Recombinant DNA Technology

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Recombinant DNA

- *Recombinant DNA (rDNA) is a form of artificial DNA that is created by combining two or more sequences that would not normally occur together through the process of gene splicing.*
- *Recombinant DNA technology is a technology which allows DNA to be produced via artificial means. The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.*

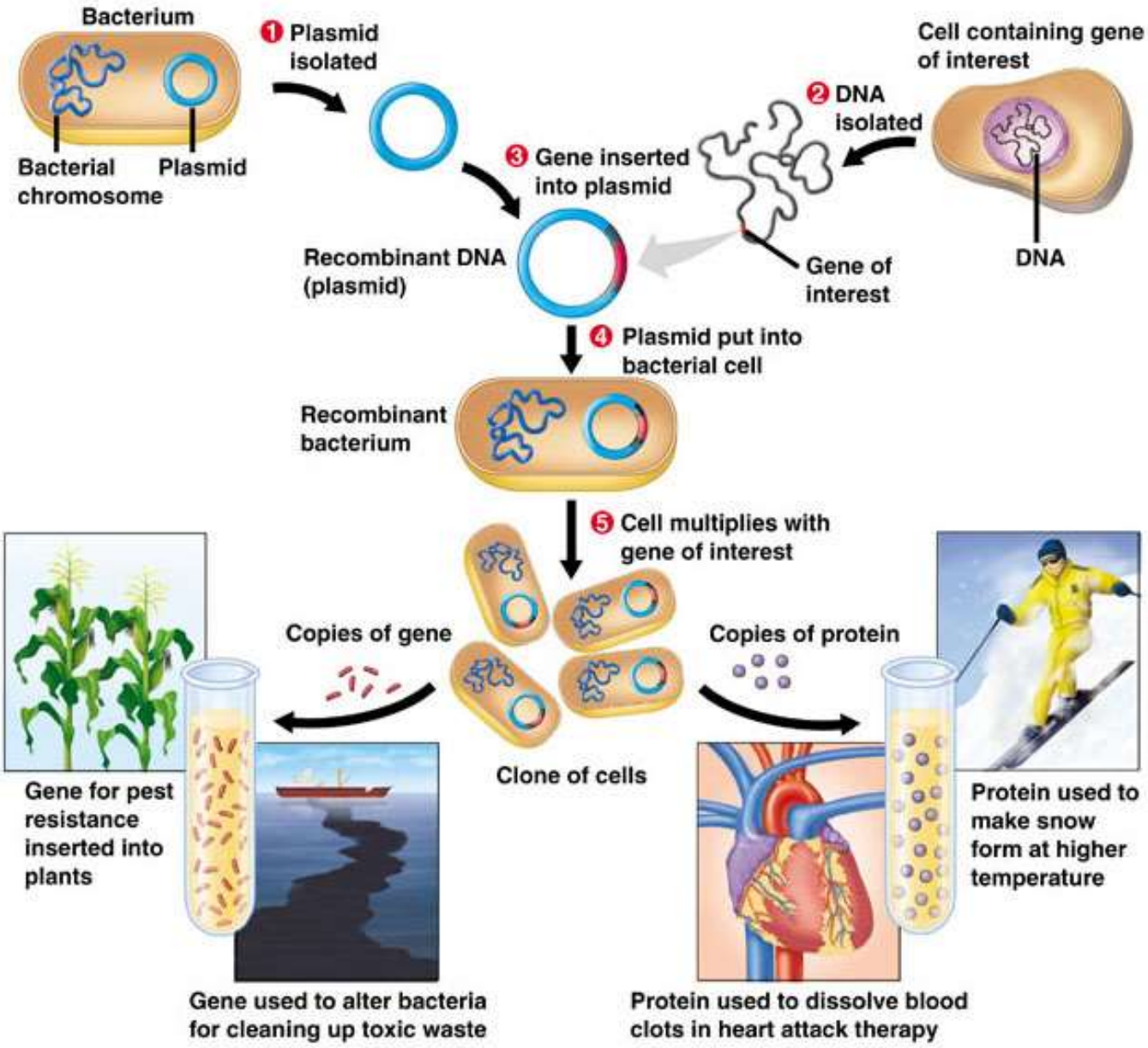
Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by two scientists named Boyer and Cohen in 1973.

What is Recombinant DNA Technology?

- Recombinant DNA technology is a technology which allows DNA to be produced via artificial means.
- The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.
- It is an area of medical science that is just beginning to be researched in a concerted effort.

- Recombinant DNA technology works by taking DNA from two different sources and combining that DNA into a single molecule. That alone, however, will not do much.
- Recombinant DNA technology only becomes useful when that artificially-created DNA is reproduced. This is known as DNA cloning.

Brief Introduction



Six steps of Recombinant DNA

1. Isolating (vector and target gene)
2. Cutting (Cleavage)
3. Joining (Ligation)
4. Transforming
5. Cloning
6. Selecting (Screening)

Six basic steps are common to most recombinant DNA experiments

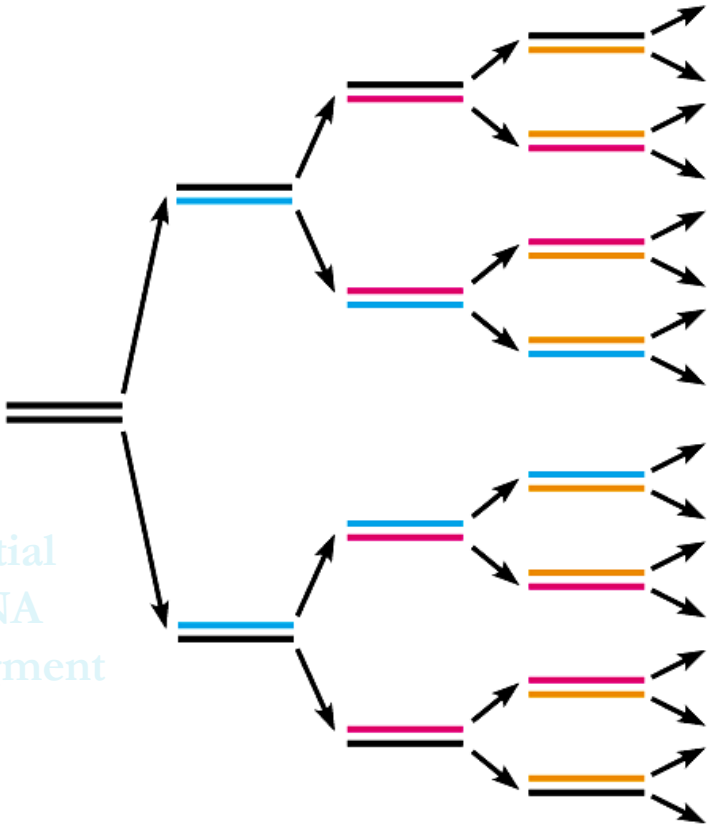
1. *Isolation and purification of DNA*.

   Both vector and target DNA molecules can be prepared by a variety of routine methods. In some cases, the target DNA is synthesized *in vitro*.

Polymerase chain reaction (PCR)

- A technique called the polymerase chain reaction (PCR) has revolutionized recombinant DNA technology. It can amplify DNA from as little material as a single cell and from very old tissue such as that isolated from Egyptian mummies, a frozen mammoth, and insects trapped in ancient amber.

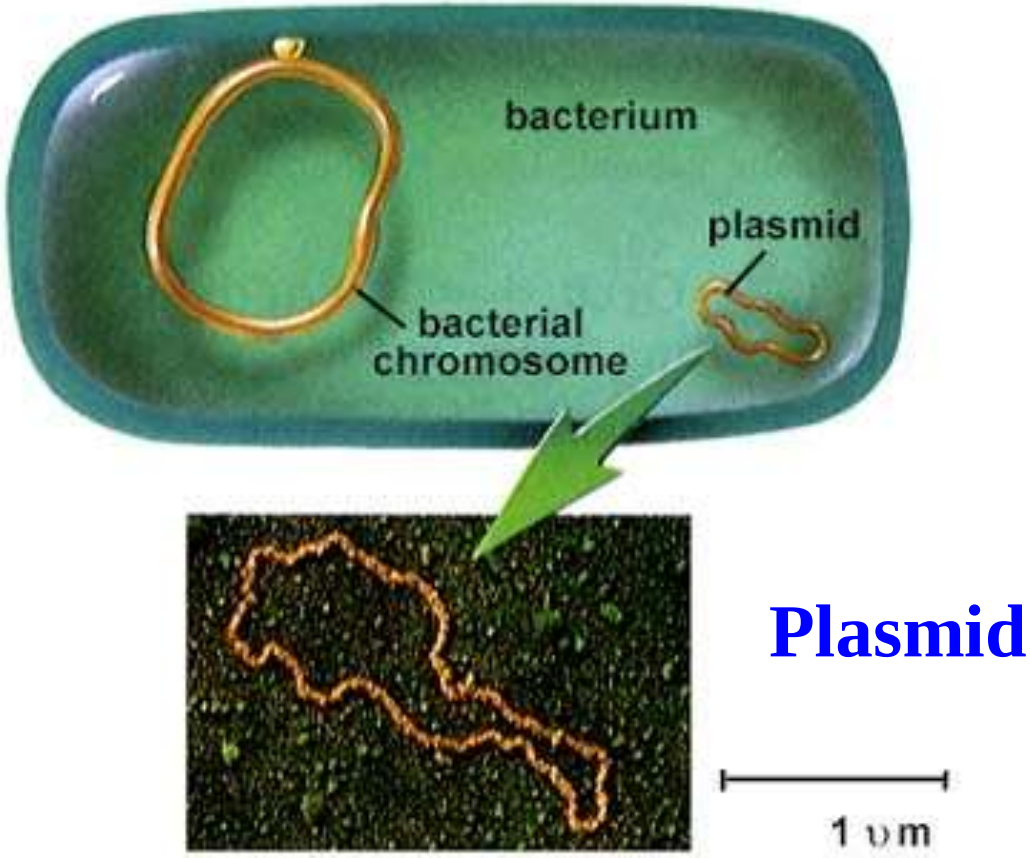
- method is used to amplify DNA sequences
- The polymerase chain reaction (PCR) can quickly clone a small sample of DNA in a test tube



2. *Cleavage of DNA at particular sequences*. As we will see, cleaving DNA to generate fragments of defined length, or with specific endpoints, is crucial to recombinant DNA technology. The DNA fragment of interest is called *insert* DNA. In the laboratory, DNA is usually cleaved by treating it with commercially produced nucleases and *restriction endonucleases*.

Types of vector

1. Plasmid Vectors
2. Bacteriophage Vectors
3. Virus vectors
4. Shuttle Vectors--can replicate in either prokaryotic or eukaryotic cells.
5. Yeast Artificial Chromosomes as Vectors



Plasmid

3. *Ligation of DNA fragments*.

A recombinant DNA molecule is usually formed by cleaving the DNA of interest to yield insert DNA and then ligating the insert DNA to vector DNA (*recombinant DNA*). DNA fragments are typically joined using *DNA ligase* (also commercially produced).

- T4 DNA Ligase

4. *Introduction of recombinant DNA into compatible host cells*. In order to be propagated, the recombinant DNA molecule (insert DNA joined to vector DNA) must be introduced into a compatible host cell where it can replicate. The direct uptake of foreign DNA by a host cell is called genetic transformation (or transformation). Recombinant DNA can also be packaged into virus particles and transferred to host cells by transfection.

5. *Replication and expression of recombinant DNA in host cells*.

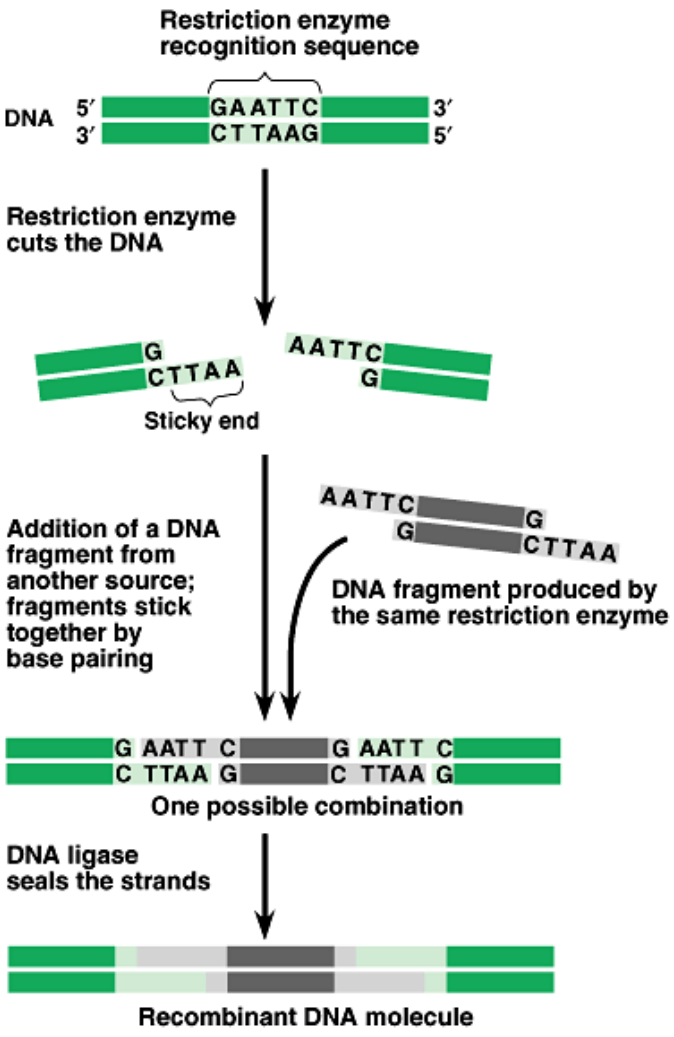
Cloning vectors allow insert DNA to be replicated and, in some cases, expressed in a host cell. The ability to clone and express DNA efficiently depends on the choice of appropriate vectors and hosts.

6. *Identification of host cells that contain recombinant DNA of interest*. Vectors usually contain easily scored genetic markers, or genes, that allow the selection of host cells that have taken up foreign DNA. The identification of a particular DNA fragment usually involves an additional step—screening a large number of recombinant DNA clones. This is almost always the most difficult step.

Enzymes

1. Restriction endonuclease, RE
2. DNA ligase
3. Reverse transcriptase
4. DNA polymerase, DNA pol
5. Nuclease
6. Terminal transferase

Restriction enzymes cleave DNA



- The same sequence of bases is found on both DNA strands, but in opposite orders. GAATTC CTTAAG
- This arrangement is called a palindrome. *Palindromes are words or sentences that read the same forward and backward.*
- form **sticky ends**: single stranded ends that have a tendency to join with each other (the key to **recombinant DNA**)

Restriction Enzymes Cut DNA Chains at Specific Locations

- Restriction enzymes are endonucleases produced by bacteria that typically recognize specific 4 to 8bp sequences, called *restriction sites*, and then cleave both DNA strands at this site.
- Restriction sites commonly are short *palindromic sequences*; that is, the restriction-site sequence is the same on each DNA strand when read in the 5′ → 3′ direction.

Restriction enzymes

- Restriction enzymes are named after the bacterium from which they are isolated
  - For example, *Eco* RI is from *Escherichia coli,* and *Bam* HI is from *Bacillus amyloliquefaciens* . The first three letters in the restriction enzyme name consist of the first letter of the genus (E) and the first two letters of the species (co). These may be followed by a strain designation (R) and a roman numeral (I) to indicate the order of discovery (eg, *Eco*RI, *Eco*RII).

Blunt ends or sticky ends

- Each enzyme recognizes and cleaves a specific double-stranded DNA sequence that is 4–7 bp long. These DNA cuts result in blunt ends (eg, *Hpa* I*)* or overlapping (sticky) ends (eg, *Bam*H I*)* , depending on the mechanism used by the enzyme.
- Sticky ends are particularly useful in constructing hybrid or chimeric DNA molecules .

Identification of Host Cells Containing Recombinant DNA

- Once a cloning vector and insert DNA have been joined *in vitro*, the recombinant DNA molecule can be introduced into a host cell, most often a bacterial cell such as *E. coli*.
- In general, transformation is not a very efficient way of getting DNA into a cell because only a very small percentage of cells take up recombinant DNA. Consequently, those cells that have been successfully transformed must be distinguished from the vast majority of untransformed cells.

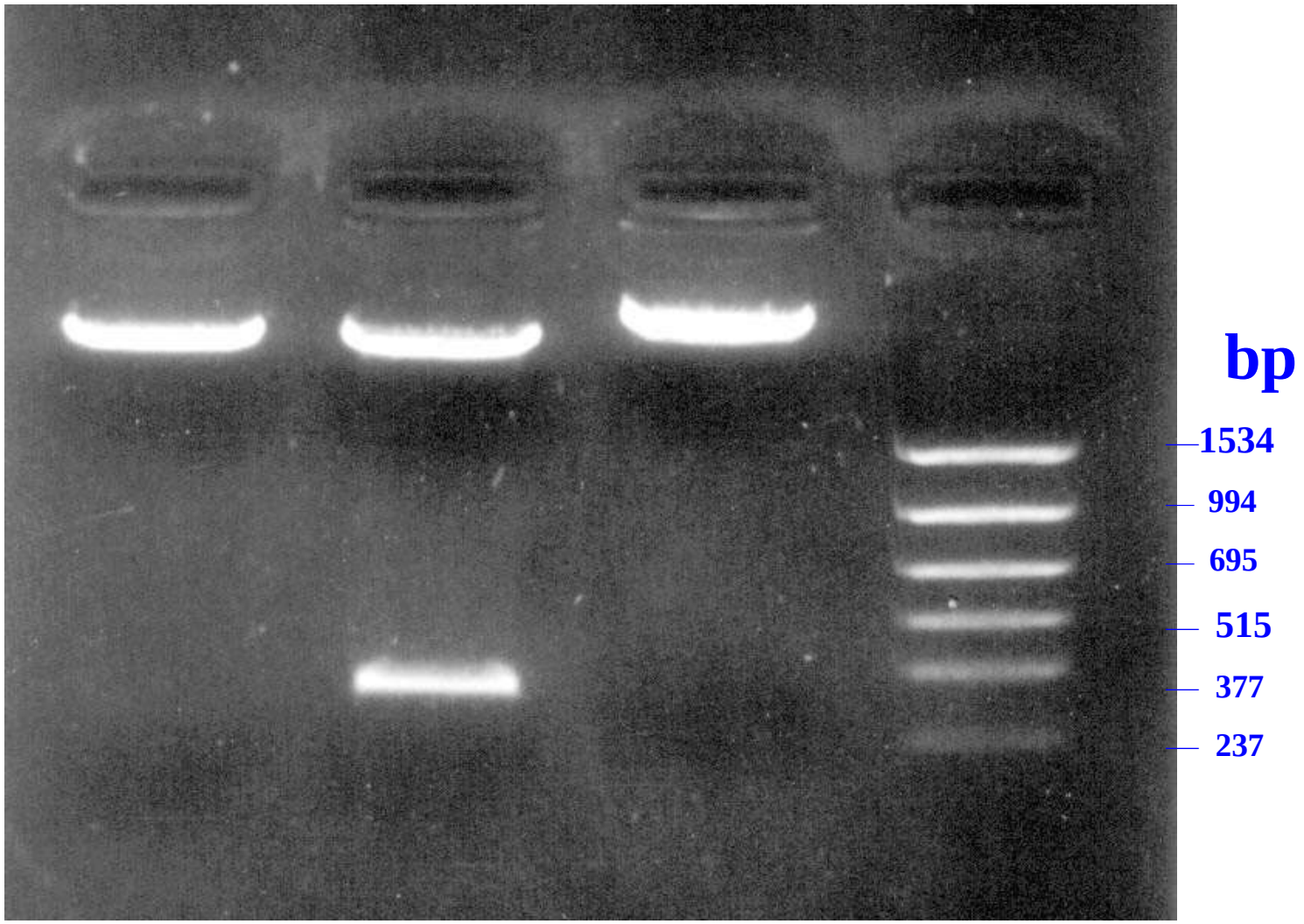
- Identification of host cells containing recombinant DNA requires genetic selection or screening or both.
- In a *selection*, cells are grown under conditions in which only transformed cells can survive; all the other cells die.
- In contrast, in a *screen*, transformed cells have to be individually tested for the presence of the desired recombinant DNA.
- Normally, a number of colonies of cells are first selected and then screened for colonies carrying the desired insert.

Selection Strategies Use Marker Genes (Primary screening)

- Many selection strategies involve selectable marker genes— genes whose presence can easily be detected or demonstrated. *amp*^{R}
- Selection or screening can also be achieved using insertional inactivation.

Screening (Strategies)

1. Gel Electrophoresis Allows Separation of Vector DNA from Cloned Fragments
2. Cloned DNA Molecules Are Sequenced Rapidly by the Dideoxy Chain-Termination Method
3. The Polymerase Chain Reaction Amplifies a Specific DNA Sequence from a Complex Mixture
4. Blotting Techniques Permit Detection of Specific DNA Fragments and mRNAs with DNA Probes



Gel Electrophoresis

negative charged DNA run to the anode

Application

1. **Analysis of Gene Structure and Expression**
2. **Pharmaceutical Products**
   - **Drugs**
   - **Vaccines**
3. **Genetically modified organisms (GMO)**
   - **Transgenic plants**
   - **Transgenic animal**
4. **Application in medicine**

Application in medicine

- Human Gene Therapy
- Diagnosis of genetic disorders
- Forensic Evidence

REFERENCE

- Text book of Biochemestry by Dr. U. Satyanarayana and Dr. U. Chakrapani page no. 578-616

THANK YOU!