

process is called **cloning** (the process of formation of multiple identical copies of DNA).

Construction of a Recombinant DNA

- Plasmid (autonomously replicating, circular, extra-chromosomal DNA) is isolated.
- Plasmid DNA acts as a **vector** since it is used to transfer the piece of DNA attached to it to the host.
- Plasmid DNA also contains genes responsible for providing antibiotic resistance to the bacteria.
- Plasmid DNA was cut with a specific restriction enzyme ('molecular scissors' – that cut a DNA at specific locations).
- The DNA of interest (to be inserted) was also cut with the same restriction enzyme.
- The DNA of interest is hybridised with the plasmid with the help of DNA ligase to form a **Recombinant DNA**.
- Recombinant DNA is then transferred to a host such as *E.coli*, where it replicates by using the host's replicating machinery.
- When *E.coli* is cultured in a medium containing antibiotic, only cells containing recombinant DNA will be able to survive due to antibiotic resistance genes and one will be able to isolate the recombinants.

Restriction Enzymes as Tools of RDT

- Restriction enzymes are specialised enzymes that recognise and cut a particular sequence of DNA.
- Nucleases are of two types:
 - Endonucleases – Cut the DNA at specific positions within the DNA
 - Exonucleases – Cut the DNA at the ends (Remove the nucleotides at the ends of the DNA)
- Every restriction enzyme identifies different sequences (Recognition sequences). Over 900 restriction enzymes have been isolated, all of which recognise different sequences.
- Recognition sequences are **pallindromic**- Pallindromes are the