



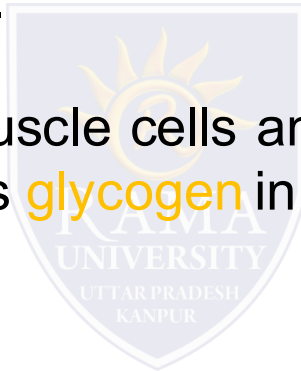
FACULTY OF ENGINEERING & TECHNOLOGY  
DEPARTMENT OF BIOTECHNOLOGY

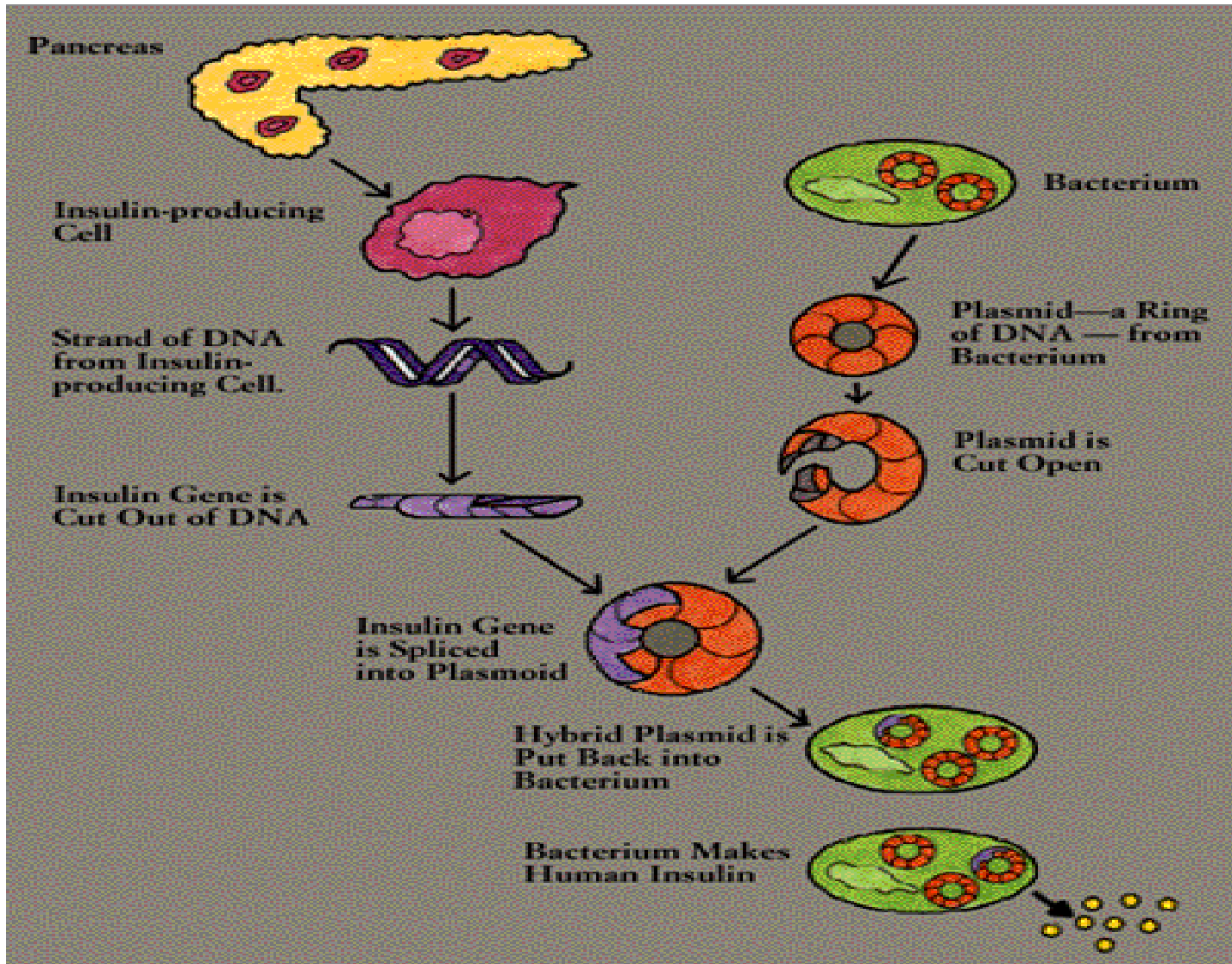
# Production of recombinant Insulin

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

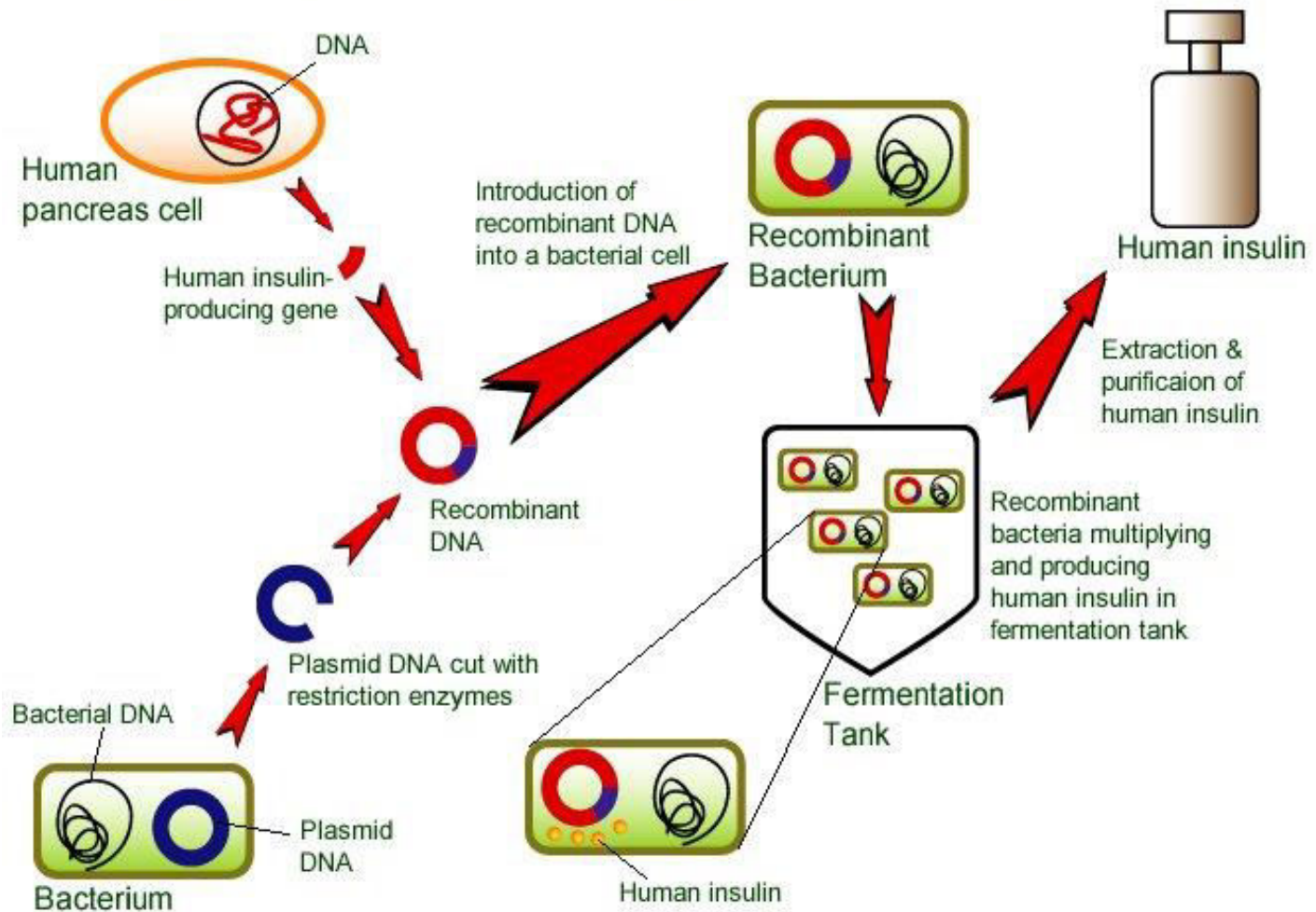
Insulin is secreted by the **Islets of Langerhans** of pancreas which catabolizes glucose in blood.

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.





# 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 secretory 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.

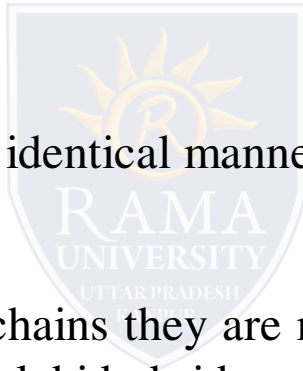
⦿ There are several strategies for producing insulin from bacteria, but the most successful is to synthesize the A and B separately and then join them together.

## Production of Recombinant Insulin

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

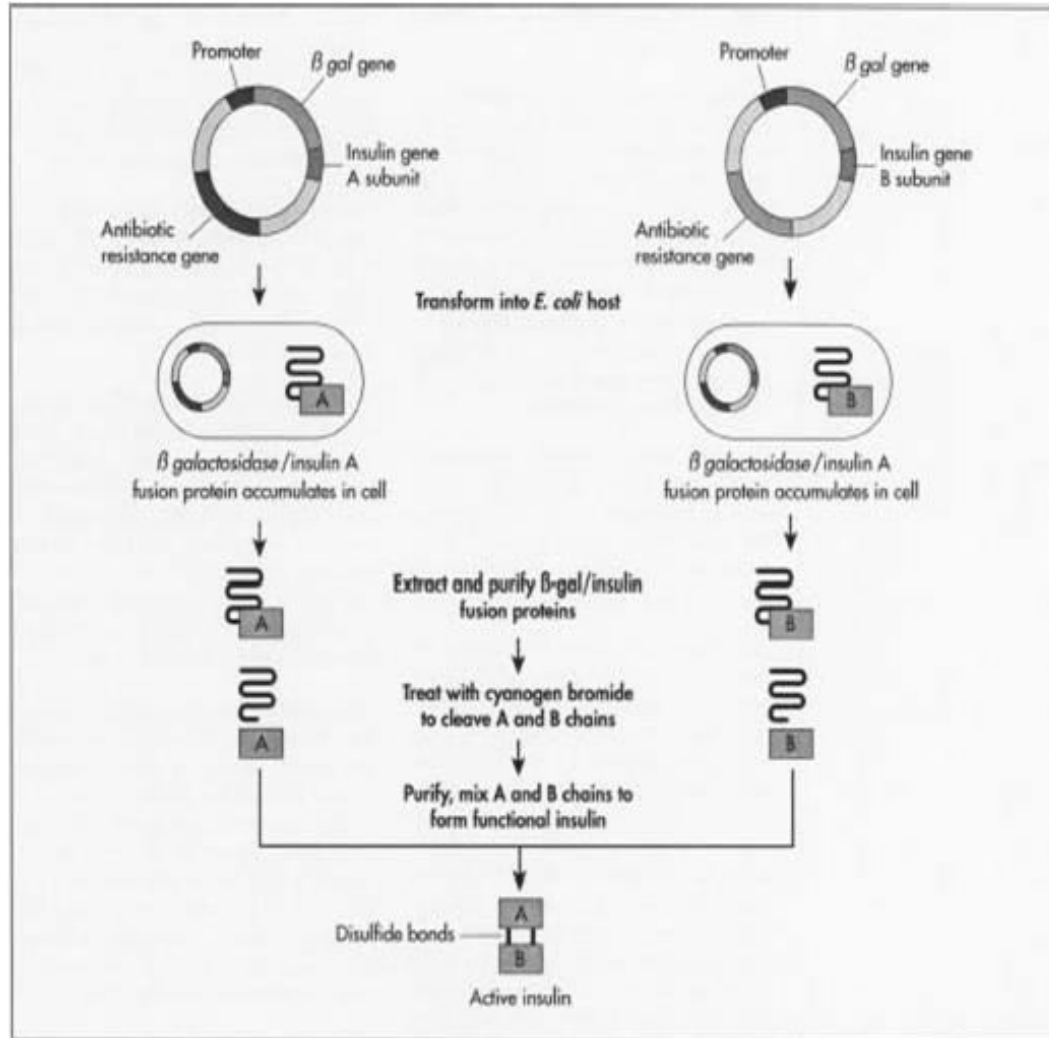
The B chain is produced in an identical manner.

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.

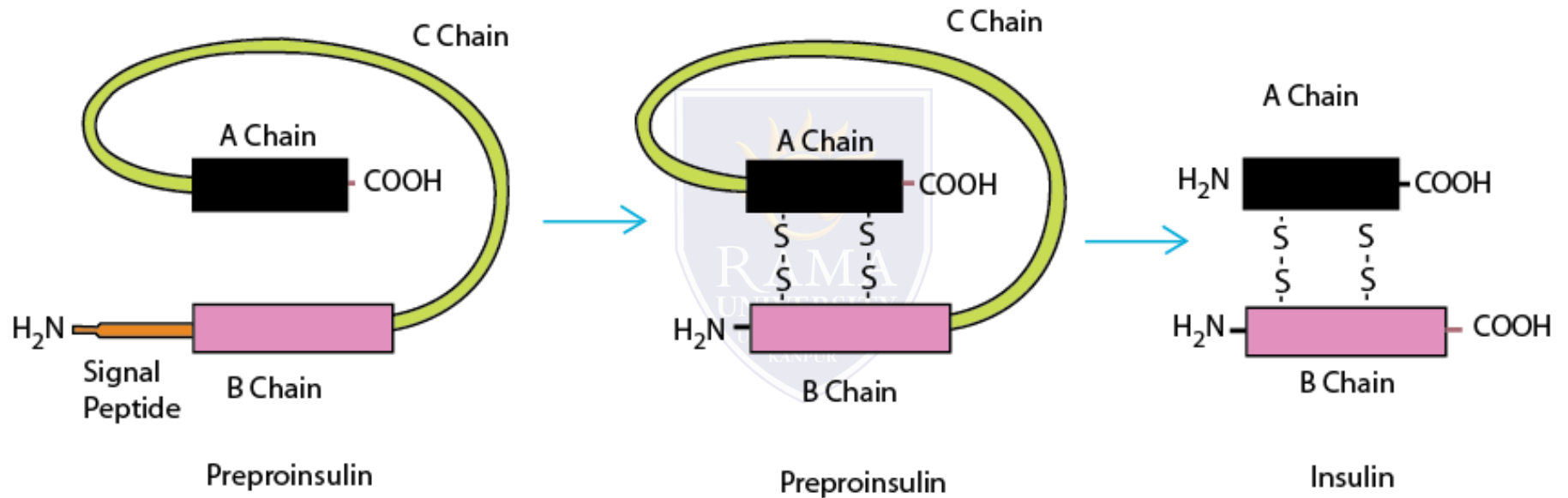


# Production of Recombinant Insulin

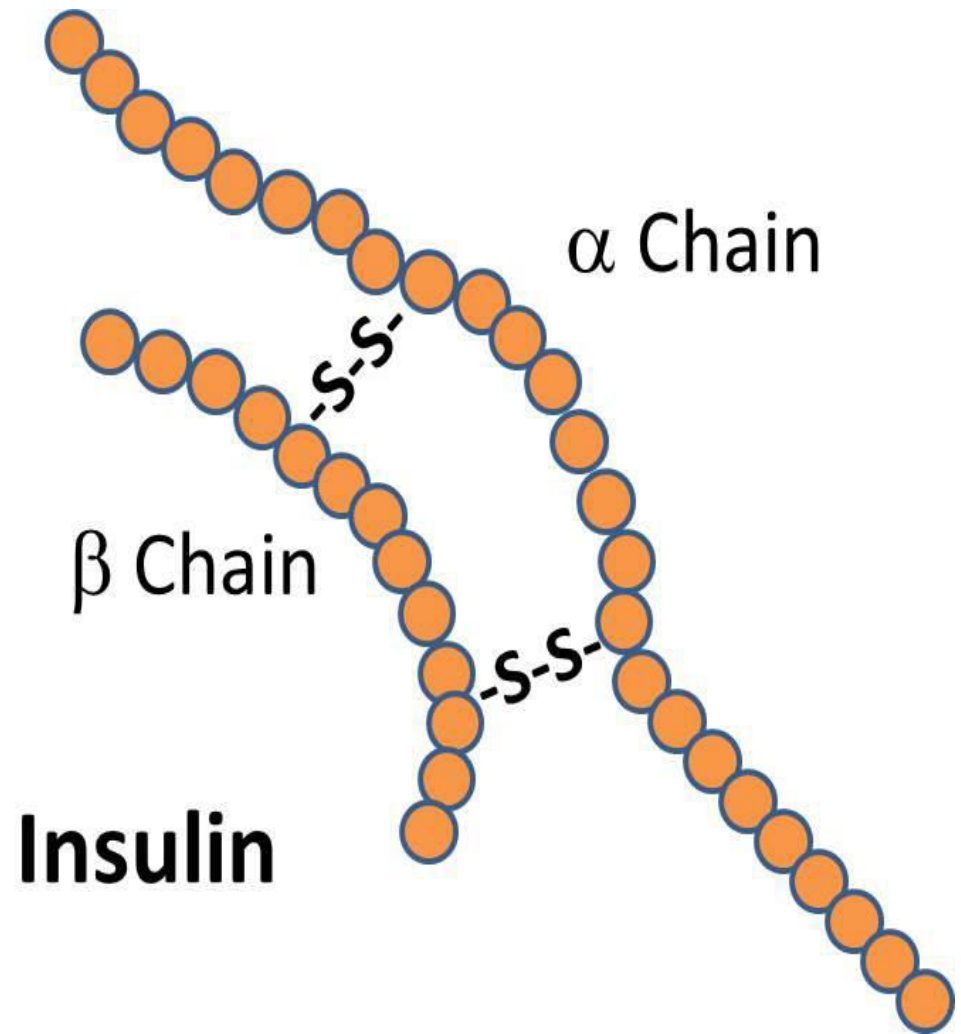
## Production of Recombinant Insulin



## Production of Recombinant Insulin





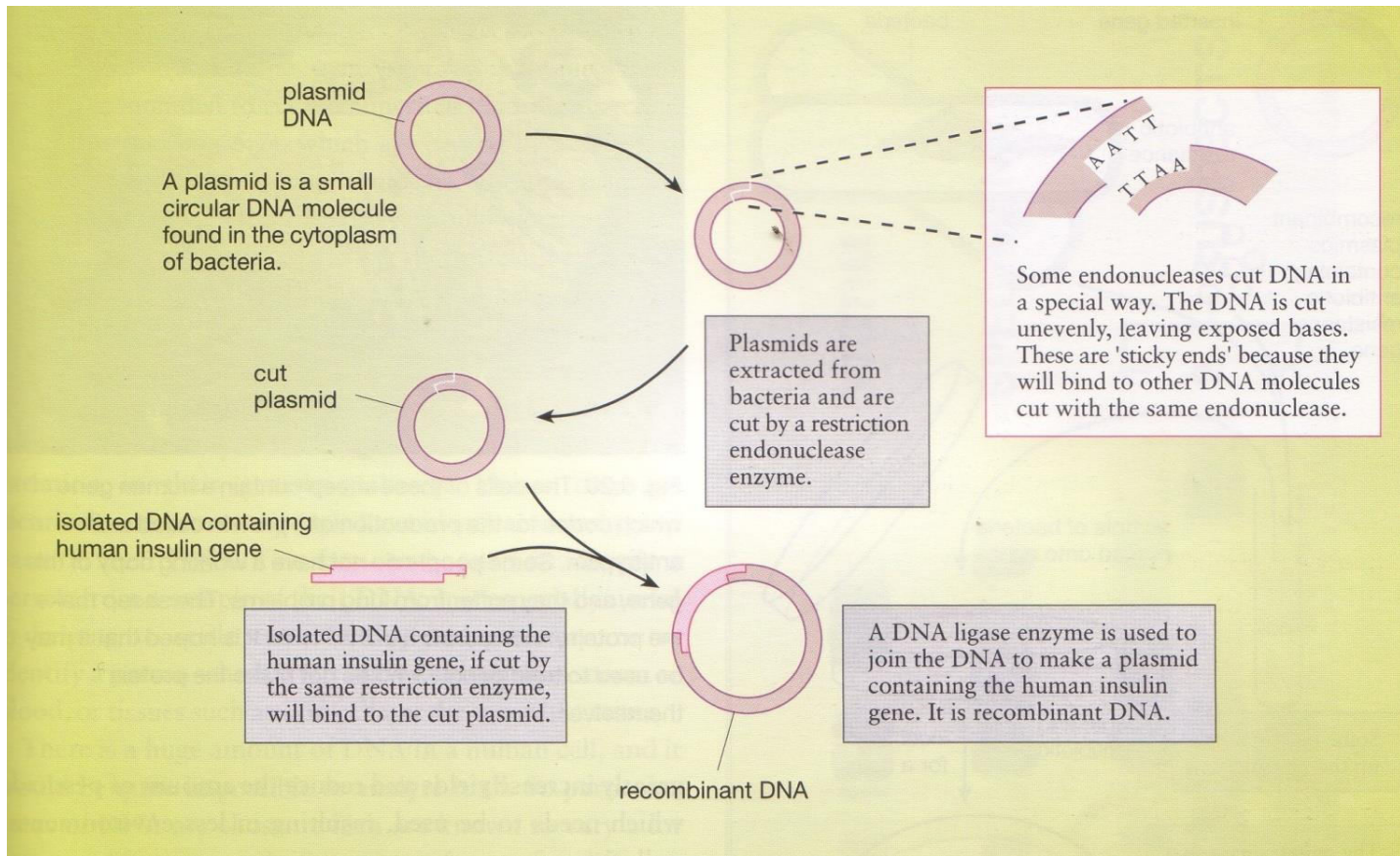


$\alpha$  Chain

$\beta$  Chain

**Insulin**

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



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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**.

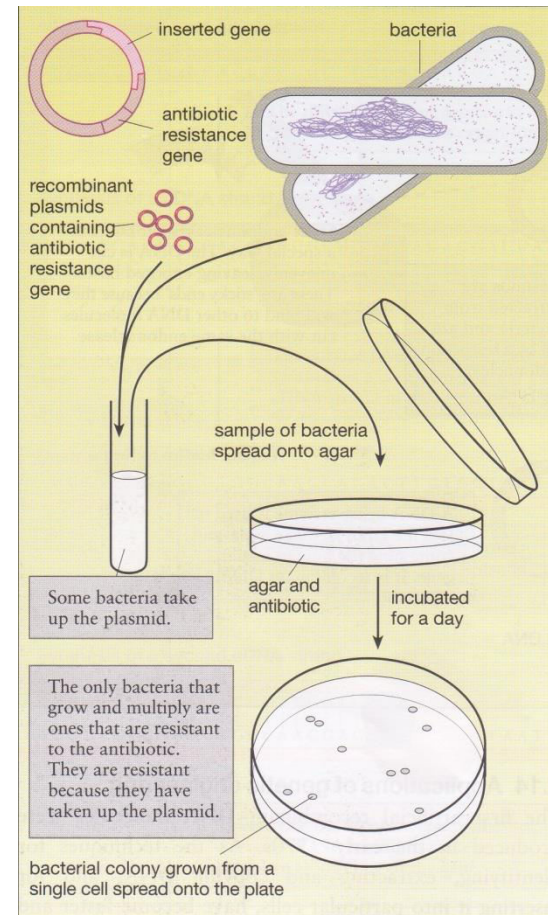


Fig. 5.19 Identifying transformed bacteria.

# Insulin Production by Bacteria

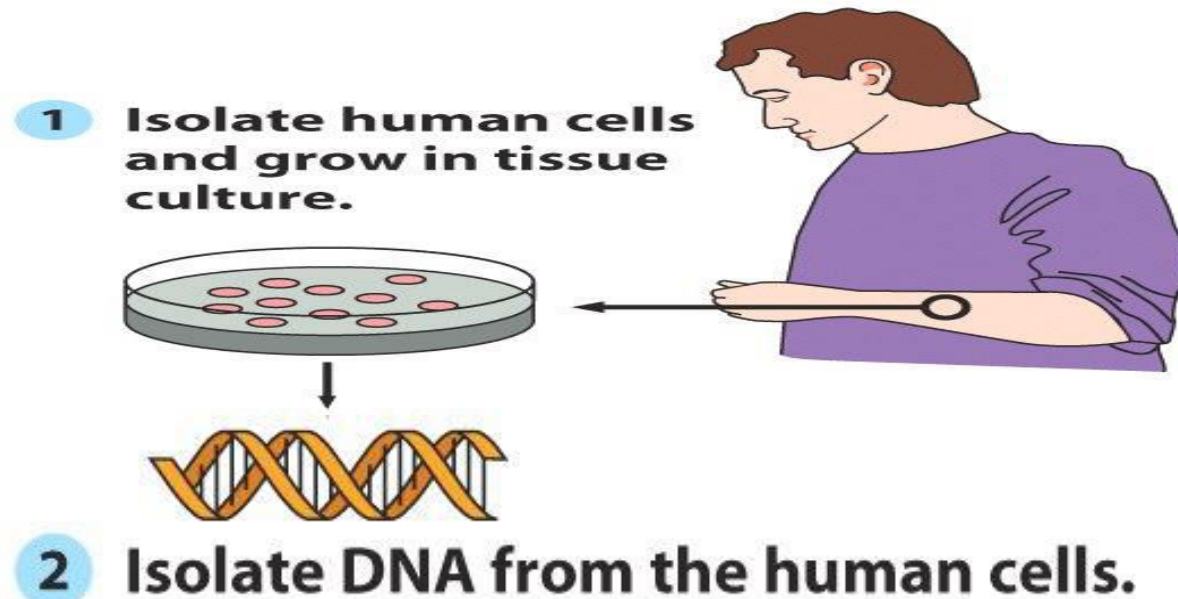


Figure 4-3 (1) Biology Today, 3/e (© 2004 Garland Science)

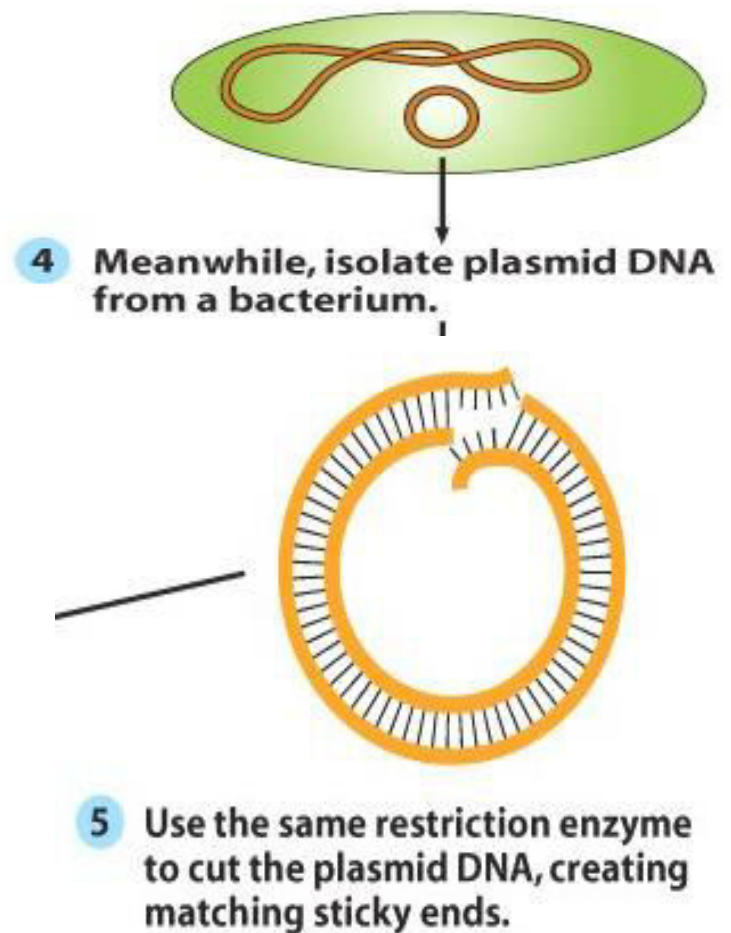
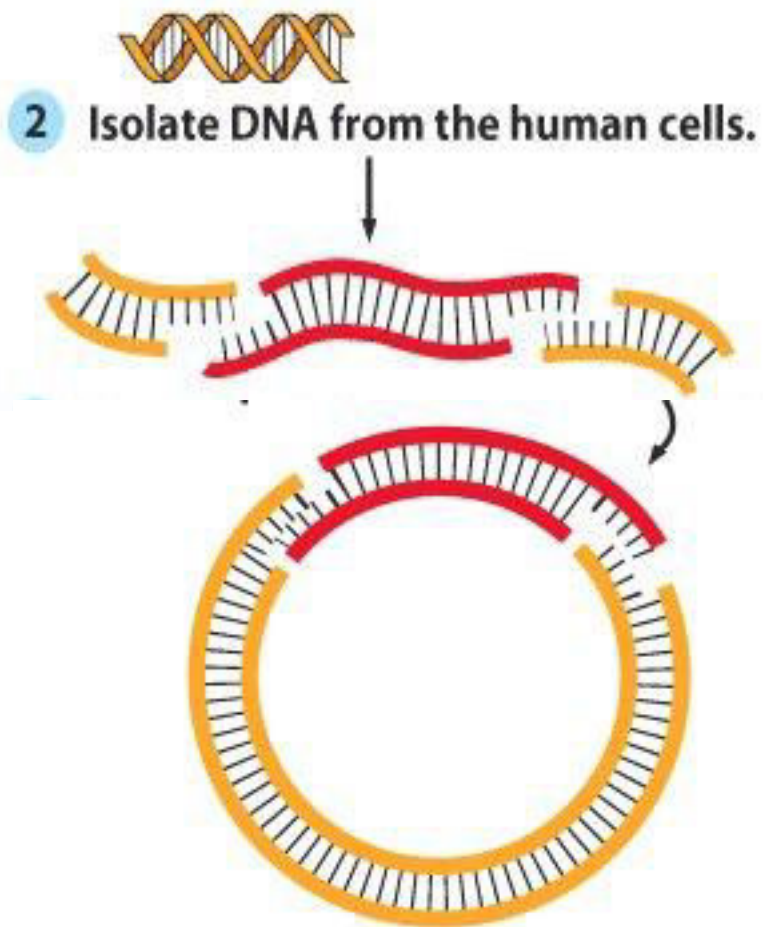
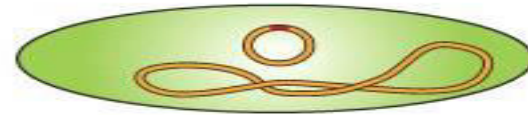
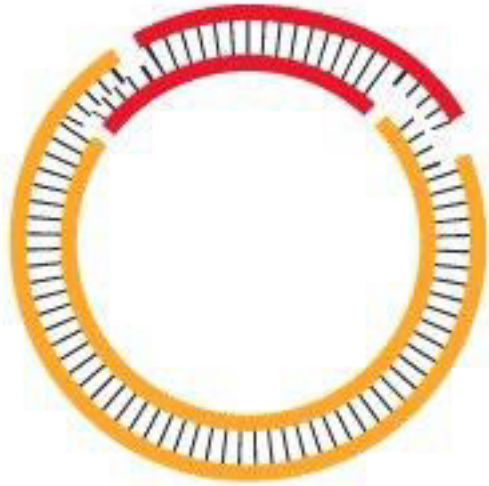


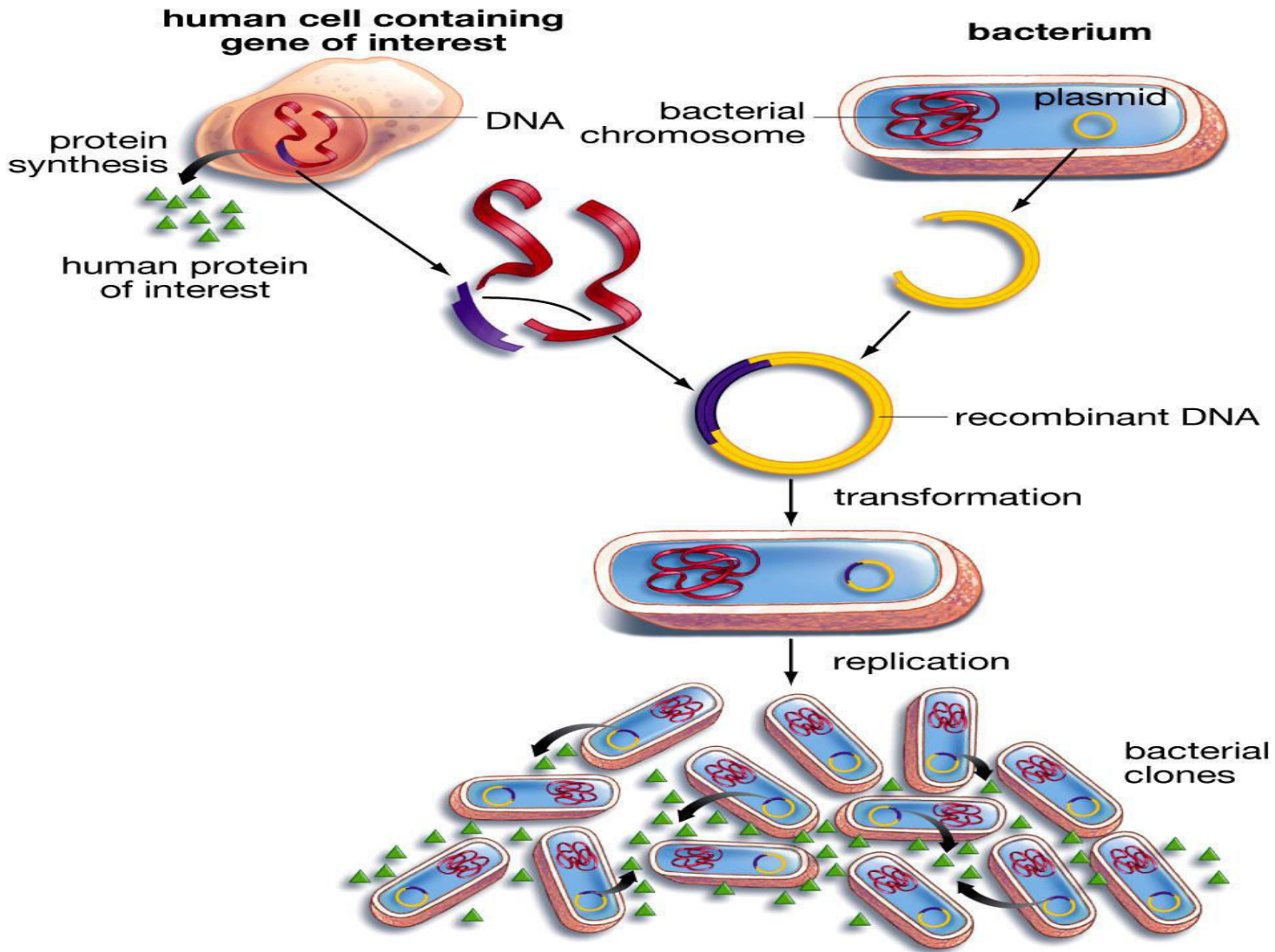
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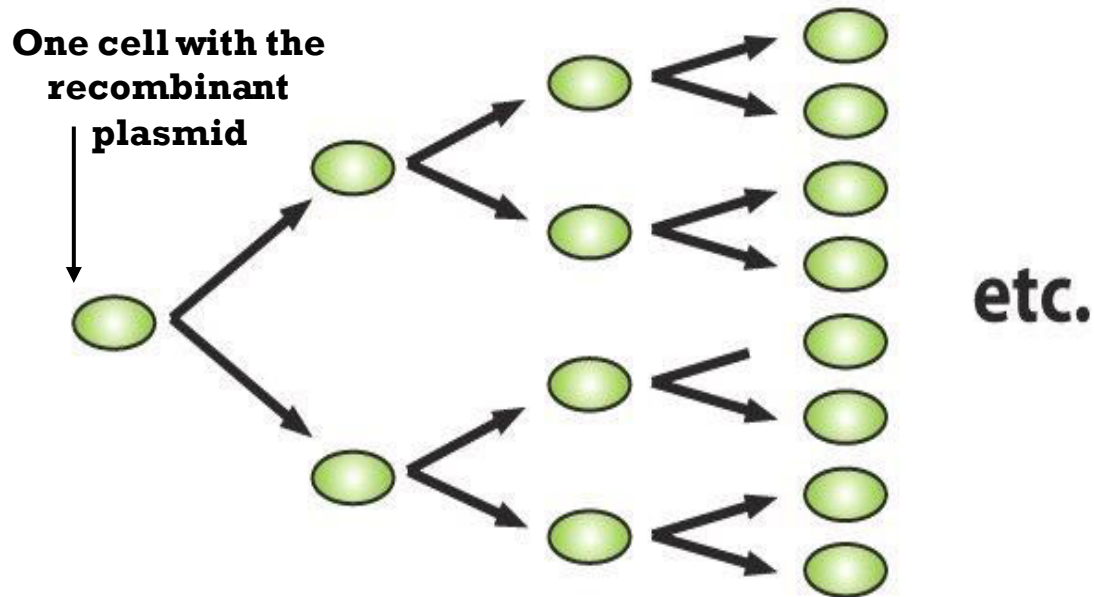




- 7** Allow new bacteria to incorporate the recombinant plasmid into the bacterial cell, then screen bacteria to find the ones that have incorporated the human gene for insulin.







## 8 Grow trillions of new insulin-producing bacteria.

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