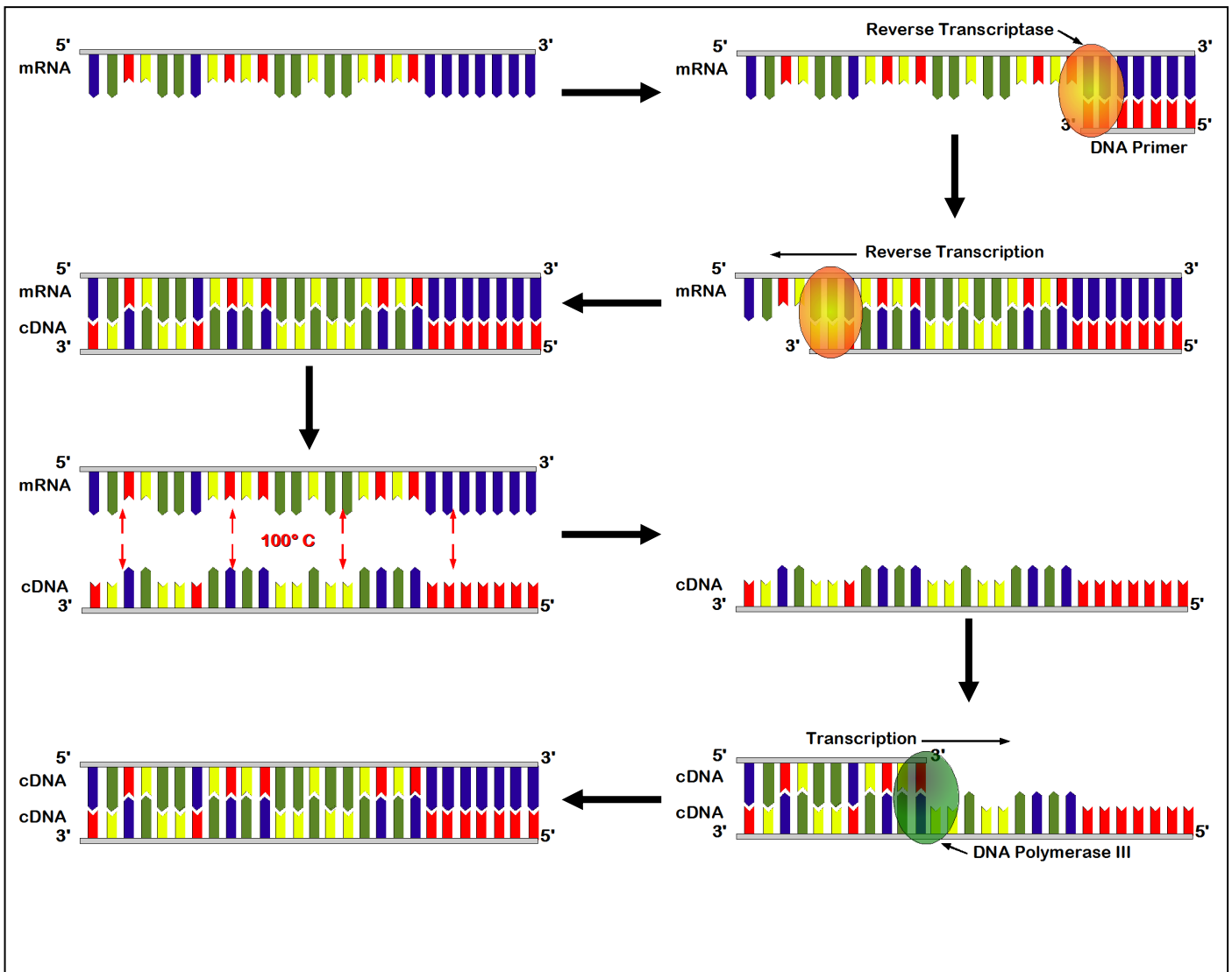


Spotlight on Biotechnology

Since people with Type 1 diabetes are unable to produce insulin, it is necessary for them receive regular insulin shots. For many years this insulin came from the pancreatic cells of cows and pigs. In recent years though, it has come from bacteria! Since bacteria do not naturally produce or use insulin, it was necessary to use recombinant DNA techniques to insert the human insulin gene into bacteria. Here is how it works!

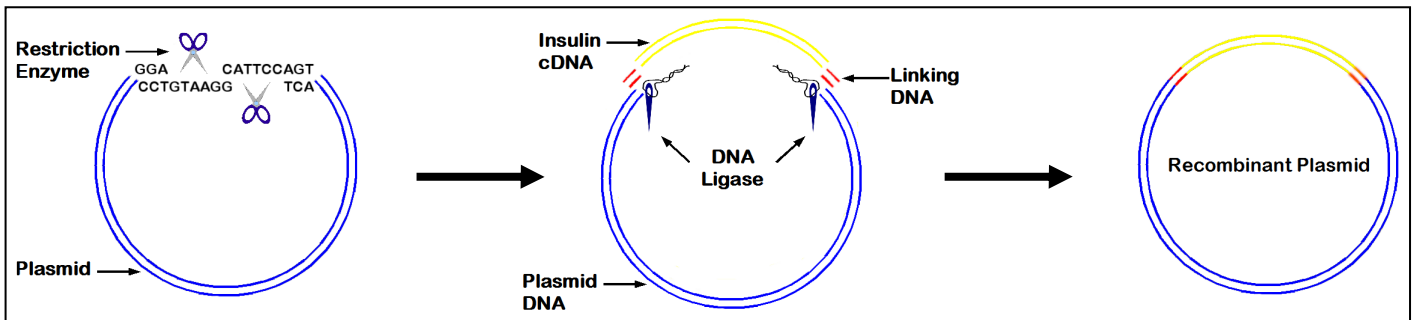
Step 1: Gene Isolation

Searching through the entire Human Genome for the insulin gene would take a very long time. It is much easier to begin with the mRNA from the cells that normally produce insulin, the beta-cells in the pancreas. The mRNA is then transformed to cDNA through the use of reverse transcriptase resulting in a DNA copy of the insulin gene.



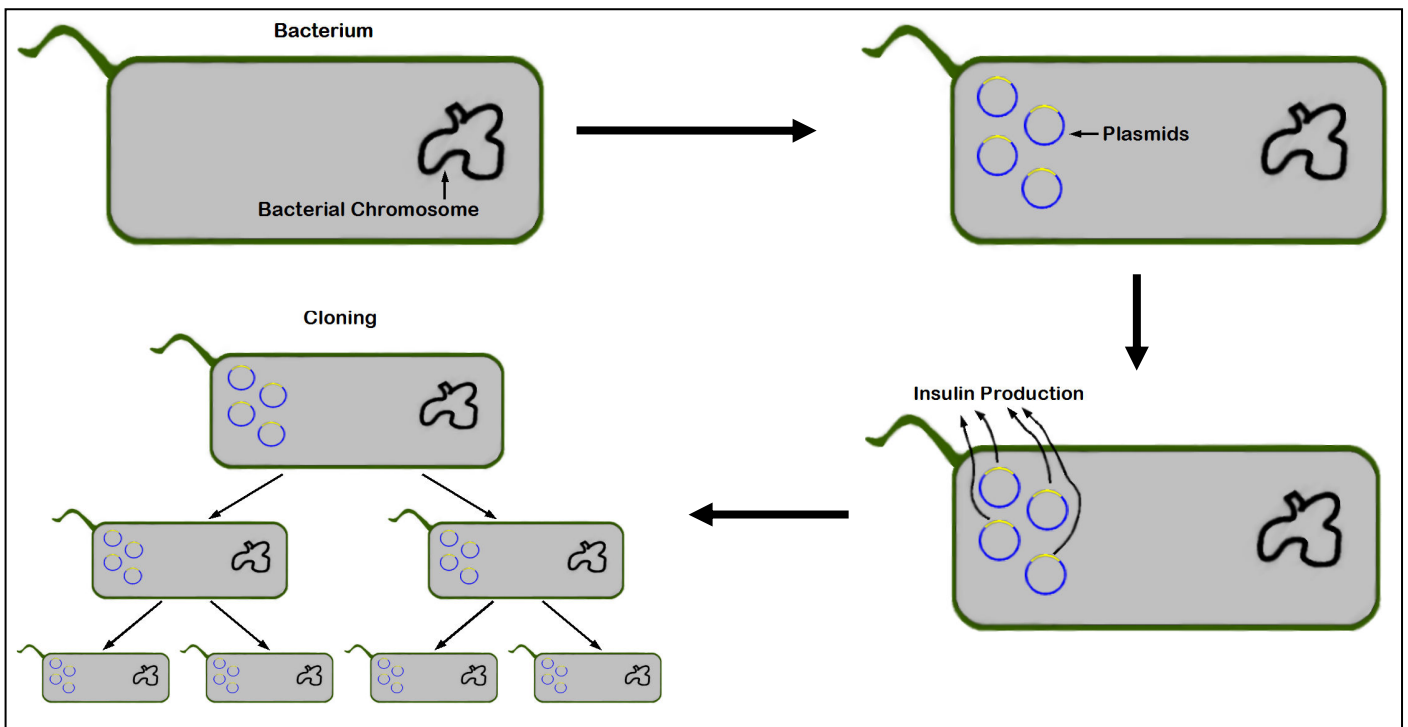
Step 2: Creation of a Plasmid Vector

Plasmids are circular segments of DNA, found in both bacteria and yeast, which are independent of the chromosomes and capable of replication. Restriction enzymes are used to cut across the two strands of DNA and leave loose ends to which the cDNA can be attached. Special linking DNA sequences are added to connect the plasmid DNA with the human insulin cDNA. Ligation enzymes are used to seal the plasmid which now includes the cDNA sequence. The plasmid is now readily able to be inserted into a bacterial host.



Step 3: Cloning the Gene

The plasmid enters a bacterium and reproduces itself, yielding up to dozens of copies. When the bacterial cell divides, the plasmids are split between the two daughter cells. These daughter cells then produce more plasmids which are then passed on to their daughter cells, etc. With cells dividing as fast as every 20 minutes, a bacterium containing human cDNA encoding insulin will, in a relatively short time, produce many millions of similar cells (clones) containing the same human gene.



Step 4: Production of Insulin

The bacteria are then grown under conditions that will lead to optimal insulin production. The insulin is then collected, isolated, and purified. In actuality, this process produces a precursor of insulin (proinsulin) and not insulin itself. It is therefore necessary to use enzymes to cleave the proinsulin protein at specific points to leave behind human insulin.

