

Chapter 14 - Microbes and Genetic Engineering

Restriction Enzyme (Restriction Endonuclease) - Its function is to destroy foreign DNA entering a cell.

- Cuts between specific base pairs
- Used in protection against viral DNA infections in bacteria
- Ex. BamHI, EcoRI, HindIII
- Leave "Sticky Ends" which are joined by **Ligase** to form a recombinant DNA molecule

Problems with Recombination of Eukaryotic to Prokaryotic

Introns - still intact that prokaryotes cannot read

However, prokaryotes can use mRNA because it's already been tailored and introns have been cut out.

Reverse Transcription

mRNA + DNA Polymerase + Reverse transcriptase = cDNA (Complimentary DNA)

Problems with Reinserting back into mammalian Sources

- Must reinsert at Promoter region

Cloning

- Insulin
- Growth Hormone
- Interferon
- AIDS vaccine
- HepB Vaccine
- Clotting factor

Most Common Technique for Recombination

1. Extract DNA
2. Add DNA fragment to Plasmid
3. Add ligase

Cloning a Gene, then Identifying the Bacteria that have acquired the Gene (Fig. 14.11)

1. Isolate a small amount of the pure culture for which you want to clone.
2. Inject into another mammal.
3. That mammal will develop antibodies to the culture.
4. Label the antibodies with a radioisotope for identification purposes.
5. Meanwhile, you plate colonies of bacteria
6. imprint colonies onto a nitrocellulose disk.
7. Expose disk to agent that will lyse the cells
8. Apply antibodies from #4 to cells disk.
9. You will be able to locate the bacteria with the original pure culture because the antibodies will bind to them.