Genetics

Biology Dep.3rd Stage

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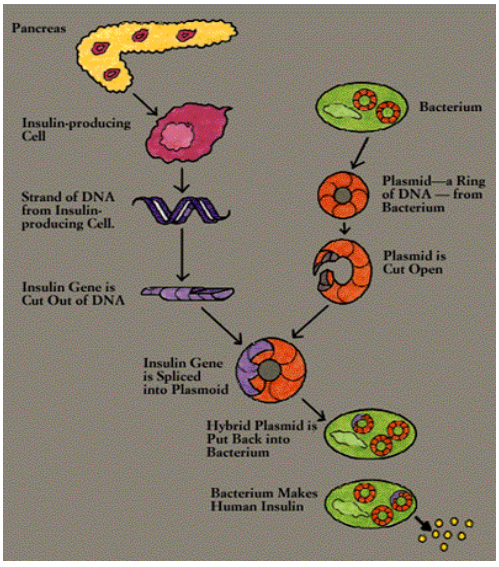
Lec-9-

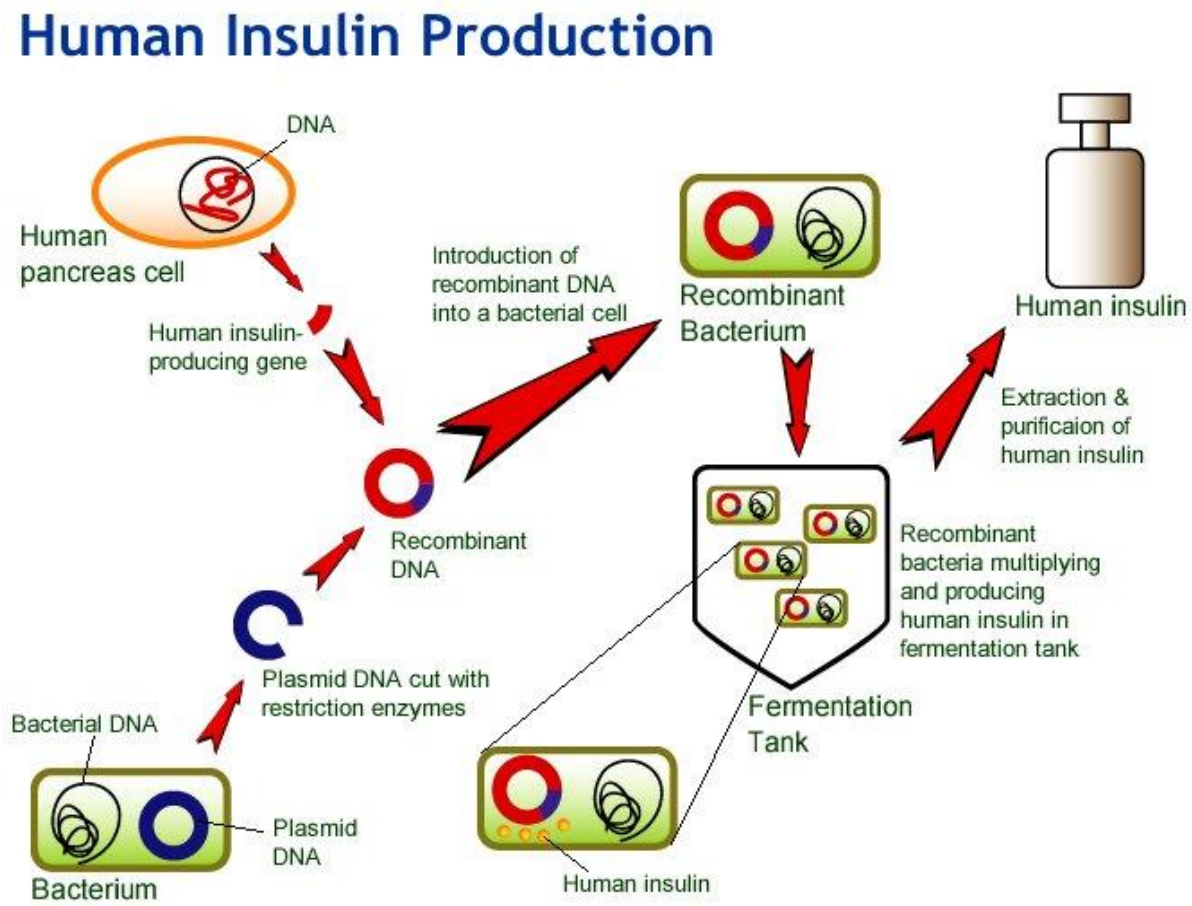
Insulin Production by recombinant DNA technology

- 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.

Insulin Production



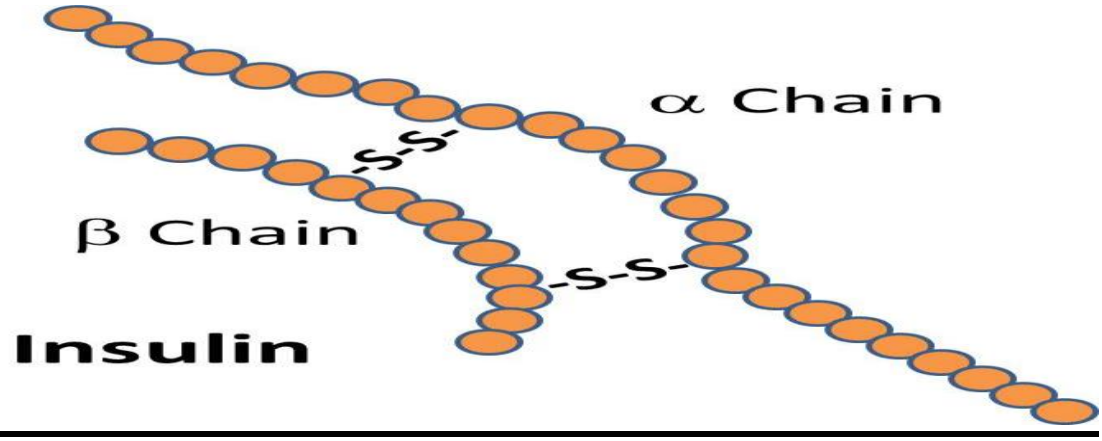


Structure

- 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.
- 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.



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

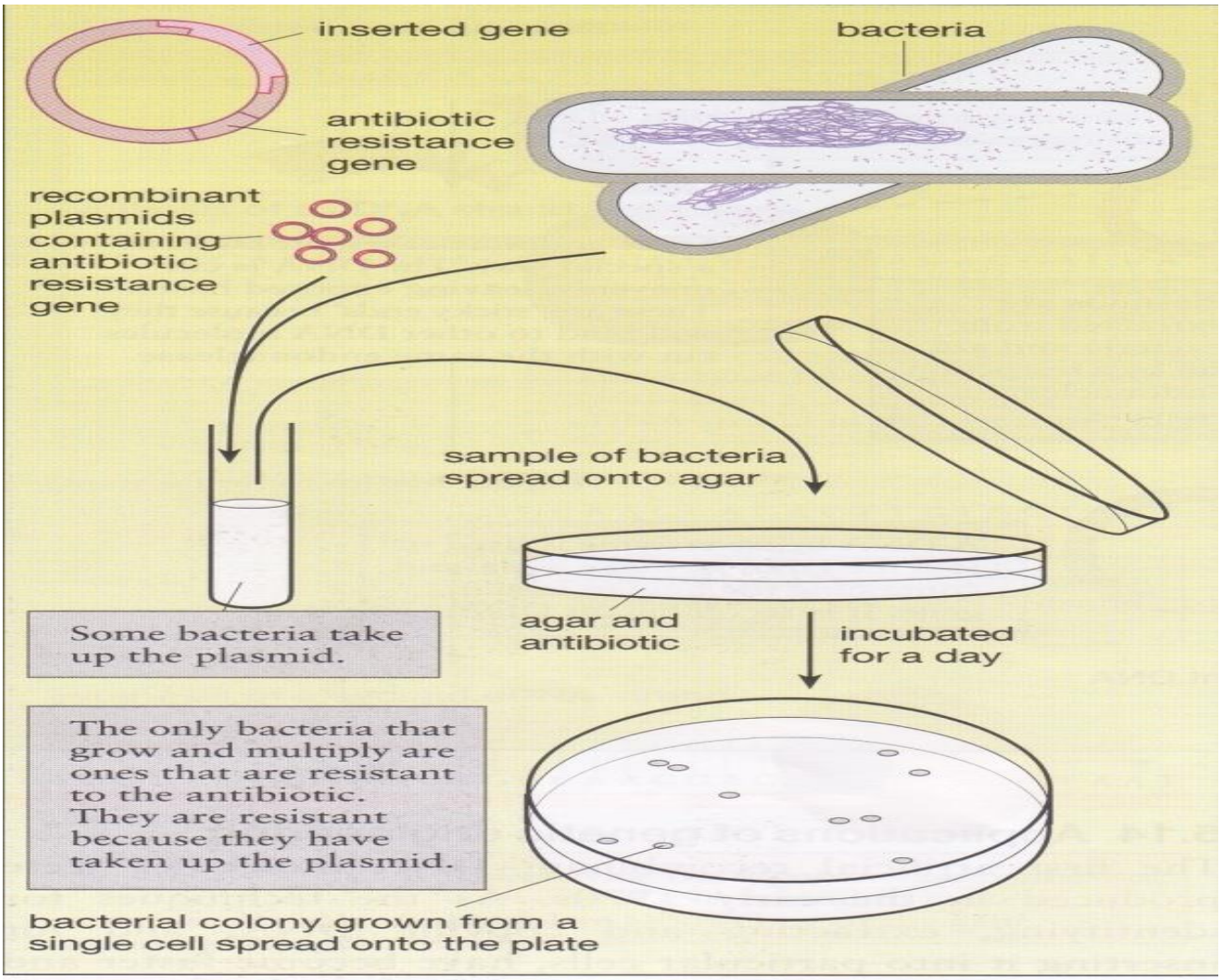
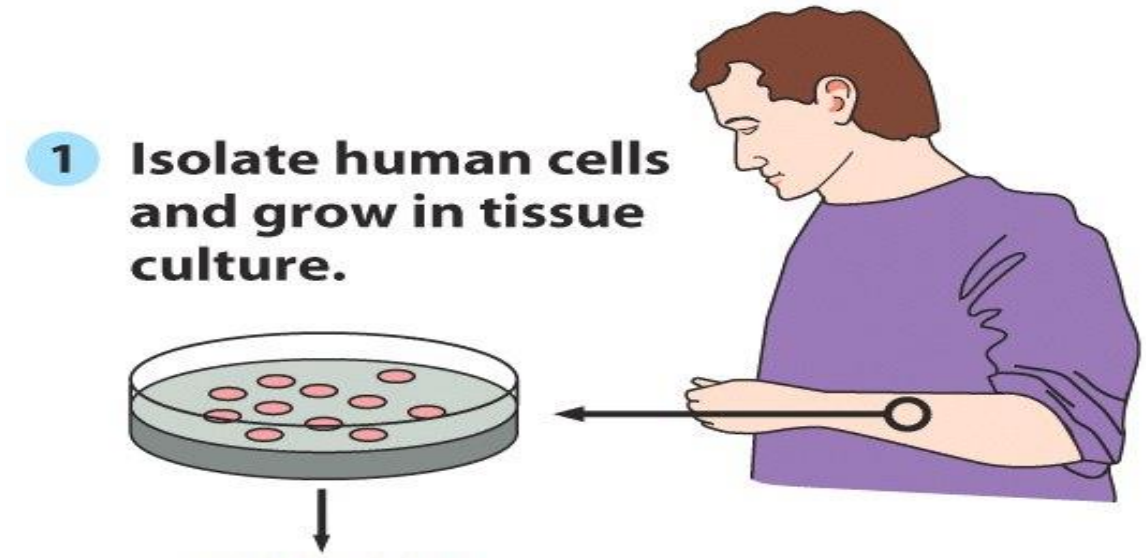


Fig. 5.19 Identifying transformed bacteria.

Human Insulin Production by Bacteria



2 Isolate DNA from the human cells.

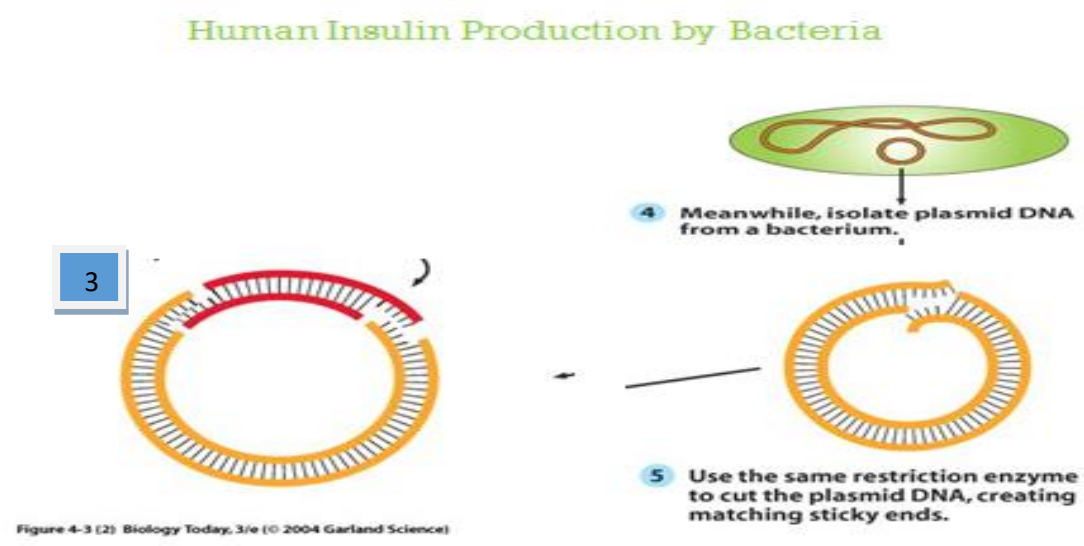
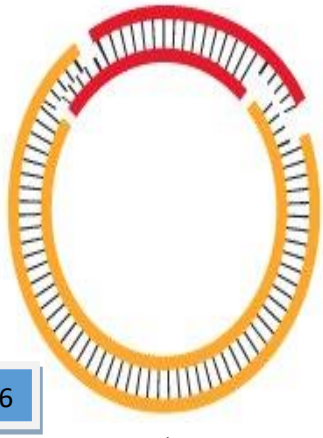


Figure 4-3 (2) Biology Today, 3/e (© 2004 Garland Science)

Human Insulin Production by Bacteria

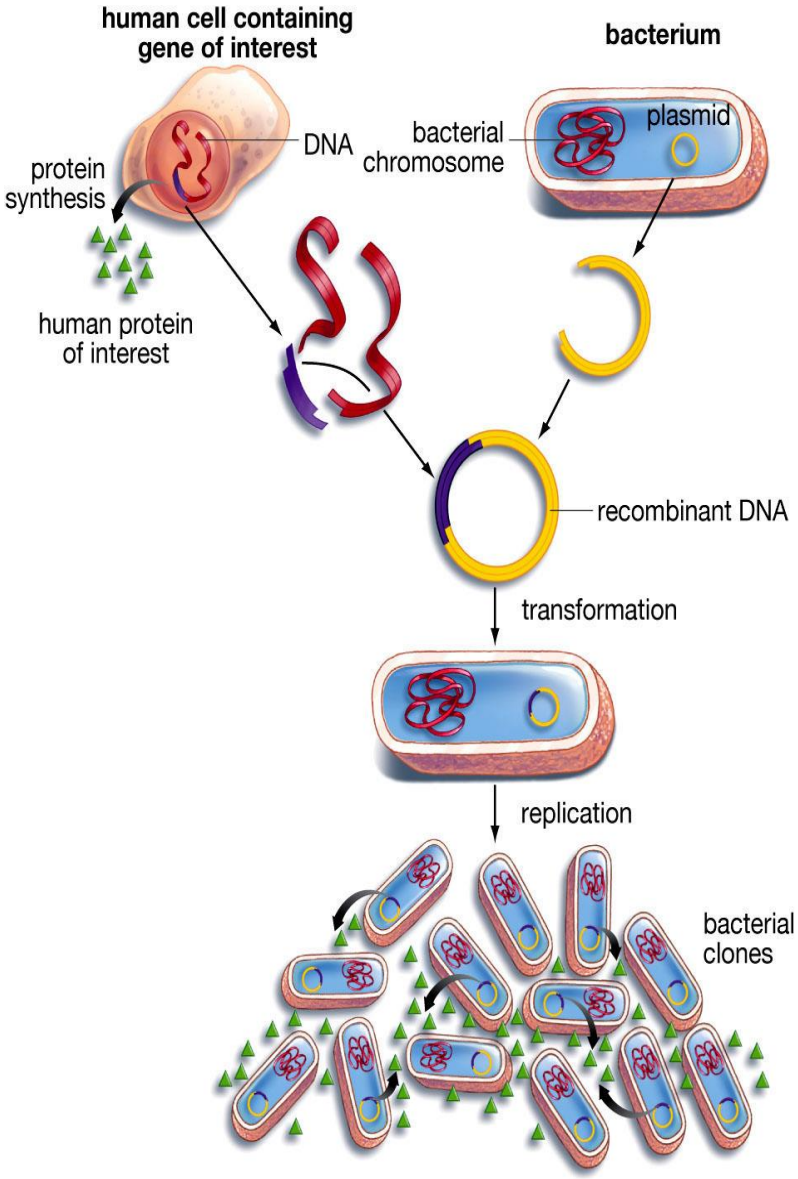


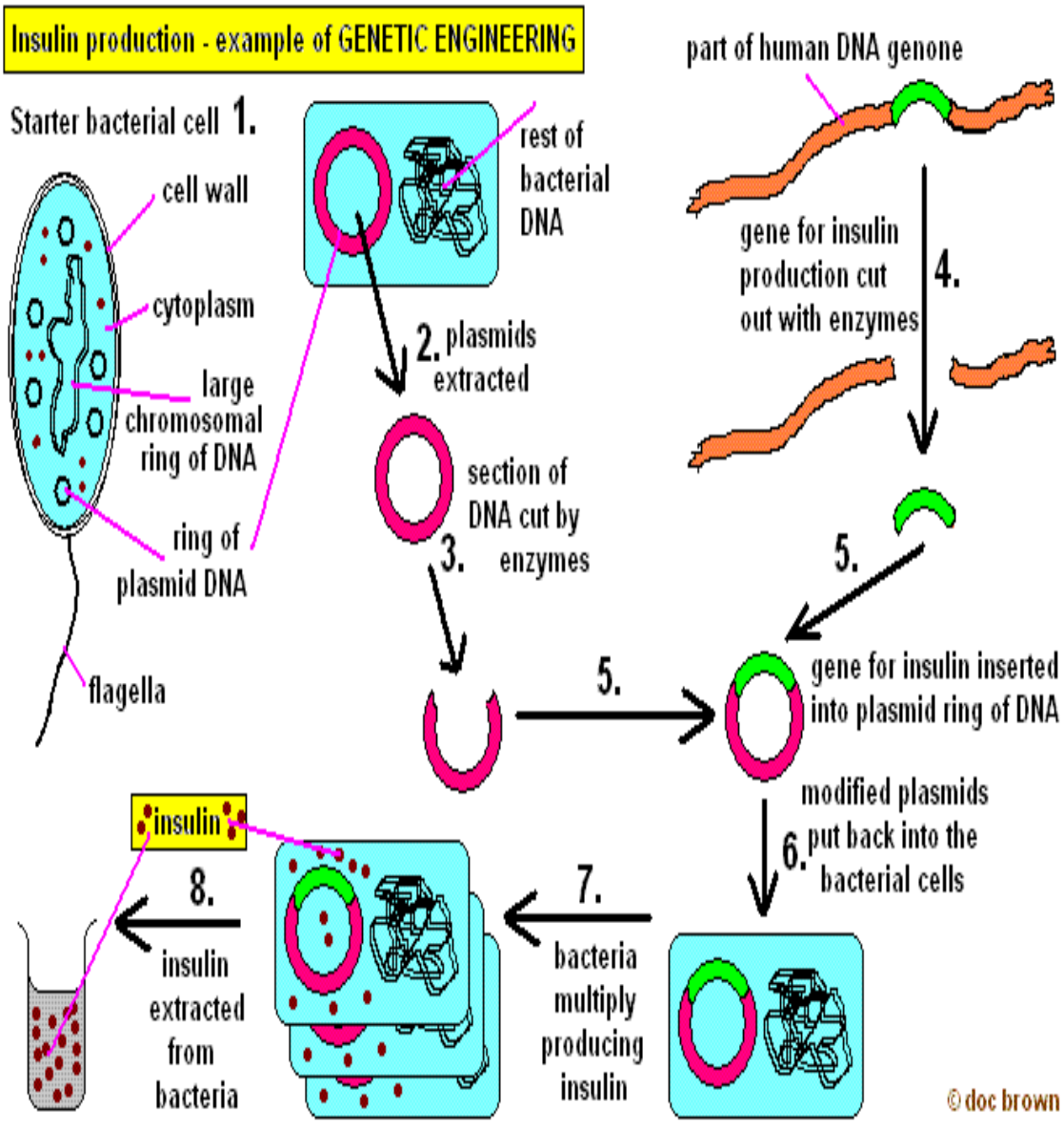
6

Mix the recombinant plasmid with bacteria.

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 Insulin Production by Bacteria




Insulin production - example of GENETIC ENGINEERING
Starter bacterial cell 1.
cell wall
cytoplasm
large chromosomal ring of DNA
ring of plasmid DNA
flagella
rest of bacterial DNA
2. plasmids extracted
section of DNA cut by enzymes
3.
part of human DNA genone
gene for insulin production cut out with enzymes
4.
5.
5.
gene for insulin inserted into plasmid ring of DNA
modified plasmids
6. put back into the bacterial cells
7.
bacteria multiply producing insulin
8.
insulin extracted from bacteria
insulin
© doc brown

Human Insulin Production by Bacteria

The final steps are to collect the bacteria, break open the cells, and purify the insulin protein expressed from the recombinant human insulin gene.