Steps involved in rDNA technology

Recombinant DNA technology, also known as genetic engineering or gene splicing, involves the manipulation and combination of DNA molecules from different sources to create new genetic combinations. The general steps involved in recombinant DNA technology:

1. **Identify the target DNA**: The first step is to identify and isolate the specific DNA sequence of interest. This could be a gene responsible for a particular trait or a specific DNA fragment.
2. **Isolate the source DNA**: Once the target DNA is identified, the next step is to obtain the source DNA that contains the desired gene or DNA fragment. The source DNA can be extracted from a variety of organisms, including bacteria, plants, animals, or even synthesized artificially.
3. **Cutting the desired DNA segment**: To manipulate the DNA, enzymes called restriction enzymes or molecular scissors are used to cut the DNA molecules at specific sites. These restriction enzymes recognize specific DNA sequences and cut the DNA at or near those sites, resulting in DNA fragments with sticky ends.
4. **Plasmid preparation**: Plasmids are small circular DNA molecules commonly found in bacteria. They can be used as vectors to carry foreign DNA. The plasmids are isolated and purified from bacterial cells or can be synthesized in the laboratory. The plasmid is also treated with the same restriction enzyme that used to cut the source DNA.
5. **Joining the DNA fragments:** The target DNA fragment is combined with the plasmid vector. The sticky ends of the DNA fragments and plasmids can pair up due to complementary base pairing. Enzymes called DNA ligases are then used to catalyze the formation of covalent bonds between the DNA fragments and the plasmid, resulting in a recombinant DNA.
6. **Introduction into host cells:** The recombinant DNA molecules are introduced into host cells, usually bacteria, through a process called **transformation**. This can be done by various processes, such as treating the host cells with calcium chloride, heat shock, electroporation, or other methods to make them competent for taking up the recombinant DNA.
7. **Selection and identification of transformed cells:** After transformation, only a small fraction of the host cells will take up and incorporate the recombinant DNA. Selective markers, such as antibiotic resistance genes, are often included in the plasmid to help identify the transformed cells. The transformed cells are then grown on a selective

medium that contains the antibiotic. Only the cells that have successfully taken up the recombinant DNA and incorporated the antibiotic resistance gene will survive and form colonies.

8. **Screening and verification**: Once transformed cells are obtained, further screening and verification steps are performed to confirm the presence of the desired recombinant DNA. Techniques such as polymerase chain reaction (PCR), DNA sequencing, or other molecular biology methods can be used to verify the presence and integrity of the inserted DNA.
9. **Expression of the gene:** If the desired gene is to be expressed, additional steps may be required to ensure proper gene expression in the host organism. This could involve the use of promoter sequences, regulatory elements, or other genetic modifications to control and enhance gene expression.

Recombinant DNA technology has revolutionized many fields of biology and has various applications, including the production of therapeutic proteins, creation of genetically modified organisms (GMOs), development of vaccines, and advancements in medical and agricultural research.

Steps involved in rDNA technology with BT gene as an example

Recombinant DNA technology, also known as genetic engineering or gene splicing, involves the manipulation and combination of DNA molecules from different sources to create new genetic combinations. BT gene, which codes for a protein called *Bacillus thuringiensis* (BT) toxin that is toxic to certain insect pests. The BT gene can be introduced into crops to provide built-in pest resistance. Here's how the steps of recombinant DNA technology would apply to the BT gene:

1. **Identify the target DNA**: In this case, the target DNA would be the BT gene sequence, which is responsible for producing the BT toxin.
2. **Isolate the source DNA**: The source of the BT gene can be the bacterium Bacillus thuringiensis itself. The bacterium can be cultured and its DNA extracted and purified.
3. **Cutting the DNA**: The BT gene sequence can be isolated by using restriction enzymes that recognize specific sites within the DNA of Bacillus thuringiensis and cut it into fragments. These restriction enzymes generate specific sticky ends.
4. **Plasmid preparation**: A plasmid vector is prepared, which will serve as the carrier for the BT gene. Plasmids can be isolated from bacteria or synthesized in the lab. The plasmid contains a selectable marker, such as an antibiotic resistance gene, to identify transformed cells.
5. **Joining the DNA fragments**: The BT gene fragments and the plasmid vector are mixed together, and their sticky ends are allowed to base pair. DNA ligase is then used to form covalent bonds between the BT gene and the plasmid, resulting in a recombinant plasmid carrying the BT gene.
6. **Introduction into host cells**: The recombinant plasmid carrying the BT gene is introduced into the host cells of the target crop, such as corn or cotton. This can be done through a process like Agrobacterium-mediated transformation, where *Agrobacterium tumefaciens* is used as a vector to deliver the recombinant plasmid into the plant cells.
7. **Selection and identification of transformed cells**: Following transformation, the host cells are grown on a selective medium containing an antibiotic that the plasmid carries resistance to. Only the transformed cells that have successfully taken up the recombinant plasmid will be able to survive and form colonies.
8. **Screening and verification**: The transformed cells are further screened and verified to confirm the presence of the BT gene. This can be achieved through techniques like PCR

or DNA sequencing, where specific primers are designed to amplify and identify the BT gene sequence.

9. **Expression of the gene**: Once the transformed cells containing the BT gene are confirmed, additional steps may be taken to ensure proper gene expression. This can involve adding promoter sequences that are recognized by the plant's own cellular machinery to initiate gene expression and produce the BT toxin.

By introducing the BT gene into the crop plants, they can produce the BT toxin, making them resistant to certain insect pests that are susceptible to the toxin. This reduces the need for chemical pesticides and offers a more targeted and environmentally friendly approach to pest control.