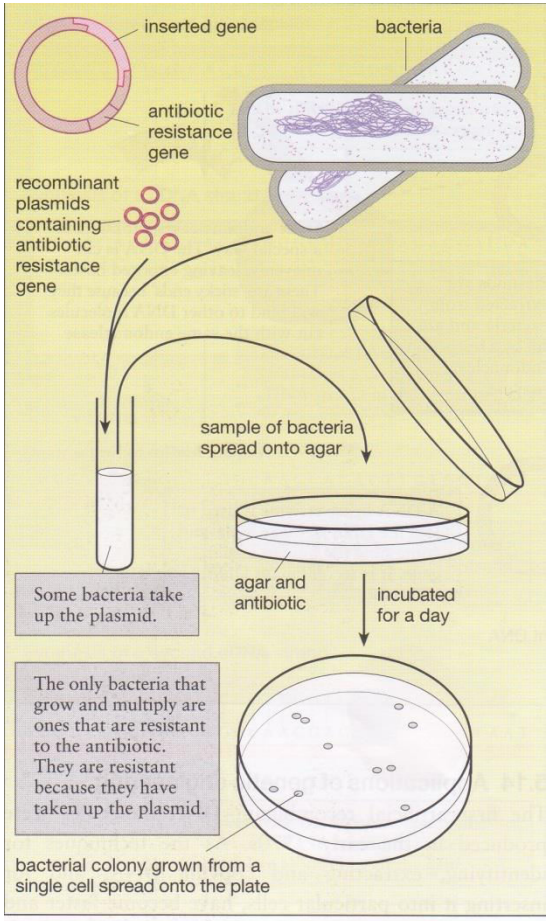


Fig. 5.19 Identifying transformed bacteria.

Insulin Production by Bacteria

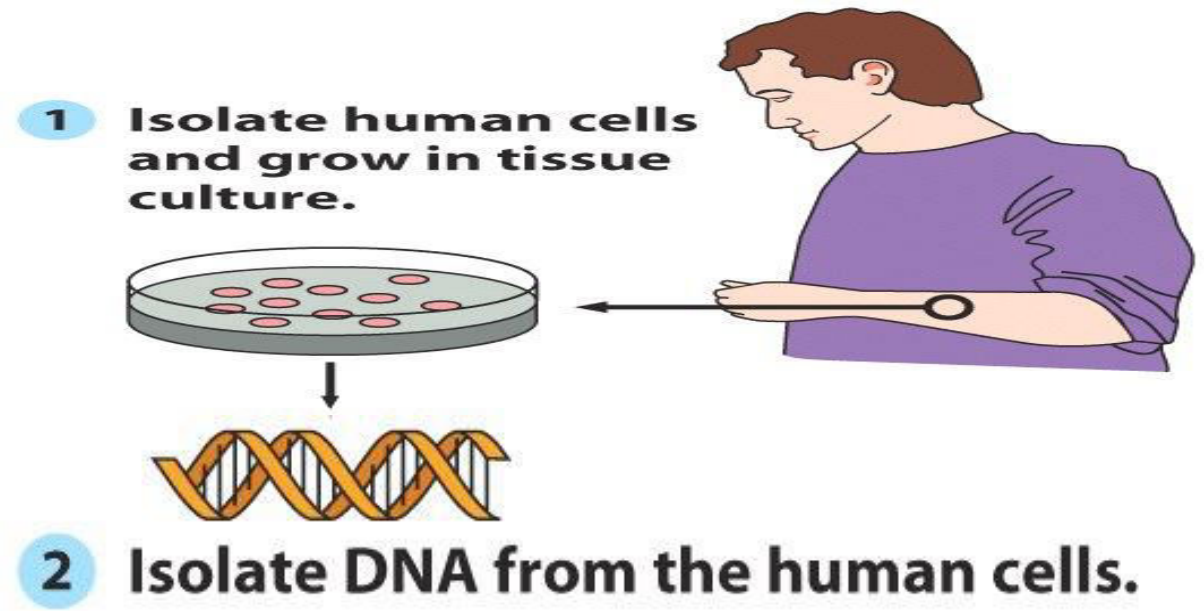


Figure 4-3 (1) Biology Today, 3/e (© 2004 Garland Science)

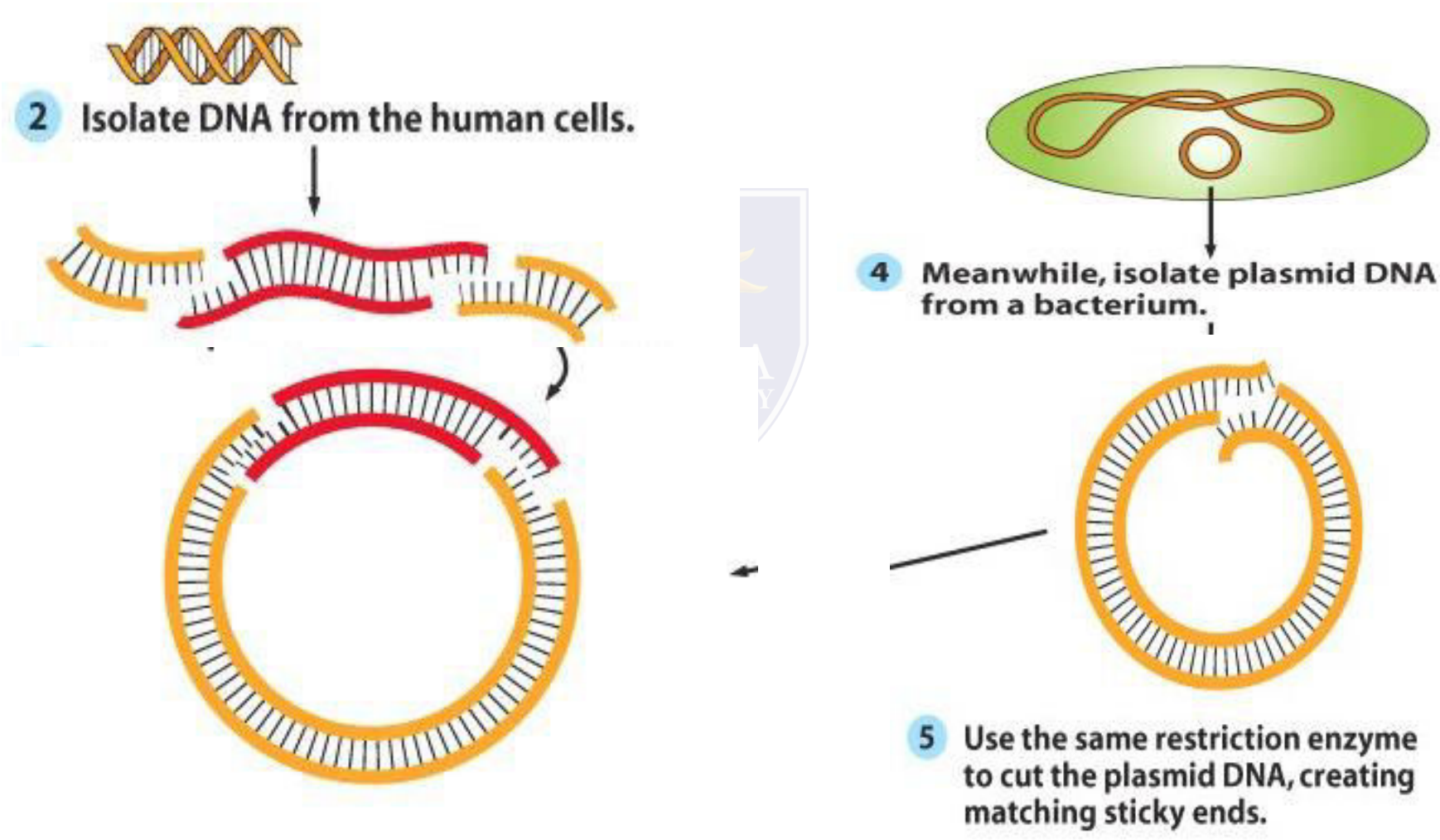
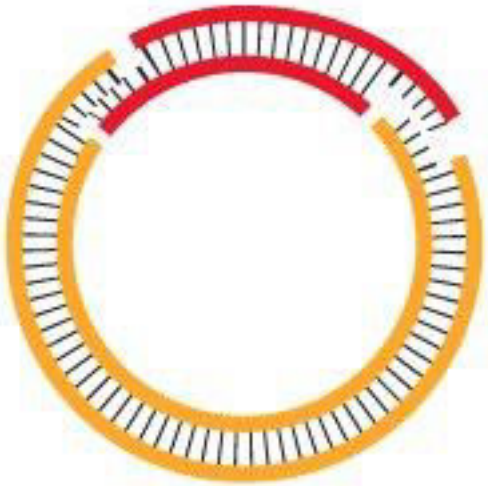
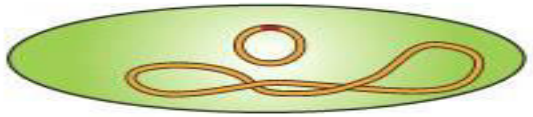
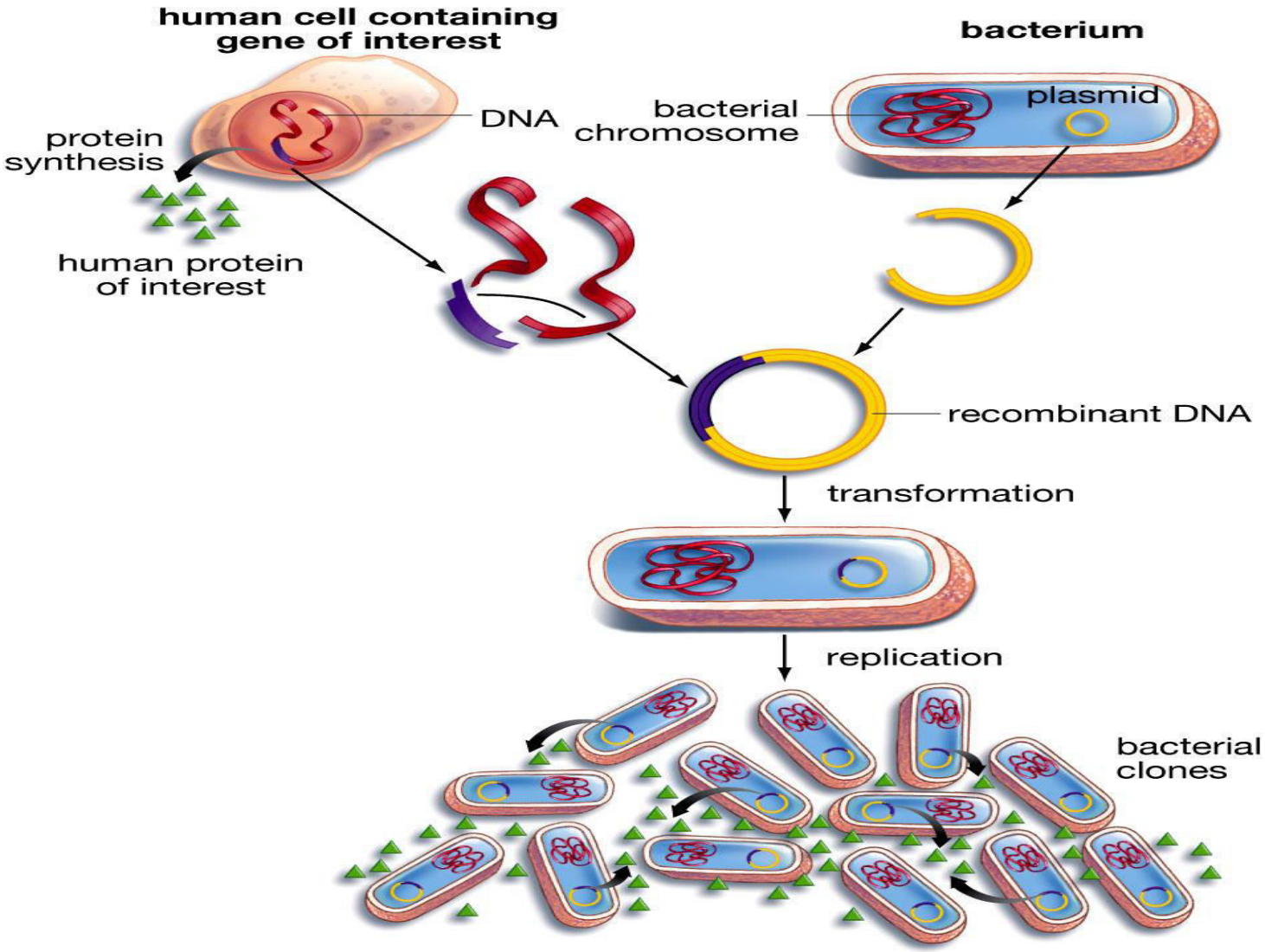


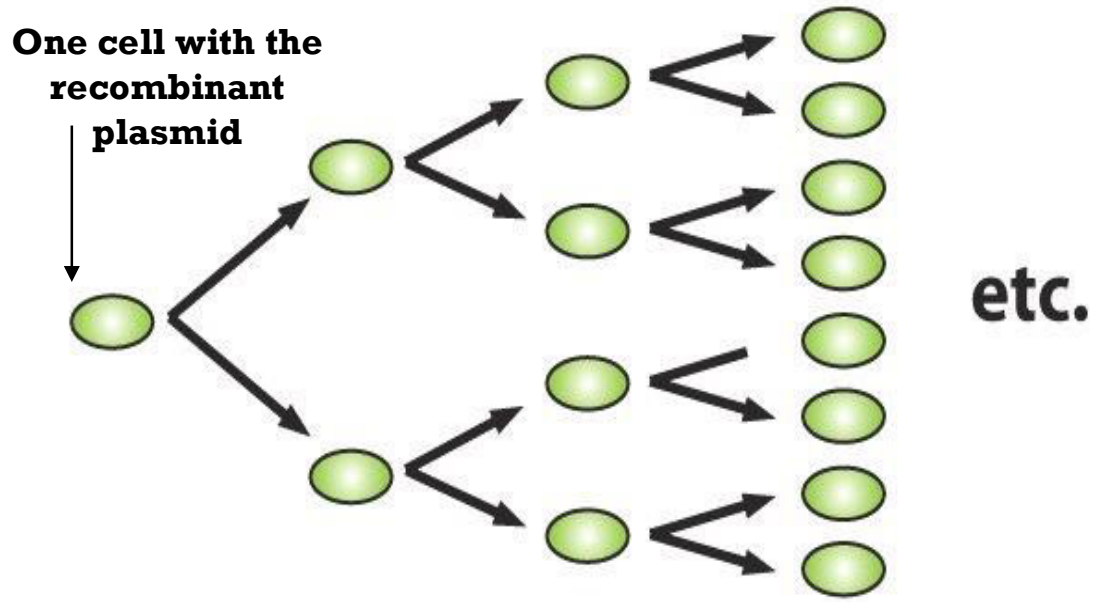
Figure 4-3 (2) Biology Today, 3/e (© 2004 Garland Science)





7 Allow new bacteria to incorporate the recombinant plasmid into the bacterial cell, then screen bacteria to find the ones that have incorporated the human gene for insulin.


human cell containing gene of interest
bacterium
DNA
bacterial chromosome
plasmid
protein synthesis
human protein of interest
recombinant DNA
transformation
replication
bacterial clones



8 Grow trillions of new insulin-producing bacteria.

Figure 4-3 (4) Biology Today, 3/e (© 2004 Garland Science)