Recombinant DNA Technology – Process and Applications

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Recombinant DNA technology (RDT), often referred to as **Genetic Engineering,** is an in-vitro (lab) method of manipulating genes (DNA fragments) by using a set of tools and techniques. The primary aim of RDT is to produce "Transgene (recombinant DNA) and its product (recombinant protein), to be applied across different fields of biotechnology.

The RDT is a continuously evolving technology due to the advancement in its tools and techniques, such as the discovery of the CRISPR-Cas9 gene editing tool.

Tools of Recombinant DNA Technology

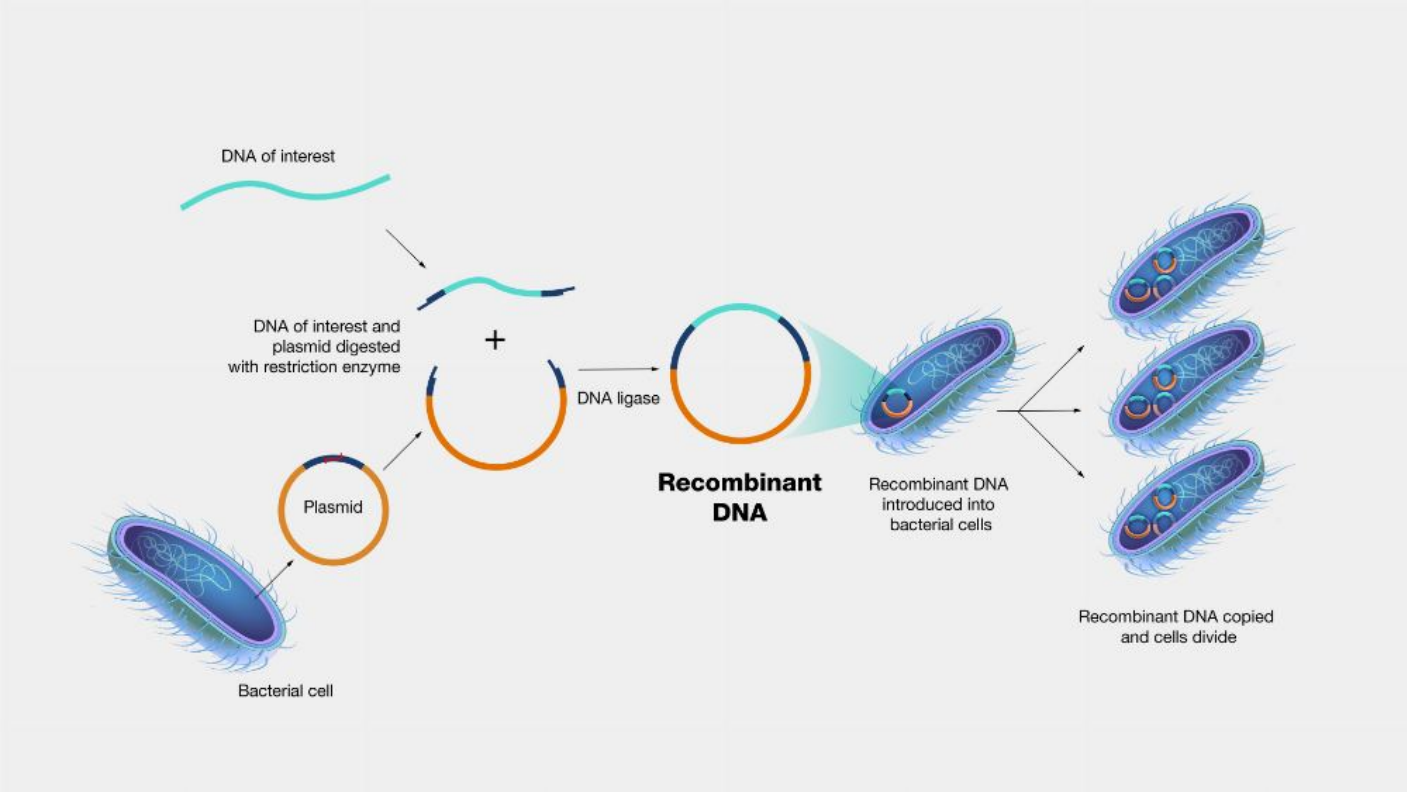
The following are the tools employed in the process involved in Recombinant DNA Technology:

Tools	Utility	Examples

Vector	They are used as **carriers** to introduce foreign DNA into a host cell	**- Plasmids** (for example, pBR322, Ti Plasmid), **- YAC** (Yeast Artificial Chromosomes), - **BAC** (Bacterial Artificial Chromosomes), - Viruses (Phages), etc.
Restriction Enzymes	It recognises specific DNA sequences and cleaves the DNA at the precise location	- EcoRI, HindIII, BamHI, etc - **CRISPR Cas9** (used primarily nowadays) - **Zinc-Finger Nuclease (ZFN)**
DNA Ligase	It joins together DNA fragments	- T4 DNA Ligase
Selectable markers	To distinguish transformed cells from non-transformed ones	- Antibiotic resistance genes, herbicide resistance genes, etc

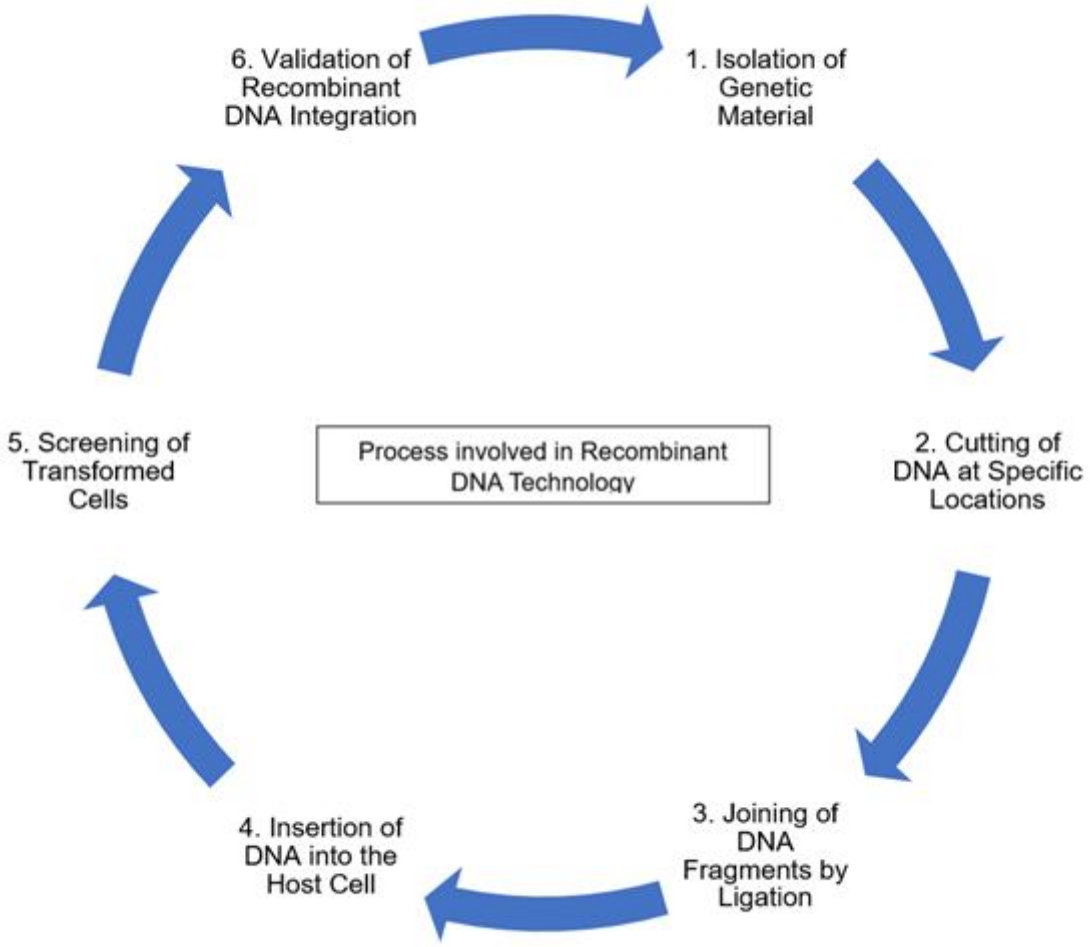
Process involved in Recombinant DNA Technology

Recombinant DNA technology undergoes through the following steps:



- **Isolation of Genetic Material:** The extraction of genetic material is performed from the source organism's DNA, such as bacteria, plants, or animals.

- **Selection of a Suitable Cloning Vector:** Vectors are carrier molecules used to introduce rDNA into a host organism.
    - **Plasmids**, which are small, circular DNA molecules, are commonly used vectors. They can replicate independently within a host cell, allowing for the propagation of the foreign DNA.
    - The vectors can also be **non-self replicating** (for example, viral vectors).
- **Cutting of DNA at Specific Locations:** It uses the specialized enzymes known as **restriction endonucleases** (RE) or **restriction enzymes,** which recognize specific DNA sequences (recognition sites) and cleave the DNA at those precise locations.
    - The RE cuts the **target DNA as well as Plasmid**, producing "sticky ends" that are complementary to each other.
- **Joining of DNA Fragments by Ligation:**
    - The isolated DNA fragments are combined with a vector, which is typically a plasmid or a viral genome modified to accept foreign DNA.
    - DNA ligase is used to catalyze the formation of phosphodiester bonds, effectively sealing the gaps and fusing the DNA fragments with the vector.
    - Now the rDNA is ready for gene transfer (for in-vivo gene cloning) or for the PCR.
- **Gene Transfer:** There are several methods which are employed in the process of Gene Transfer- physical, chemical, and biological. RDT uses the biological means of gene transfer.
    - **Physical methods: Gene gun** or Biolistics, **Electroporation, Microinjection**, etc., which make the direct entry of the rDNA into the host's cell.
    - **Chemical methods:** Using **Lipofection, calcium phosphate**, etc. make it easier for the rDNA to enter into the host's cell.
    - **Biological methods:** This is an indirect method of gene transfer, using vectors (for example, bacteria) as a means.
- **Gene Cloning:** Once inside the host, the rDNA replicates itself independently (due to self-replicating **plasmid)**. This is called Gene Cloning. It can also be done using the PCRmethod for amplifying a gene of interest.
- **Polymerase Chain Reaction (PCR):** PCR is a tool that allows for the amplification of the target DNA sequences outside the cell. It needs much less time than the traditional cloning methods.
- **Selection and Screening of Transformed Cells:** This step involves identifying and isolating cells that have successfully taken up the **recombinant DNA**.
    - **Selectable markers**, such as **antibiotic resistance genes** carried by the vector, are often used to distinguish transformed cells from non-transformed ones.
- **Validation of Recombinant DNA Integration:** To ensure that the recombinant DNA has integrated into the host genome as intended, various techniques may be employed.
    - For example, **nucleic acid hybridization, blue-white screening**, etc.



Applications of Recombinant DNA Technology

Recombinant DNA Technology stands as a cornerstone of modern science with far-reaching applications across numerous fields.

- **Advancement in Medicine**:
  - It enables the production of vital **biopharmaceuticals**, particularly **therapeutic proteins** like **insulin**, growth hormone, and clotting factors.
  - **Customized Therapeutics** enables the production of personalised medicines tailored to an individual's genetic makeup, leading to more effective and targeted treatments.
- **Gene Therapy:**
  - It offers the potential to treat genetic disorders by replacing or repairing **faulty genes.**
  - It is helpful in treating a wide range of diseases, like **cystic fibrosis**, **muscular dystrophy**, and certain types of **cancer**.
- **Recombinant Vaccines:** RDT can be used to develop **vaccines** for a variety of diseases, using vectors like bacteria, yeasts, viruses (phage), etc.
- **Immunotherapy:** RDT contributes to the development of **immunotherapies** for example, **T-cell therapy**, which harnesses the body's own immune system to target and **destroy cancer cells.**
- **Agricultural Advancements:**

- It is used to cultivate **Genetically modified (GM) crops** that have transformed agriculture. Example: **BT cotton**.
- These crops possess traits like **pest resistance**, **drought tolerance**, and improved **nutritional content**.

- **Bioremediation and Environmental Protection:**
  - Environmental biotechnology indicates that **Genetically-modified** microbes such as bacteria, yeast and filamentous fungi can **remove heavy metals** from aqueous solutions.
  - For example, **Escherichia coli strain JM109** has the ability to **remove mercury** from contaminated water or soil.
- **Targeted Drug Delivery:**
  - It enables the design and production of drug-delivery systems that can precisely target specific tissues or cells within the body.
  - This increases the effectiveness of treatments while minimizing side effects.
- **Molecular diagnosis (RDT plus PCR):**
  - It plays a critical role in diagnostic techniques, allowing for the detection of specific DNA sequences associated with diseases or pathogens.
  - It is helpful in early detection and monitoring of various conditions.
- **Industrial Applications:**
  - It is used in the production of recombinant enzymes to produce **sugar**, **cheese,** biofuels, important chemicals, etc.
