Production of Human Insulin with Recombinant DNA Technology

Human insulin was one of the first examples of a medicine being produced by modern biotechnology. Before genetically engineered human insulin was available, people with diabetes relied on insulin from cows and pigs. However animal insulin is slightly different from human insulin and may cause adverse effects or allergies. In the 1980s, human insulin became increasingly available through the use of recombinant DNA technology.

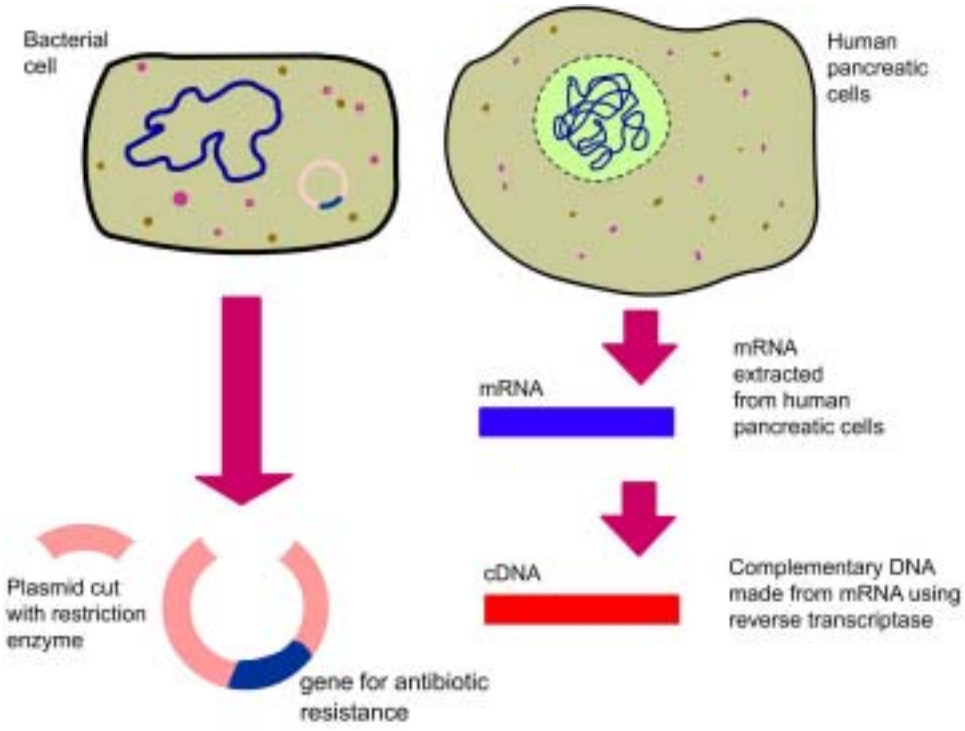
1. Structure of human insulin

Insulin consists of two separate polypeptide chains (A and B chains) which are held together by two disulphide bonds. The A chain contains 21 amino acids and the B chain contains 30 amino acids.

2. Obtaining the human insulin gene

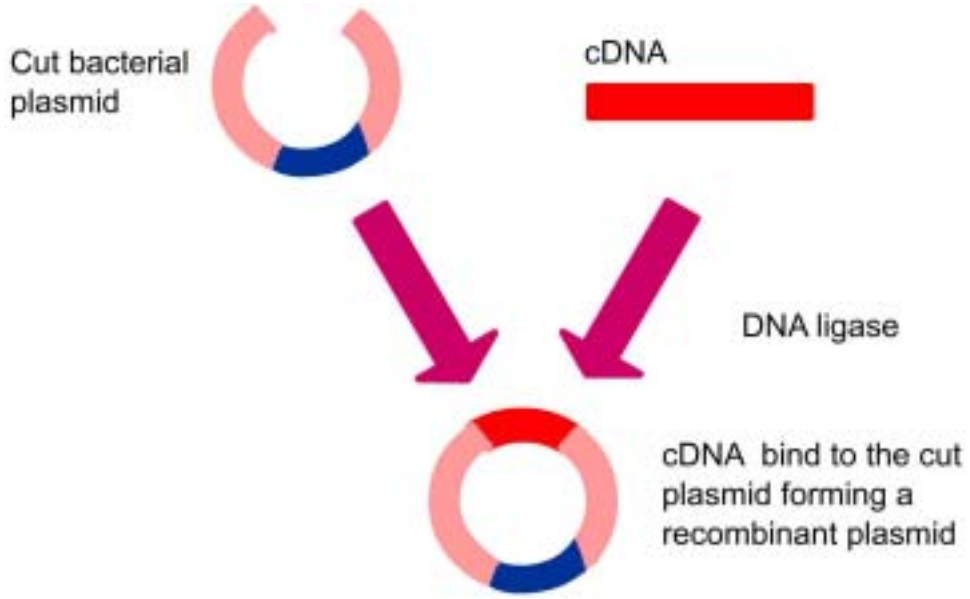
Human insulin gene can be obtained by making a complementary DNA (cDNA) copy of the messenger RNA (mRNA) for human insulin. Messenger RNAs from human pancreas beta cells are isolated and purified. Special enzyme (reverse transcriptase) is used to make cDNA from the insulin mRNA. The single-stranded DNA is then used to make double-stranded DNA molecules.

Plasmids that carry a gene for antibiotic resistance, are often used as vectors in recombinant DNA technology. The bacterial cloned cells can grow in culture medium containing antibiotics, so that only those cells with recombinant DNA can survive in the cell culture. Bacterial cells that do not contain recombinant DNA cannot grow.



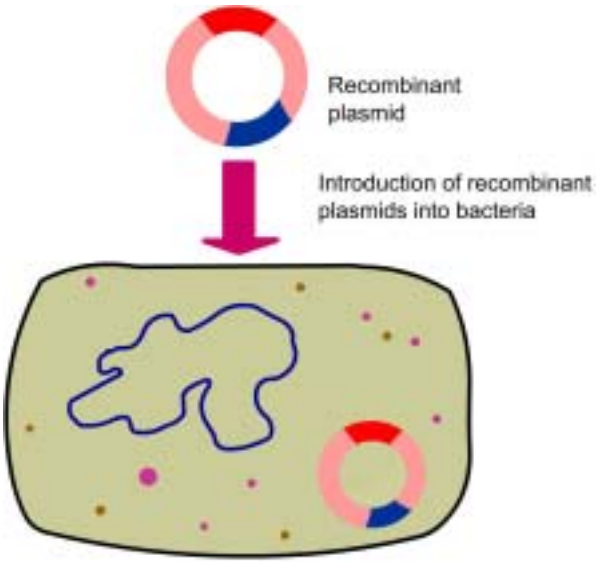
3. Joining the human insulin gene into a plasmid vector

The bacterial plasmids and the cDNA are mixed together. The human insulin gene (cDNA) is inserted into the plasmid through complementary base pairing at sticky ends. DNA ligase is used to join the ends together, thereby creating a recombinant plasmid with a human insulin gene in it.



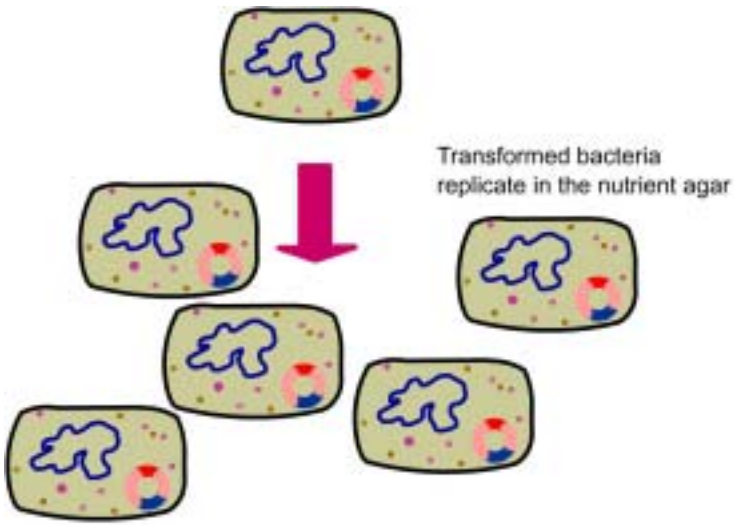
4. Introducing the recombinant DNA plasmids into bacteria

The bacteria *Escherichia coli* is used as the host cell. If *E. coli* and the recombinant plasmids are mixed together in a test-tube, some of the bacteria will take up the recombinant DNA plasmids and they are said to be transformed.



5. Selecting the bacteria which have taken up the correct piece of DNA

The bacteria are spread onto nutrient agar in a petri dish. The agar also contains substances such as an antibiotic which allows growth of only the transformed bacteria. By repeated cell division, each transformed bacterium gives rise to a visible spot called a colony with millions of identical cells.



If the transformed bacterial clones are allowed to grow in the liquid medium, the bacteria will turn on its protein synthesis machinery to synthesise human insulin.