Recombinant DNA technology

is a set of molecular techniques for locating, isolating, altering, and studying DNA segments. The term *recombinant* is used because frequently the goal is to combine DNA from two distinct sources. Genes from two different bacteria might be joined, for example, or a human gene might be inserted into a viral chromosome. Commonly called **genetic engineering,** recombinant DNA technology now encompasses an array of molecular techniques that can be used to analyze, alter, and recombine virtually any DNA sequences.

Recombinant DNA Techniques

In the sections that follow, we will examine some of the following techniques of recombinant DNA technology and see how they are used to create recombinant DNA molecules:

1. Methods for locating specific DNA sequences
2. Techniques for cutting DNA at precise locations
3. Procedures for amplifying a particular DNA sequence billions of times, producing enough copies of a DNA sequence to carry out further manipulations
4. Methods for mutating and joining DNA fragments to produce desired sequences
5. Procedures for transferring DNA sequences into recipient cells

Cutting and Joining DNA Fragments

The key development that made recombinant DNA technology possible was the discovery in the late 1960s of **restriction enzymes** (also called **restriction endonucleases**) that recognize and make double-stranded cuts in the sugar–phosphate backbone of DNA molecules at specific nucleotide sequences. These enzymes are produced naturally by bacteria, where they are used in defense against viruses. In bacteria, restriction enzymes recognize particular sequences in viral DNA and then cut it up. A bacterium protects its own DNA from a restriction enzyme by modifying the recognition sequence, usually by adding methyl groups to its DNA.

Three types of restriction enzymes have been isolated from bacteria. Type I restriction enzymes recognize specific sequences in the DNA but cut the DNA at random sites that may be some distance (1000 bp or more) from the recognition sequence. Type III restriction enzymes recognize specific sequences and cut the DNA at nearby sites, usually about 25 bp away. Type II restriction enzymes recognize specific

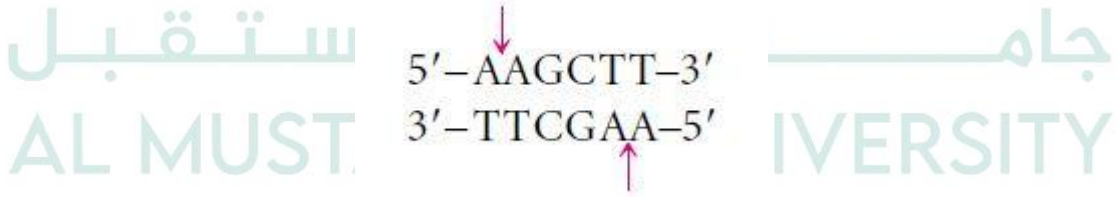
sequences and cut the DNA within the recognition sequence. Virtually all work on recombinant DNA is done with type II restriction enzymes refers to type II enzymes.

More than 800 different restriction enzymes that recognize and cut DNA at more than 100 different sequences have been isolated from bacteria.

Type	Activity of Enzyme	ATP Required	Cleavage Site
I	Cleavage and methylation	Yes	Random sites distant from recognition site
II	Cleavage only	No	Within recognition site
III	Cleavage and methylation	Yes	Random sites near recognition site

Each restriction enzyme is referred to by a short abbreviation that signifies its bacterial origin. The sequences recognized by restriction enzymes are usually from 4 to 8 bp long; most enzymes recognize a sequence of 4 or 6 bp. Most recognition sequences are palindromic— sequences that read the same forward and backward.

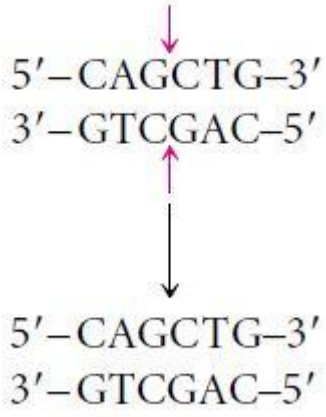
Notice in Table 18.2 that the sequence on the bottom strand is the same as the sequence on the top strand, only reversed. All type II restriction enzymes recognize palindromic sequences. Some of the enzymes make staggered cuts in the DNA. For example, *Hin*dIII recognizes the following sequence:



*Hin*dIII cuts the sugar–phosphate backbone of each strand at the point indicated by the arrow, generating fragments with short, single-stranded overhanging ends:

Such ends are called **cohesive ends** or sticky ends; because they are complementary to each other and can spontaneously pair to connect the fragments. Thus DNA fragments can be "glued" together: any two fragments cleaved by the same enzyme will have complementary ends and will pair. When their cohesive ends have paired, two DNA fragments can be joined together permanently by the enzyme DNA ligase, which seals nicks between the sugar–phosphate groups of the fragments.

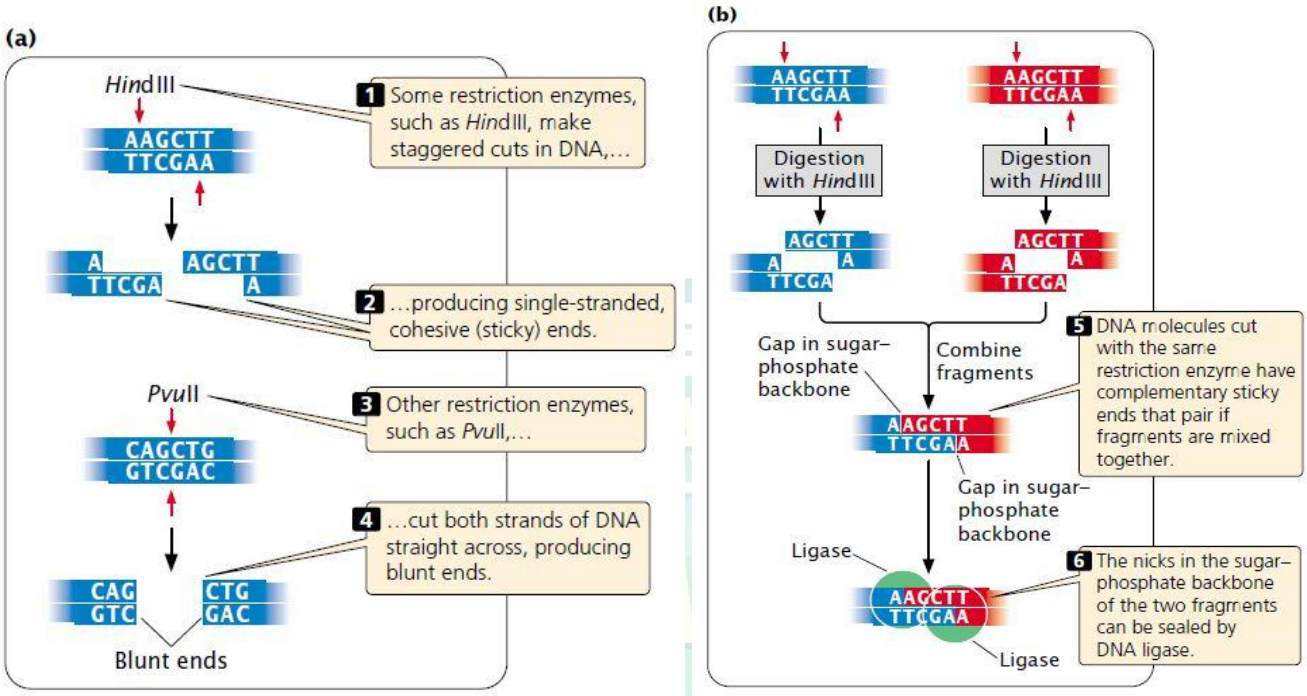
Not all restriction enzymes produce staggered cuts and sticky ends. *Pvu*II cuts in the middle of its recognition site, producing blunt-ended fragments:



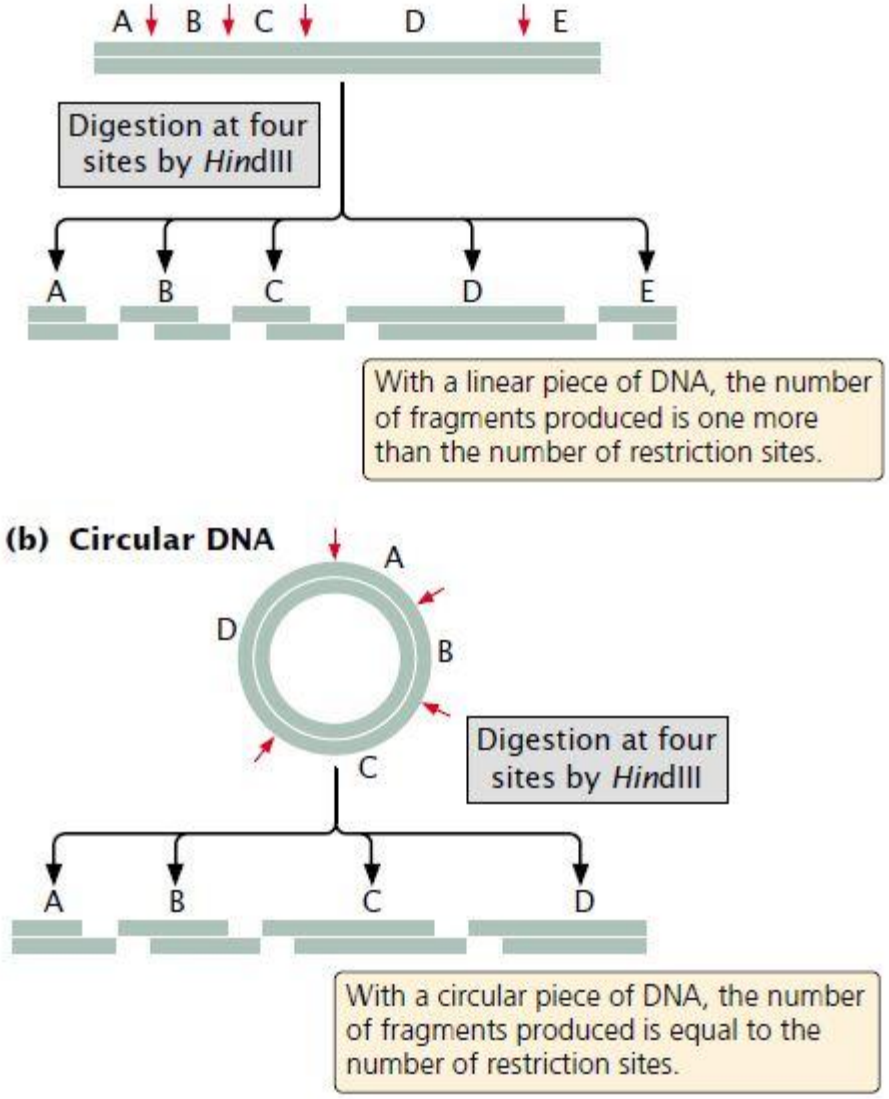
Fragments with blunt ends must be joined together in other ways, which will be discussed later.

The first three letters of the abbreviation for each restriction enzyme refer to the bacterial species from which the enzyme was isolated (e.g., *Eco* refers to *E. coli*). A fourth letter may refer to the strain of bacteria from which the enzyme was isolated (the "R" in *Eco*RI indicates that this enzyme was isolated from the RY13 strain of *E. coli*). Roman numerals that follow the letters allow different enzymes from the same species to be identified. For convenience, molecular geneticists have come up with idiosyncratic pronunciations of the names: *Eco*RI is pronounced "echo-R-one," *Hin*dIII is "hin-D-three," and *Hae*III is "hay-three."

Enzyme	Microorganism from Which Enzyme Is Isolated	Recognition Sequence	Type of Fragment End Produced
*Bam*HI	*Bacillus amyloliquefaciens*	5′–GGATCC–3′ 3′–CCTAGG–3′	Cohesive
*Cof*I	*Clostridium formicoaceticum*	5′–GCGC–3′ 3′–CGCG–5′	Cohesive
*Dra*I	*Deinococcus radiophilus*	5′–TTTAAA–3′ 3′–AAATTT–5′	Blunt
*Eco*RI	*Escherichia coli*	5′–GAATTC–3′ 3′–CTTAAG–5′	Cohesive
*Eco*RII	*Escherichia coli*	5′–CCAGG–3′ 3′–GGTCC–5′	Cohesive
*Hae*III	*Haemophilus aegyptius*	5′–GGCC–3′ 3′–CCGG–5′	Blunt
*Hind*III	*Haemophilus influenczae*	5′–AAGCTT–3′ 3′–TTCGAA–5′	Cohesive
*Hpa*II	*Haemophilus parainfluenzae*	5′–CCGG–3′ 3′–GGCC–5′	Cohesive
*Not*I	*Nocardia otitidis-caviarum*	5′–GCGGCCGC–3′ 3′–CGCCGGCG–5′	Cohesive
*Pst*I	*Providencia stuartii*	5′–CTGCAG–3′ 3′–GACGTC–5′	Cohesive
*Pvu*II	*Proteus vulgaris*	5′–CAGCTG–3′ 3′–GTCGAC–5′	Blunt
*Sma*I	*Serratia marcescens*	5′–CCCGGG–3′ 3′–GGGCCC–5′	Blunt



Restriction enzymes make double-stranded cuts in the sugar–phosphate backbone of DNA, producing cohesive, or sticky, ends.



The number of restriction sites is related to the number of fragments produced when DNA is cut by a restriction enzyme.

The sequences recognized by a restriction enzyme occur randomly within genomic DNA. Consequently, there is a relation between the length of the recognition sequence and its frequency of occurrence: there are fewer long recognition sequences than short sequences because the probability of all the bases being in the required order is less.

Restriction enzymes are the workhorses of recombinant DNA technology and are used whenever DNA fragments must be cut or joined. In a typical restriction reaction, a concentrated solution of purified DNA is placed in a small tube with a buffer solution and a small amount of restriction enzyme. The reaction

mixture is then heated at the optimal temperature for the enzyme, usually 37^0 C. Within a few hours, the enzyme cuts all the restriction sites in the DNA, producing a set of DNA fragments

Cloning Vectors

- Genetic vectors are vehicles for delivering foreign DNA into recipient cells.
- In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed.
- Vectors can replicate autonomously and typically include features to facilitate the manipulation of DNA as well as a genetic marker for their selective recognition.
- The different types of vectors available for cloning are plasmids, bacteriophages, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) and mammalian artificial chromosomes (MACs).
- The cloning vectors are limited to the size of insert that they can carry. Depending on the size and the application of the insert the suitable vector is selected for a particular purpose.

Essential Characteristics of Cloning Vectors

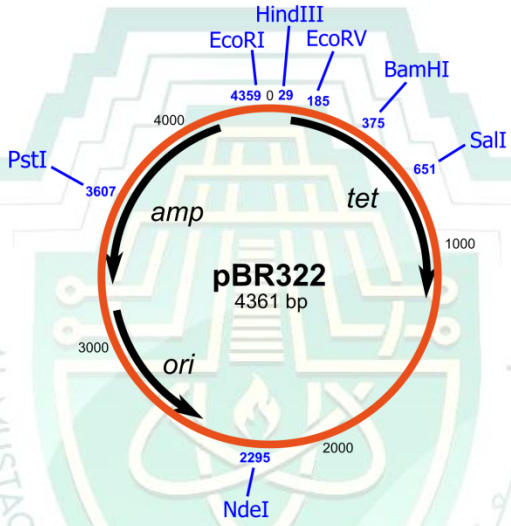
Regardless of the selection of a vector, all vectors are carrier DNA molecules. These carrier molecules should have few common features in general such as:

- It must be self-replicating inside host cell.
- It must possess a unique restriction site for RE enzymes.
- Introduction of donor DNA fragment must not interfere with replication property of the vector.
- It must possess some marker gene such that it can be used for later identification of recombinant cell (usually an antibiotic resistance gene that is absent in the host cell).
- They should be easily isolated from host cell.

Plasmids

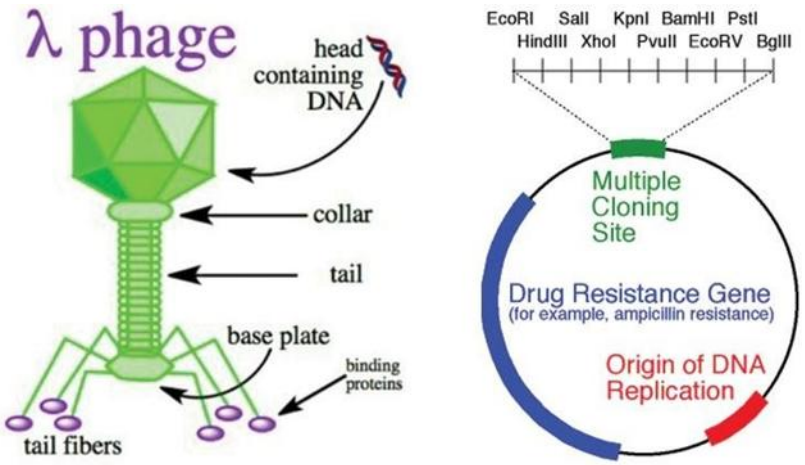
- Plasmids are extra chromosomal circular double stranded DNA replicating elements present in bacterial cells.
- Plasmids show the size ranging from 5.0 kb to 400 kb.
- Plasmids are inserted into bacterial calls by a process called transformation.
- Plasmids can accommodate an insert size of up to 10 kb DNA fragment.

- Generally, plasmid vectors carry a marker gene which is mostly a gene for antibiotic resistance; thereby making any cell that contains the plasmid will grow in presence of the selectable corresponding antibiotic supplied in the media.
- Plasmids have separated origin of replication and multiple cloning site (MCS)
- Example: Agrobacterium plasmid vector, pBR322, and pUC.



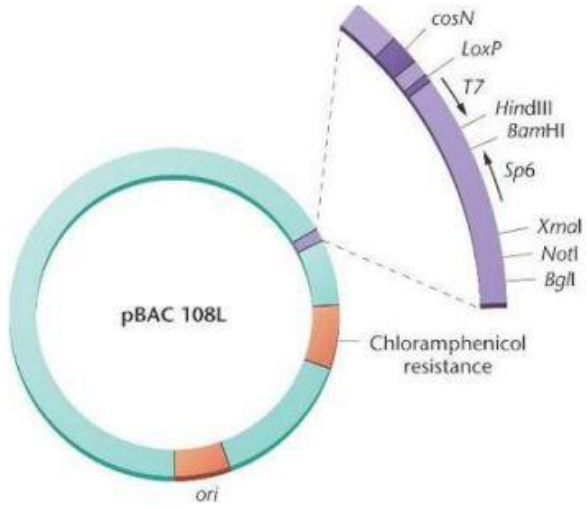
Bacteriophage

- The viruses that infect bacteria are called bacteriophage. These are intracellular obligate parasites that multiply inside bacterial cell by making use of some or all of the host enzymes.
- Bacteriophages have a very high significant mechanism for delivering its genome into bacterial cell. Hence it can be used as a cloning vector to deliver larger DNA segments.
- Most of the bacteriophage genome is non-essential and can be replaced with foreign DNA.
- Using bacteriophage as a vector, a DNA fragment of size up to 20 kb can be transformed.
- Several bacteriophages like Lambda (ʎ) and M13 are used as cloning vectors.



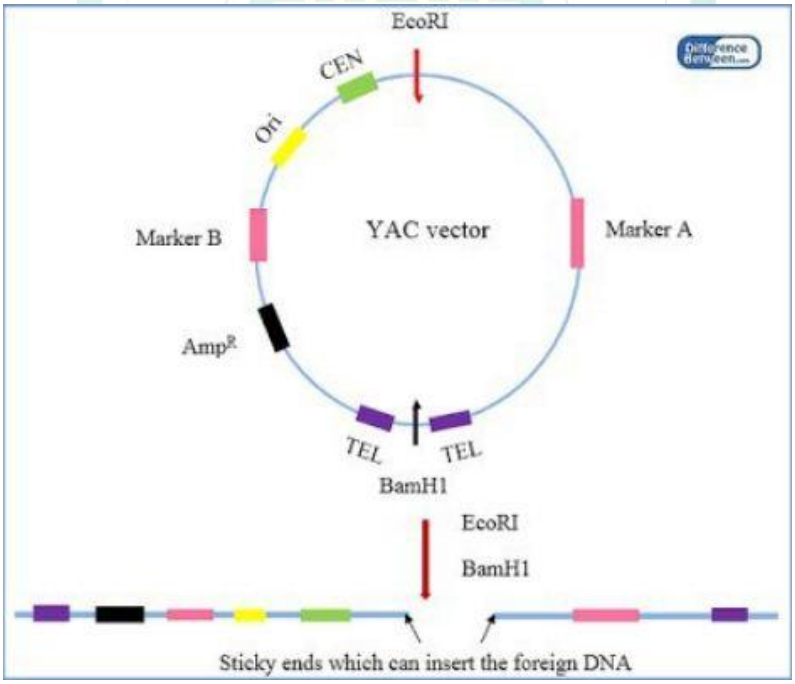
Bacterial artificial chromosomes (BACs)

- Bacterial artificial chromosomes (BACs) are simple plasmid which is designed to clone very large DNA fragments ranging in size from 75 to 300 kb.
- BACs basically have marker like sights such as antibiotic resistance genes and a very stable origin of replication (ori) that promotes the distribution of plasmid after bacterial cell division and maintaining the plasmid copy number to one or two per cell.
- BACs are basically used in sequencing the genome of organisms in genome projects (example: BACs were used in human genome project).
- Several hundred thousand base pair DNA fragments can be cloned using BACs.



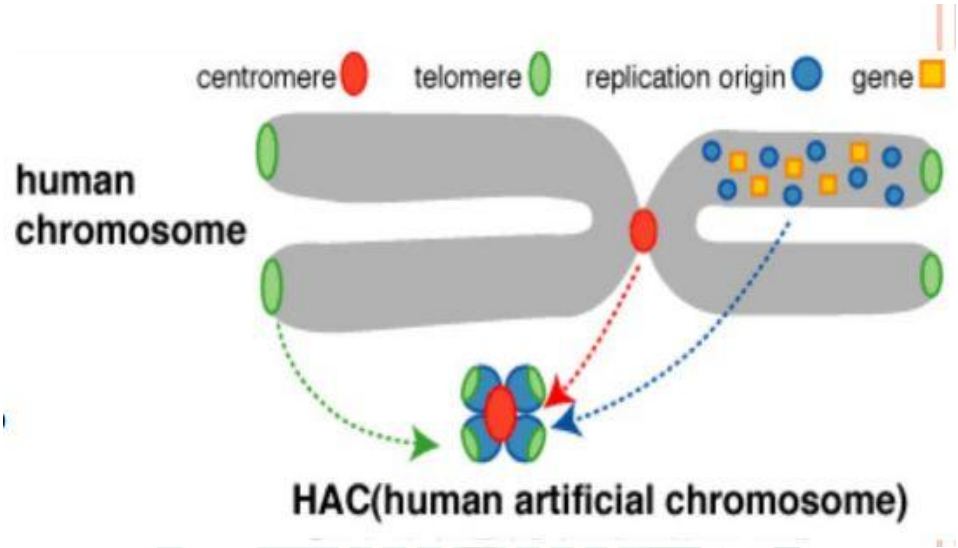
Yeast artificial chromosomes (YACs)

- YACs are yeast expression vectors.
- A very large DNA fragments whose sizes ranging from 100 kb to 3000 kb can be cloned using YACs.
- Mostly YACs are used for cloning very large DNA fragments and for the physical mapping of complex genomes.
- YACs have an advantage over BACs in expressing eukaryotic proteins that require post translational modifications.
- But, YACs are known to produce chimeric effects which make them less stable compared to BACs.



Human artificial chromosomes (HACs)

- Human artificial chromosomes (HACs) or mammalian artificial chromosomes (MACs) are still under development.
- HACs are micro-chromosomes that can act as a new chromosome in a population of human cells.
- HACs range in size from 6 to 10 Mb that carry new genes introduced by human researchers.
- HACs can be used as vectors in transfer of new genes, studying their expression and mammalian chromosomal function can also be elucidated using these micro-chromosomes in mammalian system.



Other Types of Vectors

All vectors may be used for cloning and are therefore cloning vectors, but there are also vectors designed especially for cloning, while others may be designed specifically for other purposes, such as transcription and protein expression.

1. **Expression Vectors**

Vectors designed specifically for the expression of the transgene in the target cell are called expression vectors, and generally have a promoter sequence that drives expression of the transgene. Expression vectors produce proteins through the transcription of the vector's insert followed by translation of the mRNA produced.

2. **Transcription Vectors**

Simpler vectors called transcription vectors are only capable of being transcribed but not translated: they can be replicated in a target cell but not expressed, unlike expression vectors. Transcription vectors are used to amplify their insert.

Uses of Vectors

Vectors have been developed and adapted for a wide range of uses. Two primary uses are:

(1) To isolate, identify and archive fragments of a larger genome

(2) To selectively express proteins encoded by specific genes.

Vectors were the first DNA tools used in genetic engineering, and continue to be cornerstones of the technology.